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BIOMEDICAL RESEARCH ON TRACE ELEMENTS

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Program and Abstracts of the 17th Annual Meeting

日本微量元素学会

Japan Society for Biomedical Research on Trace Elements

The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006)

第17回 日本微量元素学会

Program and Abstracts

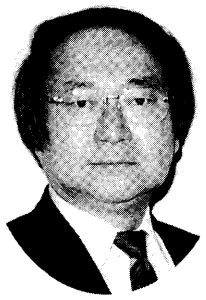
プログラム・アブストラクト集

Chairman of the Organizing Committee:
Yasuaki ARAKAWA (Professor, The University of Shizuoka)
Date: Wednesday, July 12 – Friday, July 14, 2006
Place: GranShip (Shizuoka Convention & Art Center)
Shizuoka, Japan

会長: 荒川 泰昭 (静岡県立大学教授)
会期: 2006年7月12日(水)～14日(金)
会場: グランシップ・静岡

Organized by: Japan Society for Biomedical Research on Trace Elements
Cooperated by:
The Japanese Society for Hygiene, Japan Society for Occupational Health,
The Chemical Society of Japan, The Japan Society for Analytical Chemistry,
The Pharmaceutical Society of Japan, The Japanese Society of Toxicology,
The Japanese Society of Immunotoxicology, Japan Trace Nutrients Research Society,
A Strongpoint for driving 21 Century COE Program, Shizuoka Research Institute
Supported by: Japan Health Nutrition Food Association

主催:日本微量元素学会
共催:日本衛生学会、日本産業衛生学会、日本化学会、日本分析化学会、日本薬学会、
日本トキシコロジー学会、日本免疫毒性学会、日本微量栄養素研究会、文部科学省21世紀
COEプログラム推進拠点、静岡総合研究機構
後援:(財)日本健康・栄養食品協会



Welcome Message

Yasuaki ARAKAWA
Chairman, the Organizing Committee
(Professor, The University of Shizuoka)

It is a great pleasure for me to open the 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE 2006) at Shizuoka and to welcome all of you to this conference. This conference is an interdisciplinary scientific meeting dealing with mechanism analysis, toxic assessment and countermeasure of injurious factors and maintenance of homeostasis in normal biofunction for maintenance and promotion of health and prevention of disease, and further with clinical diagnosis and medical treatment for disease in the fields of health hygienics, medical service, biology, drug manufacture, chemistry, food, nutrition, environment and analysis etc.

This conference is designed to integrate the recent advances made by various disciplines as remarked above in the rapidly expanding field of trace elements. By bringing together investigators ranging in their interests from chemistry to medicine, we have attempted to present a fairly comprehensive coverage.

Particularly in this 17th conference, we are going to focus our attention on the several main themes as follows; 1) Isogenies of Drugs and Food, and Trace Elements, 2) Essentiality and Toxicity of Trace Elements, 3) Disease and Medical Treatment and Trace Elements, 4) Metal Allergy, 5) Ecosystem and Environment and Trace Elements, 6) Metal Complexes and Organism, 7) Biofunctions (Immune System, Brain Nerve System, Endocrine System) and Trace Elements, including the fields of an interaction between trace elements and organism, biological activity and function of trace elements, cell death (necrosis and appoptosis) and trace elements, health function claims (nutrient function claims, enhanced function claims) and medical claims and trace elements, structure and property of metal and metal-complexes, analysis of trace elements, design of functional metal molecule and metal drug, development of new metal-complex and metal-probe, trace element-associated bio-chemical reaction, etc.

Research on trace elements is entering a period of mushrooming growth. Therefore, it is highly desirable that interested scientists gather to report new advances, to review processes, to discuss conflicting findings and divergent interpretations regarding trace elements, and to make more meaningful plans for the future phases and directions of research.

We hope that your participation in this meeting will be rewarding to you and that sharing ideas with colleagues will help deepen understanding and friendship.

Shizuoka is located in the central part of the main island of Japan. The surrounding area provides excellent opportunities for participants to enjoy visits to scenic and interesting spots such as Mt. Fuji or the hot springs of the Izu Peninsula. I wish all participants of the 17th BRTE 2006 a pleasant stay in Shizuoka and an enriching experience, both personal and professional.

Finally, I wish to express my appreciation to the members of the council who assisted in making this 17th conference, to the moderators, speakers, and other participants, and to the dedicated staffs of the Secretariat for their valuable contributions. I would like to thank many companies for the financial support and cooperation we received in organizing and conducting this 17th conference.

History of Annual Meeting of JSBRTE

日本微量元素学会の歴史

Time 回	Year 開催年	Organizing Chairman 会　長	Place 開催地
1	1990年(H2)	Hiroshi TOMITA (Nihon University) 冨田　寛 (日本大・医)	Tokyo 東京
2	1991年(H3)	Tadashi OKADA (Osaka University) 岡田　正 (大阪大・医)	Osaka 大阪
3	1992年(H4)	Shuichi KIMURA (Tohoku University) 木村　修一 (東北大・農)	Sendai 仙台
4	1993 年(H5)	Kazuo NOMIYAMA (Jichi Medical University) 野見山一生 (自治医科大)	Tochigi 栃木
5	1994年(H6)	Ichiro MATSUDA (Kumamoto University) 松田　一郎 (熊本大・医)	Kumamoto 熊本
6	1995年(H7)	Osamu WADA (Tokyo University) 和田　攻 (東京大・医)	Tokyo 東京
7	1996年(H8)	Kenji SOUDA (Kyoto University) 左右田　健次 (京都大・化研)	Kyoto 京都
8	1997年(H9)	Takafumi MORISHIMA (Nihon University) 森嶋　隆文 (日本大・医)	Tokyo 東京
9	1998年(H10)	Kazuo SAITO (Hokkaido University) 斎藤　和雄 (北海道大・医)	Sapporo 札幌
10	1999年(H11)	Fumiaki MARUMO (The University of Tokyo) 丸茂　文昭 (東京医科歯科大)	Tokyo 東京
11	2000年(H12)	Hiroki HARAGUCHI (Nagoya University) 原口　紘忯 (名古屋大・工)	Nagoya 名古屋
12	2001年(H13)	Yasuyuki ARAKAWA (Nihon University) 荒川　泰行 (日本大・医)	Tokyo 東京

13	2002年(H14)	Kazuo SUZUKI (Chiba University) 鈴木 和夫 (千葉大・薬)	Chiba 千葉
14	2003年(H15)	Yoji TAKAGI (Osaka University) 高木 洋治 (大阪大・医)	Osaka 大阪
15	2004年(H16)	Tsugutoshi AOKI (Toho University) 青木 継稔 (東邦大・医)	Tokyo 東京
16	2005年(H17)	Hiromu SAKURAI (Kyoto Pharmaceutical University) 桜井 弘 (京都薬大)	Kyoto 京都
17	2006年(H18)	Yasuaki ARAKAWA (The University of Shizuoka) 荒川 泰昭 (静岡県立大)	Shizuoka 静岡

ORGANIZATION

Organized by : Japan Society for Biomedical Research on Trace Elements
Cooperated by :
The Japanese Society for Hygiene, Japan Society for Occupational Health, The Chemical Society of Japan, The Japan Society for Analytical Chemistry, The Pharmaceutical Society of Japan, The Japanese Society of Toxicology, The Japanese Society of Immunotoxicology, Japan Trace Nutrients Research Society, A Strongpoint for driving 21 Century COE Program, Shizuoka Research Institute,
Supported by : Japan Health Nutrition Food Association

主催：日本微量元素学会
共催：日本衛生学会、日本産業衛生学会、日本化学会、日本分析化学会、日本薬学会、日本トキシコロジー学会、日本免疫毒性学会、日本微量栄養素研究会、文部科学省 21 世紀 COE プログラム推進拠点、静岡総合研究機構
後援：(財) 日本健康・栄養食品協会

Organizing Committee

実行委員会

Chairman 会長	Yasuaki ARAKAWA 荒川 泰昭	The University of Shizuoka 静岡県立大学
Members 委員	Hiroyuki YANAGISAWA 柳沢 裕之	Saitama Medical University 埼玉医科大学
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	Masayoshi YAMAGUCHI 山口 正義	The University of Shizuoka 静岡県立大学
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	Masashi TSUNODA 角田 正史	Kitazato University 北里大学
	Atsushi TAKEDA 武田 厚司	The University of Shizuoka 静岡県立大学
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	Yoshikazu MATSUDA 松田　芳和	Japan Clinic Co.,Ltd 日本クリニック（株）
	Masayuki KAJI 加治　正行	Health & Welfare Bureau, Shizuoka City 静岡市保健福祉局
	Tsutomu SATO 佐藤　　努	The University of Shizuoka 静岡県立大学
Secretary 事務局	Takao KURIYAMA 栗山　孝雄	The University of Shizuoka 静岡県立大学

BRTE2006 Program at a glance

Wednesday July 12, 2006: the first day

Conference Room	Room 1	Room 6	Room 9
	Medium Hall (1, 2F)	1002 (10F)	901 (9F)
9:00—			
10:00—			Committee
12:00—	Doors Open		
12:30—	Opening Address		
13:00—	Civil Extension Special Lecture Tomita H Arakawa Y Takagi Y		Committee
13:40—			
14:20—			
15:00—	Break		
15:10—	Civil Extension Special Lecture Hosoya N		
15:50—	Closing Address		
16:00—			
16:20—		Director•ate	
18:20—			

BRTE2006 Program at a glance

Wednesday July 12, 2006: the first day

Room 10	Room 11	Room 12	Conference Room
902 (9F)	905 (9F)	906 (9F)	
			—9:00
			—10:00
Committee			
			—12:00
			—13:00
Committee			
			—15:00

BRTE2006 Program at a glance

Thursday July 13, 2006: the second day

Conference Room	Room 1 Medium Hall (1, 2F)	Room 2 1001-2(10F)	Room 3 1001-1(10F)	Room 4 910(9F)
8:30—	Doors Open			
9:10—	Opening Address			
9:15—		Topic Lecture (Lecture I-V) Mihara H Fujishiro H Yoshikawa Y Kobayashi Y Nakanishi T Chair: Sakurai T Hasegawa T	Session I (Lecture 1-4) Chair: Nishimuta M	Session IV (Lecture 12-14) Chair: Ebara M
10:00—				
10:15—			Session II (Lecture 5-8) Chair: Chiba M	Session V (Lecture 15-17) Chair: Kawahara M
10:45—				Session VI (Lecture 18-20) Chair:Yamaguchi M
11:15—			Session III (Lecture 9-11) Chair: Endo F	
11:30—				Session VII (Lecture 21-22) Chair: Ikeda M
11:45—				
12:00—				
13:00—	Lunch			
14:00—	Focus Lecture Otsuki T, Nakano T Chair: Yoshida T			
14:40—	Educational Lecture I Kurasawa R Chair: Yanagisawa H			
15:20—	Educational Lecture II Kodama H Chair: Suzuki K			
15:30—	Break			
16:10—	Special Lecture I Amemiya T Chair: Ishikawa H			
16:50—	Special Lecture II Ikezawa Z Chair: Ohsawa M			
17:10—	Souvenir Photographing			
18:30—	Move			
20:30—	Social Gathering Hotel Associa Shizuoka Terminal Sunpu (3F)			

BRTE2006 Program at a glance

Thursday July 13, 2006: the second day

Room 5 1003(10F)	Room 6 1002(10F)	Room 7 1004(10F)	Room 8 904(9F)	Conference Room
				—8:30 —9:15
Exhibition Corner	Tea Corner	Greenroom for Lecturer		
Lunch			Council	—12:00 —13:00
Exhibition Corner	Tea Corner	Greenroom for Lecture		
				—16:50

BRTE2006 Program at a glance

Friday July 14, 2006: the third day

Conference Room	Room 1 Medium Hall (1, 2F)	Room 2 1001-2 (10F)	Room 3 1001-1 (10F)	Room 4 910 (9F)
8:30—	Doors Open			
9:00—		Symposium I (Lecture 1-5) Yanagisawa H Ikeda M Otaka M Moriyama M Yamazaki S Chair: Arakawa Y Takagi Y	Symposium II (Lecture 1-5) Kaji M Okuno T Takeda A Horiguchi T Kawahara M Chair: Saito T Takeda A	
11:10—		Move	Move	
11:20—	Special Lecture III Saji H Chair: Sakurai H			
12:00—	Lunch			
13:00—	Council			
13:30—	Award Lecture Sakurai T Chair: Tamura J			
14:00—	Move			
14:10—		Session VIII (Lecture 23-26) Chair: Endo G	Session XI (Lecture 34-37) Chair: Moriyama M	Session XIV (Lecture 45-46) Chair: Saji H
14:40—				Session XV (Lecture 47-49) Chair: Uchiumi A
15:10—		Session IX (Lecture 27-30) Chair: Kimura M	Session XII (Lecture 38-41) Chair: Himeno S	
15:25—				Session XVI (Lecture 50-51) Chair: Chikuma M
15:55—				Session XVII
16:10—		Session X (Lecture 31-33) Chair: Tsunoda M	Session XIII (Lecture 42-44) Chair: Maitani T	(Lecture 52-55) Chair: Haraguchi H
16:55—	Closing Address			
17:00—				

BRTE2006 Program at a glance

Friday July 14, 2006: the third day

Room 5 1003 (10F)	Room 6 1002 (10F)	Room 7 1004 (10F)	Conference Room
Doors Open			—8:30 —9:00
Exhibition Corner	Tea Corner	Greenroom for Lecturer	
Lunch			—12:00 —13:00
Exhibition Corner	Tea Corner	Greenroom for Lecturer	
			—16:00

The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Program

Civil Extension Special Lectures

The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements

COMMEMORATIVE

Civil Extension Special Lectures

"Supplements and Health"

—Isogenies of drugs and food, and trace elements—

Wednesday July 12, 2006 ; the first day
GranShip · Medium Hall · Earth (1,2 F) (Room 1)

Organized by : Japan Society for Biomedical Research on Trace Elements
Cooperated by : **FANCL CORPORATION**

The First Session

"Prescription of minerals for health · prevention · medical treatment through drugs and supplements"

Special Lecture I 13:00—13:40

A wonder relationship between a vigorous life and powers of zinc

Hiroshi TOMITA (Professor Emeritus, Nihon University · Medicine)

Special Lecture II 13:40—14. 20

Application and Practices of Trace Elements to Medical Treatments in the Spheres of Internal Medicine

Yasuyuki ARAKAWA (Professor, Nihon University · Medicine)

Special Lecture Ⅲ 14. 20—15:00

Supplementation of trace elements in surgical patients during total parental nutrition

Yoji TAKAGI (Professor, Koshien University · Nutrition)
(Professor Emeritus, Osaka University · Medicine)

The Second Session

Special Lecture Ⅳ 15. 10—15:50

"Supplements in Japan: Safety and Availability"

Norimasa HOSOYA (President, Japan Health Nutrition Food Association)
(Professor Emeritus, Tokyo University · Medicine)

The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Program

Special Lectures

Educational Lectures

Focus Lectures

Topic Lectures

Symposium Lectures

Award Lecture

Special Lectures

Thursday July 13, 2006 ; the second day

GranShip · Medium Hall · Earth (1,2 F)

Special Lecture I 15:30—16:10

Trace Elements and the Eye —Morphological Aspects—

Tsugio AMEMIYA (Professor Emeritus, Nagasaki University · Medicine)
Department of Ophthalmology and Visual Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Chairperson : Hiroshi ISHIKAWA (Asso. Professor, Nihon University · Medicine)

Special Lecture II 16:10—16.50

Pathomechanism of metal-related allergic reactions

Zenro IKEZAWA (Professor, Yokohama City University · Medicine)
Department of Environmental Immuno-Dermatology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Chairperson : Motoyasu OHSAWA (Professor, Teikyo University · Pharmaceutical Sciences)

Friday July 14, 2006 ; the third day

GranShip · Medium Hall · Earth (1,2 F)

Special Lecture III 11.20—12:00

Medical application of radioactive metal: Targeted delivery of radiometals for diagnostic imaging and therapeuty

Hideo SAJI (Professor, Kyoto University · Pharmaceutical Sciences)
Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Chairperson : Hiromu SAKURAI (Professor, Kyoto Pharmaceutical University)

Educational Lectures

Thursday July 13, 2006 ; the second day
GranShip · Medium Hall · Earth (1,2 F)

Educational Lecture I 14:00—14:40

Zinc Deficiency and its clinical features in the cases found in Kitamimaki, a rural area in Japan

Ryuuhei KURASAWA, Syujirou KUBORI
Mimaki Onsen Clinic, Nagano, Japan

Chairperson : Hiroyuki YANAGISAWA (Professor, Saitama Medical University)

Educational Lecture II 14:40—15. 20

Copper metabolism and disorders of copper transport

Hiroko KODAMA, (Professor, Teikyo University · Medicine)
Chie FUJISAWA, Yang-Hong GU, Katsuaki SHIGA
Department of Pediatrics, Teikyo University School of Medicine, Tokyo, Japan

Chairperson : Kazuo T. SUZUKI (Professor, Chiba University · Pharmaceutical Sciences)

Focus Lectures

Thursday July 13, 2006 ; the second day
GranShip · Medium Hall · Earth (1,2 F)

"Asbestos and Malignant Mesothelioma"

Focus Lecture I 13:00—13:30

Asbestos-related carcinogenesis and immunological effects

Takemi OTSUKI[1] (Professor, Kawasaki Medical School)
Yoshie MIURA[1], Megumi MAEDA[1], Hiroaki HAYASHI[1], Maolong DONG[1], Tatsumitsu NISHIMURA[1], Masafumi TOMITA[2], Hironobu KATSUYAMA[3]
[1]Department of Hygiene, Kawasaki Medical School, Kurashiki, Japan [2]Department of Medical Toxicology, Kawasaki Medical School, Kurashiki, Japan [3]Department of Public Health, Kawasaki Medical School, Kurashiki, Japan

Focus Lecture II 13:30—14.00

Malignant Mesothelioma : Incidence and Clinical Approach

Takashi NAKANO (Professor, Hyogo College of Medicine)
Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

Chairperson : Takahiko YOSHIDA (Professor, Asahikawa Medical College)

Topic Lectures

Thursday July 13, 2006 ; the second day
GranShip · Medium Hall · Earth (1,2 F)

Topics

"Trace Element Research -Young Force-"

9:15—11:45

Topic Lecture I

Molecular Mechanism of Cadmium Transport in Mammalian Cells

○**Hitomi FUJISHIRO**[1], Satomi Okugaki[1], Keisuke Kubota[1], Shingo Kakibe[1], Masahiko Satoh[2], Seiichiro Himeno[1]

[1]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan
[2]School of Pharmacy, Aichi Gakuin University, Nagoya, Japan

Topic Lecture II

Selenoprotein Biosynthesis and Selenium-Specific Enzymes

○**Hisaaki MIHARA**[1], Suguru KUROKAWA[1], Rie OMI[1], Tatsuo KURIHARA[1], Ken HIROTSU[2], Nobuyoshi ESAKI[1]

[1]Institute for Chemical Research, Kyoto University, Kyoto, Japan [2]Graduate School of Science, Osaka City University, Osaka, Japan

Topic Lecture III

α-Glucosidase inhibitory effect and action mechanism of anti-diabetic zinc and vanadium compounds

○**Yutaka YOSHIKAWA**[1], Ryoko HIRATA[1], Hiromu SAKURAI[1]

[1]Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan

Topic Lecture IV

Metabolic pathway of arsenic based on analytical toxicology

○**Yayoi Kobayashi**[1], Toru Hayakawa[1], Xing Cui[1], Seishiro Hirano[1]

[1]Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Japan

Topic Lecture V

Potential Toxicity of Organoltin Compounds Functioning as Nuclear Receptor Ligands

○**Tsuyoshi NAKANISHI**[1]

[1]Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University

Chairperson : Teruaki SAKURAI (Asso.Professor, Tokushima Bunri University)
Tatsuya HASEGAWA (Yamanashi Institute of Environmental Sciences)

Symposium Lectures

Friday July 14, 2006 ; the third day
GranShip · Conference Room 1001-2 (10F) (Room 2)

Symposium I

"The search for new availability of zinc in clinical medicine"

9:00—11:10

Lecture I 9:00—9:25

Zinc physiology

○**Hiroyuki YANAGISAWA**[1]

[1]Department of Health Science & Preventive Medicine, Faculty of Medicine, Saitama Medical University, Saitama, Japan

Lecture II 9:25—9:50

The role of zinc in taste disorders

○**Minoru IKEDA**[1], Akihiro IKUI[1]

[1]Department of Otolaryngology, Nihon University School of Medicine

Lecture III 9:50—10:15

Induction of heat shock proteins and mucosal protection by zinc derivatives in the gastrointestinal mucosa

○**Michiro OTAKA**[1], Sumio WATANABE[1]

[1]Department of Gastroenterology, Akita University School of Medicine, Akita, Japan

Lecture IV 10:15—10:40

Significance of evaluation of serum zinc concentrations in C-viral chronic liver disease

○**Mitsuhiko MORIYAMA**[1], Yasuyuki ARAKAWA[1]

[1]Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan

Lecture V 10:40—11:05

Zinc and skin diseases

○**Sohji YAMAZAKI**[1]

[1]Department of Dermatology, Dokkyo University School of Medicine, Tochigi, Japan

Chairperson : Yasuyuki ARAKAWA (Professor, Nihon University・Medicine)
Yoji TAKAGI (Professor, Koshien University・Nutrition)

Symposium Lectures

Friday July 14, 2006 ; the third day
GranShip · Conference Room 1001-1 (10F) (Room 3)

Symposium II

"The search for new toxicity of trace elements"

9:00—11:10

Lecture I 9:00—9:25

Blood lead levels in Japanese children: Effects of passive smoking

○**Masayuki KAJI**[1,2], Kazunori BABA[2]

[1]Health and Hygiene Department, Health and Welfare Bureau, Shizuoka City, Japan

[2]Division of Endocrinology and Metabolism, Shizuoka Children's Hospital, Japan

Lecture II 9:25—9:50

Study on detoxification and utilization of selenomethionine: a role of cystathionine γ-lyase in selenomethionine metabolism

○**Tomofumi OKUNO**[1], Tatsuya HASEGAWA[2], Tomoyuki KUBOTA[1], Tomonori KURODA[1], Shinji MOTOBAYASHI[1], Hitoshi UENO[1], Katsuhiko NAKAMURO[1]

[1]Faculty of Pharmaceutical Sciences, Setsunan University [2]Yamanashi Institute of Environmental Sciences, Yamanashi, Japan

Lecture III 9:50—10:15

Hippocampal Cell Death by Synaptic Zinc

○**Atsushi TAKEDA**[1]

[1]Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Japan

Lecture Ⅳ 10:15—10:40

Analysis of induction mechanism of imposex caused by tributyltin and triphenyltin in gastropod molluscs

○**Toshihiro HORIGUCHI**[1], Hiroaki SHIRAISHI[1], Tomohiro NISHIKAWA[1], Fujio SHIRAISHI[1], Jun-ichi NISHIKAWA[2], Yasuhiko OHTA[3], Masatoshi MORITA[1]

[1]Research Center for Environmental Risk, National Institute for Environmental Studies, Tsukuba, Japan [2]Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan [3]Faculty of Agriculture, Tottori University, Tottori, Japan

Lecture Ⅴ 10:40—11:05

Aluminum and human health: an old metal but new toxicity

○**Masahiro KAWAHARA**[1]

[1]Department of Analytical Chemistry, Kyushu University of Health and Welfare

Chairperson :

Takeshi SAITO (Professor, Hokkaido University・Medicine・Health Sciences)

Atsushi TAKEDA (Asso.Professor, University of Shizuoka・Pharmaceutical Sciences)

Award Lecture

Friday July 14, 2006 ; the third day
GranShip · Medium Hall · Earth (1,2 F)

Award Lecture 13:30—14:00

Chemical Biology of Arsenicals

Teruaki SAKURAI
Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University

Chairperson : Jun'ichi TAMYRA (Professor, Gunma University Hospital, Medicine,)

The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Program

Lectures

Lectures

Thursday July 13, 2006 ; the second day
GranShip · Conference Room 1001-1 (10F) (Room 3)

Session I Maintenance · Promotion of Health and Trace Element (9:15-10:15)
Chairperson : Mamoru NISHIMUTA (National Institute of Health and Nutrition)

Lecture 1

Dietary intakes of copper, iron, manganese, and zinc for Ukrainians

○Kunio SHIRAISHI[1], Susumu KO[1], Hideki ARAE[1], Kyoko AYAMA[1], Pavlo V. ZAMOSTYAN[2], Nikolay Y. TSIGANKOV[2], Ivan P. LOS[3], Vitarly N. KORUZUN[3]

[1]Division of Dose Assessment, National Institute of Radiological Sciences [2]Research Centre for Radiation Medicine of Academy of Medical Sciences of Ukraine, Kiev, Ukraine [3]Marzeev Institute of Hygiene and Medical Ecology of the Academy of Medical Science, Kiev, Ukraine

Lecture 2

Element concentrations in blood and urine in relation to residents' drinking water in the Aral Sea region

○Momoko CHIBA[1], Astuko SHINOHARA[2], Astushi OGIHARA[3], Taeko SHIMODA[4], Satoshi SASAKI[5], Sayuri HIRAISHI[2], Miwa SEKINE[2], Yutaka INABA[2]

[1]International University of Health and Welfare, School of Pharmaceutical Sciences, Otawara, Japan [2]Juntendo University School of Medicine, Tokyo, Japan [3]Waseda University School of Human Science, Tokorozawa, Japan [4]Tokyo Health Care University, Tokyo, Japan [5]National Institute of Health and Nutrition, Tokyo, Japan

Lecture 3

Differences in the Serum Zinc Level of Rural and Urban Residents in Tomi City

○Shujiro KUBORI[1], Ryuhei KURASAWA[1], Shinpei OKADA[2], Hiroharu KAMIOKA[3], Miho KOGIRIMA[4]

[1]Mimaki Onsen Clinic, Nagano, Japan [2]Laboratory of Physical Education and Medicine, Nagano, Japan [3]Tokyo University of Agriculture, Tokyo, Japan [4]Doshisha Women's College, Kyoto, Japan

Lecture 4

Mathematical approach to estimate mineral requirements from population data

○Katsuhiko YOKOI[1]

[1]Department of Human Nutrition, Seitoku University Graduate School, Chiba, Japan

SESSION II Ecosystem, Environment and Trace Elements (10:15-11:15)

Chairperson : Momoko CHIBA (International University of Health and Welfare・Pharmaceutical Sciences)

Lecture 5

Contamination by lead and its human health effects in India, Vietnam and Cambodia

○Tetsuro AGUSA[1], Takashi KUNITO[2], Karri RAMU[1], Paromita CHARABORTY[3], Chhoun CHAMNAN[4], Pham Thi Kim TRANG[5], Tu Binh MINH[1], Annamalai SUBRAMANIAN[1], Hisato IWATA[1], Pham Hung VIET[5], Touch Seang TANA[6], Shinsuke TANABE[1]

[1]Center for Marine Environmental Studies, Ehime University, Matsuyama, Japan [2]Faculty of Science, Shinshu University, Matsumoto, Japan [3]St. Thomas Boys School, Howrah, India [4]Inland Fisheries Research and Development Institute, Department of Fisheries, Phnom Penh, Cambodia [5]Center for Environmental Technology and Sustainable Development, Hanoi National University, Hanoi, Vietnam [6]Social and Cultural Observation Unit, Office of the Council of Ministers, Phnom Penh, Cambodia

Lecture 6

Effect of vanadium-containing ground water from Mt. Fuji on human health: Mortarity data around Mt. Fuji

○Yoshiyuki SEKO[1], Kaori WATANABE[1], Tatsuya HASEGAWA[1]

[1]Yamanashi Institute of Environmental Sciences

Lecture 7

Effects of cadmium administration on reproductive performance in Japanese quail (Coturnix japonica)

○Shahidur Rahman[1,2], tomohiro Sasanami[1], Makoto Mori[1], Mariko Mochizuki[3]

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Gifu University, Yanagido, Gifu 501-1193 [3]Nippon Veterinary and Life Science University, Musashino 180-8602, Japan

Lecture 8

Acclimatization to dissolved lead of carp (*Cyprinus carpio*)

○TSUTOMU SATO[1], HISAKI NAKAGAWA[2], SHIN OIKAWA[2], MIDORI WATANABE[3]
[1]School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka, Japan
[2]Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University
[3]Center of Advanced Instrumental Analysis, Kyushu University

SESSION Ⅲ Disease and Trace Elements (11:15-12:00)

Chairperson : Fumio ENDO (Kumamoto University・Medical Sciences)

Lecture 9

Analysis of copper and zinc in the breast milk from mothers with Wilson's disease

○Fumiaya KAGA[1], Katsuaki SHIGA[1], Chie FUJISAWA[1], Hiroko KODAMA[1], Hiroshi TAMAI[2], Norikazu SHIMIZU[3], Kenji KOBAYASHI[4], yoko KATSUYA[4], Rizky ABDULAH[4], HIROSHI KOYAMA[4]
[1]Department of Pediatrics, Teikyo University School of Medicine, Tokyo, Japan
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[3]Department of Pediatrics, Toho University School of Medicine, Tokyo, Japan
[4]Department of Public Health, Gunma University Graduate School of Medicine, Gumma, Japan

Lecture 10

Diagnosis of patients with Menkes disease, carriers and fetuses by gene analysis

○Chie FUJISAWA[1], Katsuaki SHIGA[1], Yan-Hong GU[1], Fumiaya KAGA[1], Hiroko KODAMA[1]
[1]Department of Pediatrics, Teikyo University School of Medicine, Tokyo, Japan

Lecture 11

Wilson disease patients with atypical psychiatric symptoms

○Norikazu SHIMIZU[1], Michiko NAKAMURA[2], Tsugutoshi AOKI[1]
[1]The Second Department of Pediatrics, Toho University School of Medicine, Tokyo, Japan [2]Department of Psychiatry, Toho University School of Medicine, Tokyo, Japan

Thursday July 13, 2006 ; the second day
GranShip · Conference Room 910 (9F) (Room 4)

SESSION Ⅳ Cancer and Trace Elements (9:15-10:00)

Chairperson : Masaaki EBARA (Chiba University · Medicine)

Lecture 12

The association between trace metals and the etiology of patients with hepatocellular carcinoma

○Yoshio MASUYA [1], Masaaki EBARA[1], Shinnichirou OKABE[1], Hiroyuki HUKUDA[1], Seigo YUKISAWA[1], Masaharu YOSHIKAWA[1]

[1]Department of medicine and clinical oncology, school of medicine, chiba university

Lecture 13

Analysis of cisplatin behavior in a non-small cell lung cancer (NSCLC) cell line

○Masaki TAKAHASHI[1], Toshihiro SUZUKI[1], Atsushi YUMOTO[1], Tadayasu TOGAWA[1], Hiromitsu HABA[2], Shuichi ENOMOTO[2], Kazuto NISHIO[3], Shinzo TANABE[1]

[1]Department of Analytical biochemistry, Meiji Pharmaceutical University, Tokyo, Japan, [2]Cyclotron Center, RIKEN (The Institute of Physical and Chemical Research), Saitama, Japan [3]Support Facility of Project Ward, National Cancer Center Hospital, Tokyo, Japan

Lecture 14

Anticancer activity of high selenium broccoli sprouts in the human esophageal cell lines

○Rizky ABDULAH[1], Ahmad FARIED[2], Kenji KOBAYASHI[1], Yoko KATSUYA[1], Hiroyuki KUWANO[2], Hiroshi KOYAMA[1]

[1]Department of Public Health, Graduate School of Medicine, Gunma University, Gunma, Japan [2]Department of General Surgical Science, Graduate School of Medicine, Gunma University

SESSION Ⅴ Bio · functions and Trace Elements (10:00-10:45)

Chairperson : Masahiro KAWAHARA (Kyushu University of Health and Welfare · Pharmaceutical Sciences)

Lecture 15

Zinc action in hippocampal mossy fiber long-term potentiation

○Naomi SAKURADA[1], Atsushi TAKEDA[1], Sayuri FUKE[1], Akira MINAMI[1], Naoto OKU[1]

[1]Department of Medical Biochemistry, School of Pharmaceutical Science, University of Shizuoka, Shizuoka, Japan

Lecture 16

Zinc release in the lateral nucleus of the amygdala by stimulation of the entorhinal cortex

○SACHIE IMANO[1], Atsushi Takeda[1], Hiromasa Itoh[1], Naoto Oku[1]

[1]School of Pharmaceutical Sciences, University of Shizuoka, shizuoka, Japan

Lecture 17

Does zinc deficiency cause repeated infections in elderly? :a pilot study

○Toshiyuki UKITA[1], Toyoho MORITA[2], Jun'ichi TAMURA[1]

[1]Department of General Medicine, Gunma University Hospital, Maebashi, Japan

[2]Ninosawa Hospital, Takasaki, Japan

SESSION VI Metabolic・Regulative Functions and Trace Elements— 1

(10:45-11:30)

Chairperson : Masayoshi YAMAGUCHI (University of Shizuoka・Nutritional Sciences)

Lecture 18

Effect of cobalt and vanadium on the liver glycogen content in the streptozotocin-induced diabetic rats

○Satoru MATSUMOTO[1], Yasutomo NOMURA[2], Zhonggang FENG[1], Takao NAKAMURA[2]

[1]Department of Bio-System Engineering, Faculty of Engineering, Yamagata University, Yonezawa, Japan [2]Department of Environmental Life Science, Graduate Science of Medical Science, Yamagata University, Yonezawa, Japan

Lecture 19

Effect of LiCl evaluated by customized DNA microarray in *C. elegans*

○Ayako Inokuchi[1], Fumiyo Morita[1], Moritoshi Nihira[1], Masami Hirai[2], Koji Arizono[1]

[1]Graduate School of Environmental and Symbiotic Sciences, Prefectural University

of Kumamoto, Kumamoto, Japan [2]School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, Miyazaki, Japan

Lecture 20

Actions of lithium, magnesium and trace elements on neural stem cell differentiation

○Masaya IKEGAWA[1], Kazuto HONDA[2], Kei TASHIRO[1], Mieko KIMURA[3]

[1]Department of Genomic Medical Sciences, Graduate School of Medical Science, Kyoto Prefectural University of Medicine [2]Perkin Elmer Japan, Yokohama, Japan [3]Takeda Research Institute of Life Science, Kyoto, Japan

SESSION Ⅶ Metabolic and Regulative Functions and Trace Elements— 2

(11:30-12:00)

Chairperson : Minoru IKEDA (Nihon University・Medicine)

Lecture 21

Changes in bone mineral density of zinc-deficient rats

○Nobuko TOMINAGA[1,3], Takashi MIYAZAKI[1], Ryuichiro ARAKI[1], Makoto NODERA[1,2], Hiroyuki YANAGISAWA[1]

[1]Department of Health Science & Preventive Medicine, Faculty of Medicine, Saitama Medical University, Saitama, Japan [2]School of Medical Technology and Health, Faculty of Health & Medical Care, Saitama Medical University, Saitama, Japan [3]Division of Laboratory Animal Medicine, Faculty of Medicine, Saitama Medical University, Saitama, Japan

Lecture 22

Role of Zinc and Bone Metabolism: Zinc Enhances Synergistically Anabolic Effect of β-Cryptoxanthin on Bone Components in Rat Femoral Tissues

○SATOSHI UCHIYAMA[1,2], MASAYASHI YAMAGUCHI[1]

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Friday July 14, 2006 ; the third day
GranShip · Conference Room 1001-2 (10F) (Room 2)

SESSION Ⅷ Manifestation of Toxicity and Trace Elements (14:10-15:10)
Chairperson : Ginji ENDO (Osaka City University · Medicine)

Lecture 23

Acute renal injury and harmful systemic effects after intravenous administration of cadmium nitrate

○Emi DOTE[1], Tomotaro DOTE[1], Go NITSUI[1], Koichi KONO[1]

[1]Department of Hygiene and Public Health, Osaka Medical College, Osaka, Japan

Lecture 24

Severe hepatic damage and renal dysfunction after intravenous administration of cadmium fluoride

○Kazuya ADACHI[1], Tomotarou DOTE[1], Emi DOTE[1], Go MITSUI[1], Koichi KONO[1]

[1]Department of Hygiene and Public Health, Osaka Medical College, Takatsuki, Japan

Lecture 25

Acute renal dysfunction, metabolic acidosis and electrolyte abnormalities after intravenous injection of low concentrations of hydrofluoric acid in rats

○Go Mitsui[1], Tomotaro Dote[1], E D[1], Kazuya Adachi[1], Koichi Kono[1]

[1]Department of Hygiene and Public Health, Osaka Medical College, Takatsuki City, Osaka, Japan

Lecture 26

Inhibitory effects of dibutyltin (DBT) dichloride on cytokine productions in murine macrophage cell line J774.1

○Masashi TSUNODA[1], Kunihito YAMAMOTO[2], Kyoko Ito[1], Yoko INOUE[1], Takeo MIKI[1], Yuichiro KUDO[1], Toshihiko SATOH[1], Yoshiharu AIZAWA[1]

[1]Department of Preventive Medicine and Public Health, Kitasato University School of Medicine [2]Kitasato University School of Allied Health Sciences

SESSION Ⅸ Nutrition and Trace Elements (15:10-16:10)
Chairperson : Mieko KIMURA (Takeda Research Institute of Life Sciences)

Lecture 27

Improvement in Trace Element Deficiency with Nutritional Supervision and Guidance to Take Powder Tea Leaves

Miho SUMIDA[1], Naomi MORITA[1], Yuka MATUMOTO[1], Asumi NAGAI[1], Mai IWASE[1], Sayaka KOJIMA[1], Sonoko NINOMIYA[1], Yuka IWANO[1], Yuumi SEKO[1], ○Naomi KATAYAMA[1,2]

[1]Department of Food Science and Nutrition, Faculty of Human Life and Environmental Sciences, Nagoya Women's University [2]Nagoya University School of Medicin

Lecture 28

Effect of Administration mulberry leaves powder on improvement of anemia and serum lipid levels in the iron deficient rats

○Noriko AKAZAWA[1], Koichi SUZUKI[2], Koichiro SERA[3]

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Lecture 29

Ingested trace elements and their blood concentrations in inpatients with enteral liquid food

○Noboru SAITO[1], Naoto TABATA[2], Yoshihisa ANDOU[2], Saburou SAITO[1], Katsuko UCHINO[3], Naoko UNOSHIMA[1], Riichirou TODA[1], Izumi YAMAGUCHI[4]

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Lecture 30

Maternal zinc deficiency during lactation period modify pups' salt taste preference after weanling.

○Michio KOMAI[1], Yasunori TANAKA[1], Hitoshi SHIRAKAWA[1]

[1]Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

SESSION X Foods and Trace Elements (16:10-16:55)

Chairperson : Masashi TSUNODA (Kitasato University・Medicine)

Lecture 31

Fluoride levels in various tea-based beverages of Japan and in Chinese tea infusions

○Tetsuo SHIMMURA[1], Hirofumi NAGASE[1], Tetsuo ARATANI[1], Sadanobu KAGAMIMORI[2]

[1]Department of Environmental Health, Toyama Institute of Health, Toyama, Japan [2]Department of Welfare Promotion and Epidemiology, Faculty of Medicine, University of Toyama, Toyama, Japan

Lecture 32

Nutritional availability of selenium in selenium-enriched radish sprouts and selenium-enriched pumpkin

○Munehiro YOSHIDA[1], Kikue SANO[1], Eri ISHIYUKI[1], Kenji FUKUNAGA[1], Toshimasa NISHIYAMA[2]

[1]Laboratory of Food and Nutritional Sciences, Department of Biotechnology, Faculty of Engineering, Kansai University, Suita, Japan [2]Department of Public Health, Kansai Medical University, Moriguchi, Japan

Lecture 33

Intracellular changes of trace elements by fucoidan extracted from *OKINAWA MOZUKU*

○Hisashi TAKADA[1], Takeaki Nagamine[1], Kyoumi Nakazato[2], Takahiko Kusakabe[1], Katsuyuki Nakajima[2], Keiji Suzuki[2], Takuro Sakai[3], Masakazu Oikawa[3], Kazuo Arakawa[3], Masahiko Iha[4]

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Friday July 14, 2006 ; the third day
GranShip · Conference Room 1001-1 (10F) (Room 3)

SESSION XI Absorption and Excretion of Trace Elements (14:10-15:10)

Chairperson : Mitsuhiko MORIYAMA (Nihon University · Medicine)

Lecture 34

Effects of zinc deficiency on rat intestinal mucosa

○Makoto NODERA[1,2], Takashi MIYAZAKI[1], Ryuichiro ARAKI[1], Nobuko TOMINAGA[1,3], Hiroyuki YANAGISAWA[1]

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Lecture 35

Effect of difference in zinc source and dietary zinc level on absorption and tissue concentration of zinc in rats

○Yoshikazu MATSUDA[1], Mamiko ABE[1], Nami KOMURA[1], Tomoe KAWASHIMA[1], Kenji FUKUNAGA[2], Yasuaki ARAKAWA[3], Munehiro YOSHIDA[2]

[1]Central Research Institute, Japan Clinic Co., Ltd., Kyoto, Japan [2]Department of Biotechnology, Faculty of Engineering, Kansai University [3]Department of Hygiene and Preventive Medicine, Faculty of Health Sciences, The University of Shizuoka

Lecture 36

Analysis of molecular mechanism of a copper intestinal absorption obstruction by zinc

○Takeshi SAITO[1], Mari MIURA[2], Masaaki KURASAKI[3], Toshiyuki HOSOKAWA[4], Hiroyuki YANAGISAWA[5]

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Lecture 37

CueO, cuprous oxidase concerned in the copper efflux system of E. coli

○Yusaku UEKI[1], Yushuke KONNO[1], Kunishige KATAOKA[1], Takeshi SAKURAI[1]

[1]Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Japan

SESSION XII Movement of Trace Elements in the body (15:10-16:10)

Chairperson : Seiichiro HIMENO (Tokushima Bunri University・Pharmaceutical Sciences)

Lecture 38

Comparative study of behavior of bismuth and antimony administered intratracheally to rat

○Atsuko SHINOHARA[1], Momoko CHIBA[1,2], Hiroshi SATO[3], Kazuyuki OMAE[4], Masahide OKAMOTO[5], Koji SERIZAWA[5], Yutaka INABA[1]

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Lecture 39

Changes of elements concentration and distribution in the liver and kidney of masked palm civet (Paguma larvata) infected with sarcoptic mange

○Yoshinari SUZUKI[1], Megumi INAOKA[1], Yuji KASHIMA[2], Hifumi SAITO[3], Izumi WATANABE[1], Katsuji KUNO[1]

[1]United Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan [2]Environmental Science Department, Japan Environmental Sanitation Center, Kanagawa, Japan [3]College of Environmental Health, Azabu University, Kanagawa, Japan

Lecture 40

Serum fluoride concentrations in healthy adult populations

○Kazuyoshi ITAI[1], Toshiyuki ONODA[1], Masaru NOHARA[1], Akira OKAYAMA[2], Masaki OHSAWA[1], Kouzou TANNO[1], Kiyomi SAKATA[1]

[1]Department of Hygiene and Preventive Medicine, Iwate Medical University, Morioka,

Japan [2]Department of Preventive Cardiology, National Cardiovascular Center

Lecture 41

The study for a effect of radiation-hormesis in Misasa spa

○Sayoko OHMORI[1], Miyoko FUJII[1], Kimiko HORIUCHI[1], Masaaki MIFUNE[2]
[1]School of Social Information, Otsuma women's university, Tokyo, Japan [2]Okayama University Medical School, Tottori, Japan

SESSION XV Assessment of Safety on Trace Elements (16:10-16:55)

Chairperson : Tamio MAITANI (National Institute of Health Sciences)

Lecture 42

Safety evaluation of inorganic antimicrobial agents (1)-Analysis of inorganic antimicrobial agents used in antimicrobial products-

○Harunobu NAKASHIMA[1], Tomoko OOSHIMA[2], Takao KURIYAMA[3], Yasuaki ARAKAWA[3]
[1]Osaka Prefectural Institute of Public Health, Osaka, Japan [2]Osaka City Institute of Public Health and Environmental Sciences, Osaka, Japan [3]Department of Hygiene & Preventive Medicine, Faculty of Health Sciences, The University of Shizuoka, Shizuoka, Japan

Lecture 43

Safety evaluation of inorganic antimicrobial agents (2)-Elution of metals with artificial sweat and saliva from antimicrobial agents and processed cloths-

○Harunobu NAKASHIMA[1], Naoko MIYANO[1], Tadashi TKATUKA[2], Takao KURIYAMA[3], Yasuaki ARAKAWA[3]
[1]Osaka Prefectural Institute of Public Health, Osaka, Japan [2]Technology Research Institute of Osaka Prefecture [3]Department of Hygiene & Preventive Medicine, Faculty of Health Sciences, The University of Shizuoka, Shizuoka, Japan

Lecture 44

Safety evaluation of inorganic antimicrobial agents (3)-Influences of inorganic antimicrobial agents on indigenous microorganisms on skin -

Harunobu NAKASHIMA[1], ○Tadashi TAKATUKA[2], Takao KURIYAMA[3], Yasuaki ARAKAWA[3]
[1]Osaka Prefectural Institute of Public Health, Osaka, Japan [2]Technology Research Institute of Osaka Prefecture [3]Department of Hygiene & Preventive Medicine, Faculty of Health Sciences, The University of Shizuoka, Shizuoka, Japan

Friday July 14, 2006 ; the third day
GranShip · Conference Room 910 (9F) (Room 4)

SESSION XIV Development and Design of New Function on Trace Elements
(14:10-14:40)

Chairperson : Hideo SAJI (Kyoto University · Pharmaceutical Sciences)

Lecture 45

Antioxidant activities of synthesized selenocompounds without selenol groups

○Yoshihiro SAITO[1], Daisuke UMEMOTO[1], Ai MATSUNAGA[1], Takaji SATO[1], Masahiko CHIKUMA[1]

[1]Department of Bio-Analytical Chemistry, Osaka University of Pharmaceutical Sciences, Osaka, Japan

Lecture 46

Normalizing effect of zinc(II) complex with 6-ethoxy picolinic acid on blood glucose levels in KK-A^y mice

○Mika MORISHITA[1], Midori NISHIDE[1], Kinuyo MATSUMOTO[1], Yusuke ADACHI[2], Yutaka YOSHIKAWA[2], Hiromu SAKURAI[2], Naemi KAJIWARA[1]

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SESSION XV Trace Element-Associated Bio-Chemical Reaction－1
(14:40-15:25)

Chairperson : Akira UCHIUMI (Forman Science and Technology Laboratories Co.,Ltd.)

Lecture 47

A study on the mechanism relating to the inhibition of lipid peroxidation by copper histidine complex and Cu, Zn-superoxide dismutase activity in blood

Setsuko INOUE[1], Shigeru NAKAJIMA[1], ○Naoki SHIDA[2], Yuko AMAMIYA[2], Fumiyuki YAMAKURA[3], Keiichi IKEDA[3], Hideaki IWAI[2]

[1]Department of Health and Nutrition, Bunkyo University Women's College [2]Department of Environmental and Industrial Health, Juntendo University, School of Health and Sports Science [3]Department of Chemistry, Juntendo University, School of Medicine

Lecture 48

Alleviation by long-chain fatty acids and their salts of inhibition by oxalates of a copper enzyme, tyrosinase

○Atsushi HIRAOKA[1], Mika Shirao[2], Hiroko Kasai[3], Masayoshi Tsubuki[3]

[1]Kyorin University School of Health Sciences, Hachioji-shi, Tokyo, Japan [2]Jissen Womens College, Hino-shi, Tokyo, Japan [3]Hoshi University, Shinagawa-ku, Tokyo, Japan

Lecture 49

Effects of Hydroxy-monoazine- and -diazine-type Heterocycles on Removal of Depleted Uranium in Rats

○Akira KATOH[1], Mizuyo IKEDA[2], Mariko NAKAMURA[2], Satoshi FUKUDA[2]

[1]Department of Materials and Life Science, Faculty of Science and Technology, Seikei University, Tokyo, Japan [2]Research Center for Radiation Emergency Medicine, Natural Institute of Radiological Sciences, Chiba, Japan

SESSION XVI Trace Element-Associated Bio-Chemical Reaction－2

(15:25-15:55)

Chairperson: Masahiko CHIKUMA (Osaka University of Pharmaceutical Sciences)

Lecture 50

Aggregation of recombinant PrP provoked by coexistence of nicotinamide adenine dinucleotide coenzyme and metal

○NORIYUKI SHIRAISHI[1], Morimitsu Nishikimi[1]

[1]Department of Biochemistry, Wakayama Medical University, Wakayama, Japan

Lecture 51

Copper binding of nuclear RNA- and DNA-binding proteins $p54^{nrb}$/NonO and PSF

○Masaki WATANABE[1], Masakatsu TEZUKA[1]

[1]Department of Health Science, College of Pharmacy, Nihon University, Chiba, Japan

SESSION XVII Development of the Analytical Technique on Trace Elements

(15:55-16:55)

Chairperson : Hiroki HARAGUCHI (Nagoya University・Engineering)

Lecture 52

Detection of metallothionein isoforms in isoform-specific knock down cells by nano-speciation using 2D capillary HPLC-ICP-MS

○Takamitsu Miyayama[1], Yasumitsu Ogra[1], Kazuo Suzuki[1]

[1]Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

Lecture 53

Direct determination of ceruloplasmin in human blood serum by HPLC-inductively coupled plasma-mass spectrometry for diagnose of Wilson disease

○Kenji KOBAYASHI[1], Yoko KATSUYA[1], Rizky ABDULAH[1], Chie FUJISAWA[2], Takeaki NAGAMINE[3], Akihiro MORIKAWA[4], Masami MURAKAMI[5], Hiroko KODAMA[2], Hiroshi KOYAMA[1]

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Lecture 54

Determination of Fluoride Ion Concentrations by Flow Injection Method in Culture Medium Supplemented with NaF

○Tsutomu SATO[1], Tomoko TANAKA[1], Ken YAEGAKI[1], Kazuyoshi ITAI[2]

[1]Dept. of Oral Health, School of Life Dentistry at Tokyo, Nippon Dental University, Tokyo, Japan [2]Department of Hygine and Public Health, School of Medcine, Iwate Medical University, Morioka, Japan

Lecture 55

Metal contaminants in evacuated blood-collection tube used for trace metal assays

○Kenji KAWASAKI[1], Mitsutoshi SUGANO[1], Shozo NOMOTO[2]

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The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Abstracts

Special Lectures

Special Lecture I Trace Elements and the Eye —Morphological Aspects—

○Tsugio AMEMIYA

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1. Introduction

Some trace elements are essential for living, while some are toxic. Trace elements have been studied mainly symptomatically and biochemically. However, morphological study is very rare. The purpose of the present paper is to describe in the eye morphological aspects of deficiency and intoxication of trace elements such as Zn, Mg, Cu, Mn, Al, Se and Cr, as the abstract of a special lecture of the 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements.

2. Zinc

1) Zn deficiency

Twenty-one-day-old male Wistar Kyoto rats weighing 40-50g were fed a Zn-deficient diet containing 0.007mg Zn/100g with complete all other nutrients and were divided into the following 4 groups: 1. Control group: Zn-deficient diet+3mg Zn/100g deionized and distilled water (water). 2. Zn-deficient group: Zn-deficient diet+water. 3. Recovery group: Zn-deficient diet for 7 weeks, then Zn-deficient diet+3mg Zn/100g water. 4. Pair-fed control group: Supplied with the same amount of Zn-deficient diet and 3mgZn/100g water which Zn-deficient group took previous day. All rats were killed 5 weeks after experiments.

Results in the conjunctiva and cornea: In Zn-deficient rats, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed increased dark cells in the most superficial epithelial layer. Microvilli and goblet cells were decreased in number. Goblet cells were small and shrunken with poorly developed secretory granules. The fibrils and desmosomes were decreased in the cytoplasm of the epithelial cells. The rats recovered from Zn deficiency had many microvilli, microplicae and desmosomes in the epithelial cells in the conjunctiva and cornea. The goblet cells contained well-developed secretory granules.

TOF-SIMS detection: In the Zn-deficient group, Zn, Ca, Al and S counts were significantly lower in the conjunctiva than in controls, while K, Fe and Cl counts were significantly higher in Zn deficiency than in controls. The Cl and S count was significantly higher in the cornea of the Zn-deficient group, while Ca was significantly less in that of the Zn-deficient group.

Results in the retina: In rats fed a Zn-deficient diet for 7 weeks, photoreceptor outer segments (OS) showed the decrease of discs. The retinal pigment epithelium (RPE) contained many lamellar structures and intercellular dehiscence.

Results in the optic nerve: TEM of the optic nerve in the Zn-deficient rats showed increased unmyelinated axons, destruction of myelin and proliferation of glial cells. These changes became more prominent with time and never recovered in the recovery experiment.

Discussion: Zn is associated with many metabolisms by Zn containing enzymes, epithelial proliferation, development and epithelial movement and attachment through Zn-finger. These Zn function damages were morphologically proved.

2) Contribution of Zn to corneal epithelial wound healing

The purpose of this section is to clarify whether wound healing is promoted by Zn supplementation under normal and pathological conditions, because this thesis is still controversial.

Materials and methods: Twenty-one-day-old Wistar Kyoto rats weighing 40 to 50g were divided randomly into 4 groups. 1. Control group: a usual diet containing 4.7mgZn/100g+water. 2. Zn-deficient group: 0.007mgZn/100g diet+water. 3. Recovery group: Zn-deficient rats fed a usual diet+30mgZn/100g water immediately after corneal abrasion. 4. Zn-supplemented group: a usual diet+30mgZn/100g water.

Corneal epithelial abrasion: Under a stereomicroscope, the epithelium in all rats was completely abraded

with a razor blade from the limbus to limbus 6 weeks after birth under Nembutal anesthesia. The completeness of the abrasion was confirmed with fluoresceint test paper.
The rats were killed under Nembutal anesthesia 12h, 1, 3, 5 days, and 1 and 2 weeks after epithelial abrasion and corneas were examined by electron microscopy.
Results: At 12h after abrasion, no epithelial cells were seen in any of the corneas. However, the corneas of rats in the normal control, Zn-supplemented and recovery groups had many polymorphic cells in the stroma. On the other hand, the corneas of Zn-deficient rats had no polymorphic cells in the stroma. On the 1st day after abrasion, epithelial cells appeared in the normal rat with Zn supplementation, but no epithelial cells in the other groups. On the 3rd day epithelial cells were seen in all groups, and they had desmosomes, but these in Zn-deficient group were only a few. In corneas 2 weeks after abrasion, epithelial cells were almost regularly and compactly arranged in the control, Zn-supplemented and recovery groups, but epithelial cell layer was still less in number in the Zn-deficient group.
In all groups, histochemical activities of integrin β_4, β_5, cadherin and laminin appeared in the 3rd day after abrasion and increased in intensity until the 7th day, and then returned to normal.
Discussion: Excessive Zn apparently accelerated corneal epithelial cell regeneration and Zn deficiency made epithelial cell repair delayed. Zn is contained in many enzymes which are related to protein and collagen metabolisms, cell movement, migration, intercellular attachment, development, etc. All these dysfunctions due to Zn deficiency may delay regeneration of the epithelial cells.

3. Magnesium
Mg-deficient experiment: In the Mg-deficient group, mother rats were given free access to a Mg-deficient diet containing 0.1mgMg/100g diet and water. Infant rats were suckled by these mother rats for 21 days, and then fed the same Mg-deficient diet. Control mother rats were fed usual commercial rat pellets, and infants rats were also fed the same diet starting 21 days after birth. The routine pair-fed control experiment was done. All rats were killed at 6 weeks of age.
Results in the retina: Some RPE cells showed multifocal necrosis, breake-down of the nuclear membrane, sparse chromatin granules, breake-down of the cytoplasmic membrane and destruction of intracytoplasmic organelles.
Results in the optic nerve: Small foci of necrosis were seen in the cluster of axons in the optic nerves of Mg-deficient rats. With time of deficiency, myelinated nerve fibers were prominently decreased.
Discussion: Most intracellular Mg is associated with organic compounds and particularly with ATP. Thus, a relatively small change in the percentage of bound Mg may lead to major changes in the amount of Mg^{2+}. Multifocal necrosis in the retina and optic nerve were specific in Mg deficiency. The mechanism of multifocal necrosis is unknown, probably many causes related to Mg function affect cytoplasmic organelles and myelin. Mg deficiency may induce high Ca level which causes nitric oxide synthase in neurons and nitric cytotoxicity and damage in essentially all kinase reactions. The activity of superoxide dismutase reduces in Mg-deficient retinal tissues. Thus Mg deficiency may reduce stabilization of cell membrane and cell wall.

4. Chromium
Cr has been shown to be involved in the metabolism of glucose and lipid. Patients on total parenteral nutrition developed severe diabetic symptoms that are refractory to insulin. However, morphologic alterations due to Cr deficiency are still unknown. The purpose of this section is to show morphologic changes in the retina.
Cr-deficient experiment: The 21-day-old Wistar Kyoto rats were fed a low-Cr powdered diet which is the AIN-93G formula-based diet containing sucrose without added Cr. Control rats were fed the same formula containing 0.9295mg Cr/100g diet. Rats were killed after 7 and 18 months on a low Cr diet.
Clinical features: The blood glucose level in the rats fed a low-Cr diet more than 7 months was significantly higher than that in controls.
Results in the retina: In rats on a low-Cr diet for 7 months, the RPE contained many lamellar structures, dense bodies and ribosomes. In rats fed a low-Cr diet for 18 months, the entire retina was severely destroyed and the structural architecture scarcely remained except the RPE and inner plexiform layer.
Discussion: The retinal changes in Cr-deficiency are quite different from those seen in other trace element studies by the author and his associates. The findings in Cr-deficient rats were rather similar to

retinal diabetic changes.

5. The morphologic similarity between Mn and Cu deficiencies

1) Mn deficiency

Mn-deficient experiment: The offspring of Mn-deficient Wistar Kyoto rat parents (the second generation of Mn-deficient rats) continued to be fed a Mn-deficient solid diet containing 0.23mgMn/100g and water. Rats were examined at age 2 months.

Results in the cornea: The corneal epithelial cells of Mn-deficient rats had very few microvilli and bundles of tonofibrils in micrographs. In the basal layer of the epithelium, mitochondria were numerous, but abnormal in shape and low in electron density.

2) Cu deficiency

Cu-deficient experiment: During gestation and lactation, female Wistar Kyoto rats were given a purified diet low in Cu prescribed by the American Institute of Nutrition (1977). Control rats were given the same diet and water supplemented with 20ppm Cu. After 11 weeks on a Cu-deficient diet, the retina was examined by electron microscopy and cytochemistry.

Results in the retina: The diaminobenzidine (DAB) method of Seligman et al (1968) showed reactive products with oxidation of DAB through cytochrome-c oxidase were seen in the mitochondrial intermembrane-intracristal space in the photoreceptor inner segment (IS) of normal and Cu-deficient rats. However, the reaction to cytochrome oxidase in the IS was apparently reduced by light micrographs. DAB-OsO_4 reaction products were fewer in Cu-deficient rats than in controls. The area of reaction products measured with a computed image analyzer Zeiss IBAS by a point count method showed significantly low reactive products (low cytochrome oxidase activity) in Cu-deficient rats than controls.

Discussion: The morphological changes in Mn and Cu deficiencies appear in mitochondria, because Cu-containing cytochrome oxidase and Mn superoxide dismutase are located in mitochondria. Thus Mn and Cu deficiencies induce destruction of mitochondria which makes mitochondria increase in number for compensation.

6. Selenium

Se-deficient experiment: Twenty-one-day-old female Wistar Kyoto rats were fed a Se-deficient diet (Hafeman et al, 1974). Eyes were examined after 3, 6, 12 and 18 months.

Results in the retina: In rats fed a Se-deficient diet for 18 months, the retina was thin. The inner nuclear layer became two layers consisting mainly of bipolar and Müller cells. The photoreceptor cells were prominently decreased in number. The RPE showed scarce lipofuscin granules.

Results in the rectus muscle: No lipofuscin granules were found in the recti muscle at any period of the experiment.

Discussion: According to the author's report, OS degeneration and increased lipofuscin granules in the RPE and rectus muscle are prominent and characteristic. The fact that the discs of the OS contain much polyunsaturated lipid and vitamin E suggests that the OS disc degeneration may be due to peroxidation of the disc containing polyunsaturated lipids induced by vitamin E deficiency. The findings in vitamin E deficiency were not found in Se-deficiency. Although it has been suggested that Se deficiency and a subsequent lack of adequate level of glutathione peroxidase can lead to peroxidative damage of lipid membranes by H_2O_2, the effect of Se deficiency on the retina and rectus muscle may be different from that of vitamin E deficiency. Antioxidant effect of Se may be weaker than that of vitamin E. Se might interact with the activity of vitamin E.

7. Intoxication of aluminum and copper

1) Al excessive experiment: 1. Al-treated group: Normal 4-week-old Wistar Kyoto rats were injected in with 0.3ml of 4% $AlCl_3$ everyday for up to 16 weeks. 2. Control group: Age-equivalent normal rats were treated with physiological sodium solution instead of $AlCl_3$.

All rats were killed on the 1st day of the 8th, 12th and 16th weeks after the start of administration of $AlCl_3$ or physiological sodium solution.

Results: The serum Al level increased with the dose of Al administered; in the controls it was always less than 10μg/l, the limit of sensitivity. In rats treated with $AlCl_3$ for 8 and 12 weeks, the photoreceptor cell nuclei did not decrease, but Müller cells in outer nuclear layer were vacuolated and some has disappeared

altogether. However, rats treated with $AlCl_3$ for 16 weeks showed two thirds of that of the control rat retina in thickness: thin RPE and disappearance of the photoreceptor cells and appearance of unidentifiable cells with debris of intracytoplasmic organelles and dense irregular granules. Energy dispersive X ray analyzer detected Al at the dense irregular granules in unidentifiable cells between the inner nuclear layer and the RPE.

2) Cu excessive experiment: 1. Ten-month-old Wistar Kyoto rats were injected intraperitoneally with 5mg/ml of $CuCl_2$/Kg body weight once a week. 2. Age-equivalent control rats were injected intraperitoneally with 1ml of water. After 4 weeks the retina was examined.
Results in the retina: The inner nuclear layer of the retina showed clumps of chromatin and pyknosis in the nucleus and high density deposits were seen in secondary lysosomes in the apical portion of the RPE. Energy dispersive X ray analyzer showed iron, but not copper.
Discussion: Al deposition is proved in the brain of Alzheimer's patients. The retina is very much like that of the brain. Since Al has an affinity for the central nervous system, Al may be deposited in the retina and toxic.
Cu excess induced Fe accumulation in the retina and very similar damages to siderosis, probably affected in part by long-term Cu excess. Experimental hypercupuremia may induce secondary siderosis.

8. Interaction between Zn and vitamin A
Materials and methods: Three-week-old normal weanling Wistar Kyoto rats were used. Rats were divided into the following 5 groups. 1) Group A(+)Zn(+): normal group. 2) Group A(-): vitamin A-deficient diet (less than 0.01mg retinol/100g diet). 3) Group A(-)Zn(+): vitamin A deficient diet+water with 30mg Zn/100g. 4) Group Zn(-): Zn-deficient diet containing 0.007mgZn/100g. 5) Group Zn(-)A(+): Zn-deficient diet+3000IU retinol palmitate intraperitoneal injection/week.
Findings in the conjunctiva and cornea: In group A(-), the surface of the conjunctiva and cornea showed no microvilli and microplicae, and showed keratinization and desquamation in the superficial epithelium (Fig. 1) and less goblet cells. In group A(-)Zn(+), however, goblet cells were seen, but no secretory granules. In the conjunctiva and cornea, microvilli and microplicae were seen, but fewer than in group A(+)Zn(+) and more numerous than in group A(-). Group A(-)Zn(+) showed no keratinization. In group Zn(-), the conjunctiva and cornea had less microvilli and microplicae (Fig. 2) and decreased goblet cells, while group Zn(-)A(+) group had a little decrease in microvilli and microplicae, but showed more these than did group Zn(-). Zn(-)A(+) group had less goblet cell secretory granules in number, smaller in shape and more shrunken than in group A(+)Zn(+), but more granules than did those of group Zn(-). Keratinization never appeared in groups Zn(-) (Fig. 2) and Zn(-)A(+) (Fig.3).
Discussion: Zn has the obligatory role on the synthesis of its carrier protein, retinol-binding protein (RBP). Zn maintains the serum vitamin A level and plays a role in the release of vitamin A from the liver by influencing either the synthesis of RBP or release of the RBP-vitamin A complex from the liver. Zn deficiency can depress the synthesis of RBP in the liver and lead to a lower concentration of RBP in the plasma. Zn is necessary for the oxidative conversion of retinol to retinal. In Zn deficiency, Zn-containing alcohol dehydrogenase activity declines significantly and may impair the corneal wound healing effect. Supplementation of Zn may accelerate wound healing or delay the onset of corneal and conjunctival changes due to vitamin A deficiency in group A(-)Zn(+). The DNA-binding domain of the RAR which contains zinc-finger is susceptible to intercellularly relevant oxidizing agents. Zn plays functional role in regulating DNA protein interactions of zinc finger protein. In Zn deficiency, the structure of these proteins could be abnormal, yielding a possible explanation for the resemblance of signs of Zn deficiency to those of vitamin A deficiency. The findings in group A(-)Zn(+) showed that excessive Zn may prevent epithelial damage due to vitamin A deficiency. The concept of zinc finger proteins may explain the intriguing possibility of interaction between vitamin A and Zn.

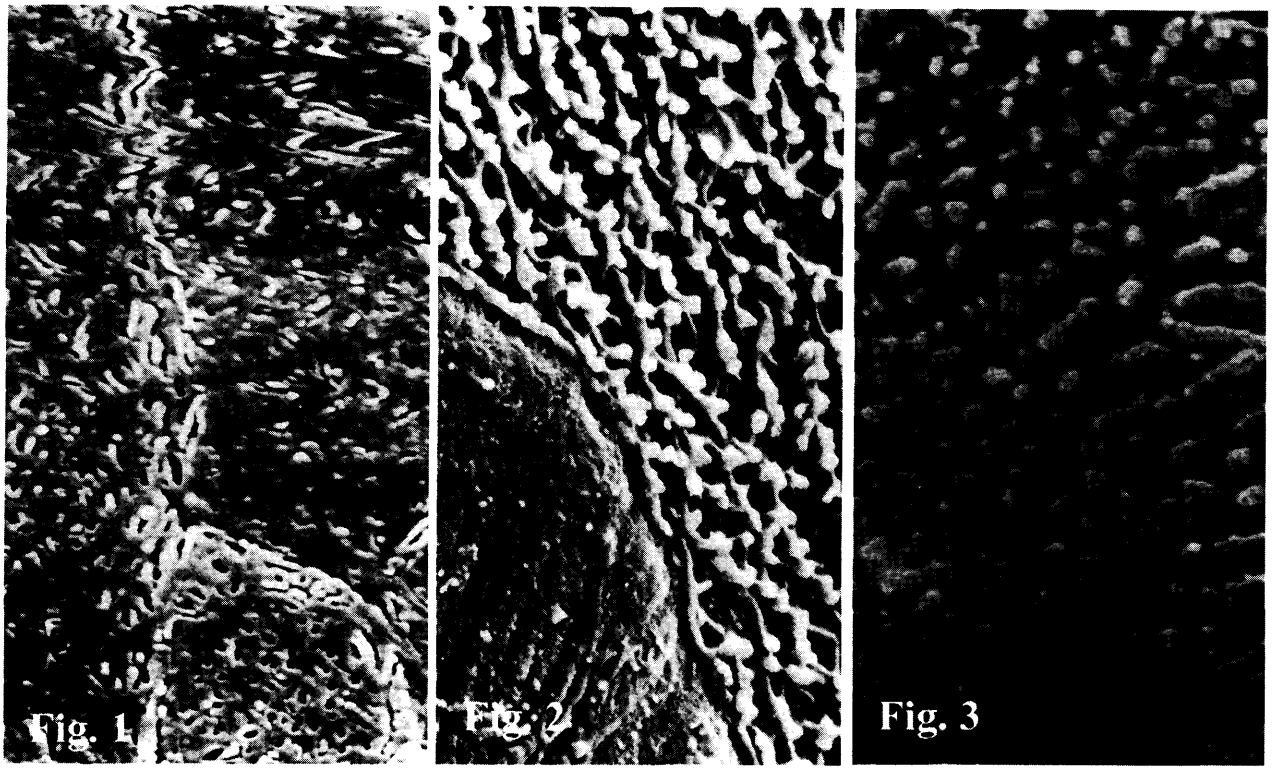

Fig. 1 The corneal surface (SEM) of an A(-) rat. Fig. 2 The corneal surface (SEM) of a Zn(-) rat. Fig. 3 The corneal surface (SEM) of an A(-)Zn(+) rat.

微量元素と眼組織 －形態学的研究－

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長崎大学 大学院 医歯薬学 総合研究科 眼科・視覚科学分野

Special Lecture II Pathomechanism of metal-related allergic reactions

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Metal-related allergic reactions are classified to allergic reactions to metals, allergy-like irritant reactions induced by metals and allergic reactions modulated by metals. Allergic reactions to metals are well-known as contact allergic reactions to metal, systemic metal allergic reactions and dental metal eruptions in dermatologic fields. In these allergic reactions to metals, the relevant T cells are supposed to recognize the metal-modified self peptides in context with self MHC molecules or metal-modified self MHC molecules. The frequency of allergy-like irritant reactions induced by patch testing with metals was higher in patients with atopic dermatitis (AD) than in non-AD patients, indicating that allergy-like irritant reactions with metals might be involved in exacerbation of AD. The diet of TBT, which was a metal with a unique immunomodulating activity, resulted in early induction of AD-like lesions induced with TNCB sensitization in DS-Nh mice. The AD-like lesions themselves induced in DS-Nh mice fed with TBT diet were severer than those in DS-Nh mice fed with control diet. Increase of S.aureus on the skin, decrease of IFN-r, and increase of IL-5 and IL-13 production were observed in DS-Nh mice treated with TNCB and fed with TBT. These effects might be interpreted as a kind of allergic reactions modulated by metals.

金属関連アレルギー反応の発症機序

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Special Lecture III Medical application of radioactive metal: Targeted delivery of radiometals for diagnostic imaging and therapeuty

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Nuclear medicine is a diagnostic technique used to visualize changes in physiological and biochemical processes throughout the distributed and interrelated systems of living tissues and organs as well as in regional anatomy. Furthermore, nuclear medicine also includes application to therapy, which is based on the radiation damage of radioactive nuclides. Drugs containing radioactive nuclide to be used in nuclear medicine are called radiopharmaceuticals. Radiopharmaceuticals are required to have certain biological properties: high radioactivity localization into target tissue and a high target/non-target tissue ratio for clear imaging or effective therapy.

Among radionuclides used in radiopharmaceuticals, some radiometals have received much attention due to their nuclear physical characteristics and widespread availability. Furthermore, since these radionuclides are isotopes of metallic elements, they have also received much attention due to their inherent capacity to coordinate with a great variety of ligands.

In some cases, the direct labeling of biologically active molecules with radiometallic nuclides is available, but there are problems. The functional groups inherent in the biologically active molecules are generally used for the binding of radionuclides, and the binding strength is insufficient for radionuclides to remain stable in vivo. Moreover, since these metallic radionuclides are not isotopes of constituent elements of bioactive molecules and drugs but of heterogeneous elements, one cannot simply replace a common constituent atom in biologically interesting compounds, such as carbon and oxygen.

Thus, the great demand for biospecific radiopharamceuticals bid for rational design of radiometallic compounds to mimic the pharmacokinetics of bioactive molecules and drugs, and evolved into the generation of the so called, "bifunctional radiopharmaceuticals" that is, a radiopharmaceutical containing a biologically active molecule together with a group with the ability to bind radiometallic nuclides. In general, a radiometallic nuclide is incorporated onto a biologically active molecule through a chelate. Research on bifuncitonal radiopharmaceuticals labeled with ^{99m}Tc, ^{111}In and ^{67}Ga started from the development of radiolabeled macromolecules for diagnostic nuclear medicine, such as albumin, antibodies against tumor-associated antigens and enzymes. In recent years, bifunctional radiopharmaceuticals have also been used for the labeling of non-protein compounds such as peptides, receptor binding agents and other drugs.

The successful use of bifunctional radiopharmaceuticals for selective targeting to diseased tissue in diagnostic nuclear medicine prompted development of radiometallic compounds for therapeutic nuclear medicine. Radionuclide therapy is a unique tumor treatment modality, which can selectively deliver radiation doses in target tissues. Furthermore, its unique characteristics include its non-invasiveness, the relative lack of immediate and late side effects, and the advantage that uptake and retention in the tumor can be pre-assessed by tracer studies. Radionuclide therapy is also of great interest to the treatment of other disorders such as thyrotoxicosis and rheumatoid arthritis and pain palliation in bone metastases. Beta- or alpha particle emitters, which provide high radiation dose, are useful as therapeutic radionuclides. Some compounds labeled with metallic radionuclides such as ^{90}Y, ^{186,188}Re, ^{89,90}Sr and ^{64,67}Cu have received attention for therapy.

Thus, the use of bifunctional radiopharmaceuticals has enabled the control of behavior of metal in the living body. In this lecture, recent progress in research of bifunctional radiopharamceuticals labeled with metallic radionuclides for diagnosis and therapy will be presented.

金属薬剤の開発と医療への応用

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The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Abstracts

Educational Lectures

Educational Lecture I Zinc Deficiency and its clinical features in the cases found in Kitamimaki, a rural area in Japan

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<<**Abstract**>> Zinc deficiency has been shown to cause impaired taste, anorexia, decubitus (bedsores, pressure ulcers), delayed healing of wounds, various types of skin lesions (including atopic dermatitis and alopecia), night blindness, hypogonadism, behavior abnormality, immune disorders, and other medical conditions. Many elderly patients with zinc deficiency visited our clinic in Kitamimaki Village, Nagano Prefecture, in the autumn of 2002. To learn why they were zinc deficient, we conducted a study in 2003 analyzing the serum zinc levels of people in the Kitamimaki area(Fig.1). The survey revealed that the area's inhabitants, especially its elderly residents, had much lower serum zinc levels than those occurring in the general public. This may indicate that zinc deficiency occurs easily in elderly people whose physical condition is poor or whose nutritional intake is insufficient. For its effects on public health and its implications for clinical practitioners, zinc deficiency is emerging as an important subject of clinical interest in an aging society. In this study we present the clinical features of 98 definite cases of zinc deficiency encountered at Kitamimaki until the end of March 2005.

[Symptoms and Signs]
Anorexia, easily recurring or poorly healing decubitus, impaired taste, some skin lesions with bulla, vesicle, crust, pustle or keratosis, burning or pain or discomfort of tongue (glossodynia), stomatitis, loss of energy, anemia, chronic diarrhea, and other ailments are frequently found in patients with zinc deficiency. The prevalence of each symptom is shown on Fig.2.

[Diagnosis]
The "normal" range of serum zinc level is usually considered to be from 65 to 110μg/dl. As we reported, serum zinc level shows daily variation, and also depends on age, gender of subject, and the length of time between the participant's consumption of a meal and the moment each sample is taken. We deduce that an individual has a zinc deficiency by observing his/her symptoms and other indicators, and by finding low serum zinc levels in the participant's morning sample. We found zinc deficiency not only in patients with serum zinc levels under 65μg/dl, but in some patients with zinc levels of the accepted range(Fig.3). In cases where there are no specific symptoms or signs of zinc deficiency, the deficiency can sometimes be identified when a participant responds positively to zinc supplements.

[Symptoms and Effect of Zinc Supplement]
1.Anorexia
Anorexia-induced zinc deficiency is caused by an inadequate intake of zinc. This symptom is in many cases relieved by taking zinc supplement for one or two weeks. Anorexia is often latent, therefore it is often recognized after recovery from the zinc deficiency.
2.Impaired Taste
This is said to be the most typical symptom of zinc deficiency. To relieve this symptom (and to relieve symptoms of anorexia as well), patients must sometimes increase their zinc supply for a few months or more. In cases where individuals suffer from both anorexia and impaired taste, it often takes more time for one to recover from impaired taste than from anorexia. This discrepancy may show that there are different mechanisms contributing to the appearance of the two symptoms.
3.Decubitus
Many factors are related to the appearance of decubitus. We believe that zinc deficiency plays an important role in the appearance, and in the healing delay, of this lesion. Zinc supply can facilitate the healing of fresh decubitus in a matter of weeks, while chronic and deeply involved skin lesions may take several months to heal. We must pay attention to other origins of decubitus as well; zinc deficiency is not the only basis for this condition.
4.Glossodynia

No specific changes to the surface of the tongue are found in many cases of glossodynia. Pain, discomfort, or burning sensations found in zinc deficient patients usually subside when zinc supplements are taken for a few months. We must distinguish cases in which people complain of these symptoms from those caused by reasons other than zinc deficiency. A few patients with glossodynia also complained to us of pain around the lips. For people with lip pain, zinc supplements were less likely to provide relief than for patients with glossodynia; the mechanism allowing this divergence is unknown.

5.Skin Lesions except decubitus

Lesions with vesicles, bullas, pustles or hyperkeratosis (sometimes called pemphigoid, pustulosis, or dyskeratosis) in patients with zinc deficiency were healed within a few months by zinc supplements. We advise dermatologists to prescribe zinc supplements to treat these lesions.

6.Loss of Energy

It is very difficult to evaluate the state of "energy loss" and its etiology. Many physical and mental factors contribute to this state. We believe that zinc deficiency may cause the energy loss that some patients complain about, and that some can be cured through simple zinc supplementation. Recovery from this state enables individuals to remain physically active in their daily lives, which is vital, especially for the elderly.

7. Diarrhea and Others

We encountered several patients with zinc deficiency who suffered from continuing or recurring diarrhea. We addressed the complaints and relieved symptoms using zinc supplements.

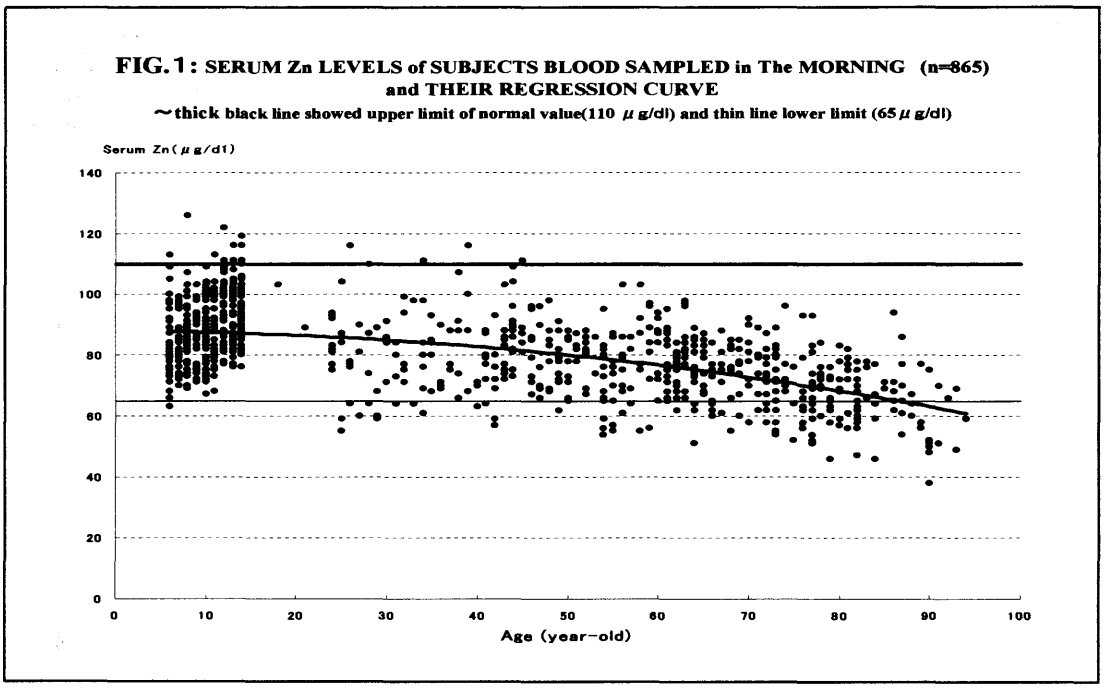
Anemia with or without iron deficiency may occur in patients with zinc deficiency.

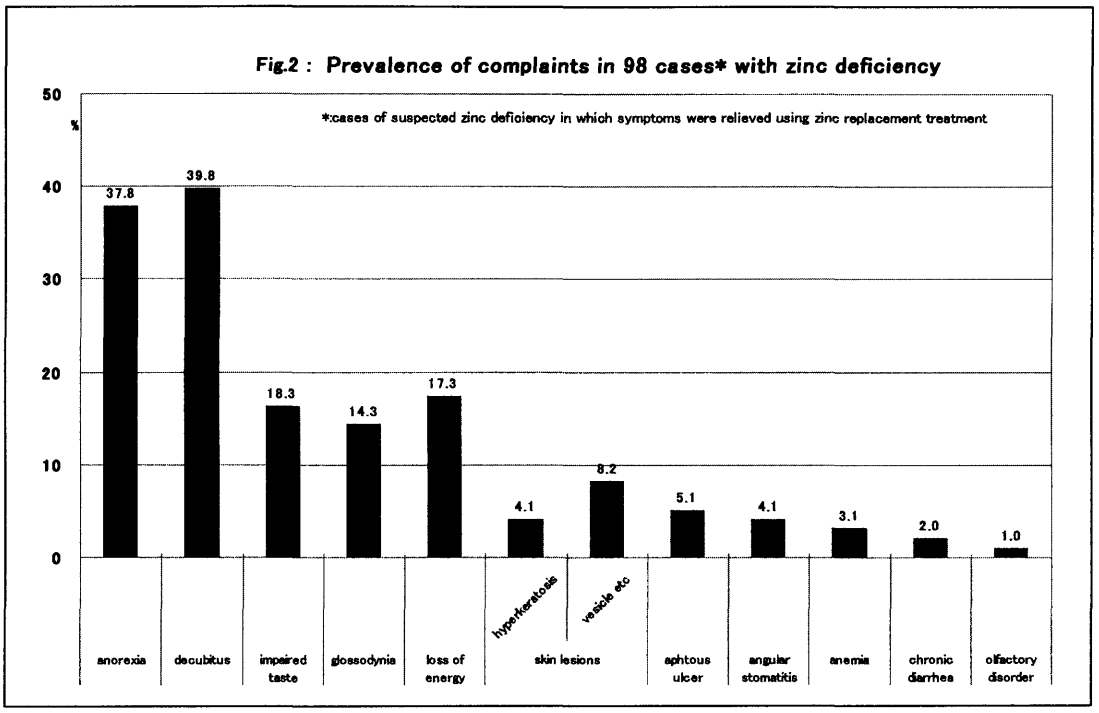
[Treatment (Zinc Supplement)]

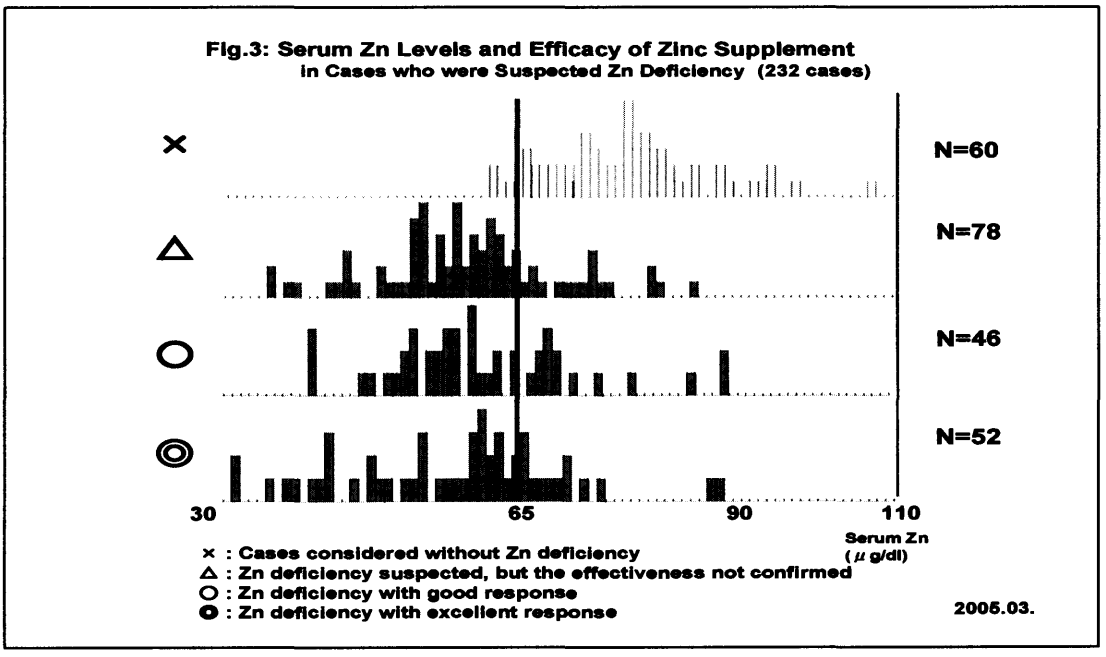
Medicine containing zinc is available in Japan in the form of polaprezinc granules (Promac: trademark) only. This medicine is currently used to treat gastric ulcers and contains 34mg zinc/g. For the treatment of zinc deficiency, 1g of the granules (34 mg zinc) a day is usually prescribed.

When treated with zinc supplements, symptoms found in patients with zinc deficiencies subside in periods of time ranging from a week to several months, depending on the type of symptom and duration of exposure to the deficiency.

We must note that serum alkaline phosphate (ALP) activity is triggered as serum zinc level rises. Because ALP is considered to be a metalloenzyme of zinc, the behavior of ALP, as regulated by zinc supply, helps us to evaluate the efficacy of zinc supplementation in addressing the symptoms of each patient.







住民調査に見る高齢者の亜鉛不足と亜鉛補充療法

○倉澤 隆平、久堀 周治郎
東御市立みまき温泉診療所

Educational Lecture II Copper metabolism and disorders of copper transport

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Introduction

Copper is an essential trace element for all living organisms, as it functions as an integral component of cuproenzymes, including cytochrome C oxidase, lysyl oxidase, dopamine β oxidase, superoxide dismutase tyrosinase, ascorbic acid oxidase and ceruloplasmin. In contrast to its necessary, when in excess, this oxidative potential can induce the production of reactive free radical which causes cellular damage. Thus, tight regulation of copper homeostasis, which is maintained by various mechanisms, including uptake, transport, storage and excretion of copper, is required. Disruptions of copper homeostasis are illustrated in two human genetic disorders, Wilson disease (WD) and Menkes disease (MD). Each disease results from the absence or dysfunction of homologous copper-transporting ATPases. The WD ATPase (ATP7B) has been recently noticed in relation to cancer. The ATP7B expresses in various kinds of cancer cells, and the ATP7B expressed malignant cells show cisplatin-resistance. Copper has been also reported to acts distinct role in the pathogenesis of neurodegenerative diseases, including amyotropic lateral sclerosis, Alzheimer disease and the prion-mediated encephalopathies. In this section, disorders of copper transport are mainly reviewed, and some problems still unsolved in these diseases are discussed.

Copper Homeostasis

Figure 1 shows copper metabolism in human. The average daily intake is about 5 mg of copper in healthy adults. About 40% of this is absorbed and an equivalent amount is returned to the gastrointestinal tract by biliary secretion. Since discovery of a responsible gene for Menkes disease in 1993, the molecular mechanism of copper in the cells has been elucidating (Fig. 2). Ctr1 acts on copper uptake into the cells. Copper in the cytsol is delivered to Cu/Zn superoxide dismutase in the cytosol, Golgi apparatus and mitochondria by Ccs2, HAH1 (ATOX1) and Cox 17, respectively, which are named generically copper chaperons. Copper-transporting ATPases transport copper from the cytosol into the Golgi apparatus (Fig. 3). Various genetic disorders of copper metabolism are characterized either depletion or accumulation of copper (Table 1). Genetic defects of copper metabolism in humans are MD and WD (Table 2).Menkes disease (MD) In the affected cells of MD, copper transport from the cytosol to the Golgi apparatus is disturbed, resulting in a reduction of copper efflux. Almost all cells including the intestine, kidney and blood brain barriers, except the hepatocytes, are affected. Orally administered copper accumulates in the intestine, resulting in the failure of copper absorption and thus a copper deficiency arises. The characteristic features of MD are caused by a reduction in the activities of several copper-dependent enzymes (Fig. 4, Table 3). The treatment generally accepted so far is subcutaneous injections of copper-histidine. This treatment prevents the neurological degeneration in some patients when the treatment is initiated soon after birth. However, when the treatment is started later, the neurological degeneration can not be prevented. Thus, early diagnosis is very important. We recently developed a screening test for MD by using urine HMA and VMA. Moreover, the treatment with copper injections can not improve the connective tissue disorders, because that injected copper can not be transported into the Golgi apparatus. To solve the connective tissue disorders, copper should be delivered into the Golgi apparatus. A combination therapy with copper injections and diethydithiocarbonate (DEDTC) was studied using macular mouse, an animal model of MD. The combination therapy improved the copper concentration, cytochrome C oxidase activity and catecholamine metabolism in the brain of macular mice. These results suggest that DEDTC facilitates the passage of copper across the blood-brain barrier and Golgi membrane and that the combination therapy may be an effective treatment for the neurological degeneration and connective tissue disorders of Menkes disease. Occipital horn syndrome (OHS)OHS also results from a mutation in ATP7A gene and shows a mild phynotype of MD. Most of the patients with OHS have splice-site mutations, which produce the normal transcript as well as abnormal transcript. The characteristic clinical features are connective tissue abnormalities. Wilson disease (WD)WD is caused by a large number of different mutations in ATP7B. R778L mutation is mostly observed in Asian patients, while the H1069Q mutation is most common in European patients. In WD, impaired biliary copper excretion leads to accumulation of copper

in the liver (Fig. 5). The accumulated copper in the liver are released into plasma, resulting in hemolysis and deposition of copper in extrahepatic tissues (Fig.6). The clinical symptoms mainly appeared as liver disease and neurological diseases. However, various other symptoms are also observed. These various symptoms sometimes make early diagnosis difficult. All the patients should be treated with chelating agents and/or zinc. Zinc has been used in patients with WD as an orphan drug in the U.S.A. In Japan, however, zinc is not yet available for WD. Patients with neurological diseases temporarily become worse during the first few weeks of the therapy with these agents. Tetrachiomolybdate has been reported not to induce the deterioration of neurological symptoms. In the case of patients with fluminant hepatitis or hemolysis, these treatments are ineffective, and liver transplantation is accepted for these patients.

Aceruloplasminemia

Aceruloplasminemia is an autosomal-recessive disease characterized by absent serum ceruloplasmin, progressive neurodegeneration and insulin-dependent diabetes in association with mutations in the ceruloplasmin gene. These symptoms result from iron accumulation in affected tissues. Recognition of this disease shows an essential role for ceruloplasimin in iron homeostasis.

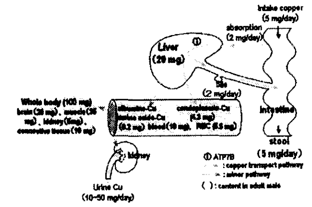


Fig. 1 Copper metabolism in humans

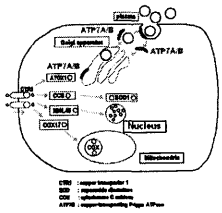


Fig. 2 Copper metabolism in the cells

Table 3 Decreased enzyme activities and symptoms in Menkes disease

cuproenzymes	symptoms
Cytochrome C oxidase (mitochondria)	Hypothermia Brain damage
Tyrosinase (cytsol)	Hypopigmentation Hair abnormalities
Dopamine β hydroxidase (secretary enzyme)	Hypotension
Lysyl oxidase (secretary enzyme)	Arterial abnormalities Bladder diverticla Loose skin and joint osteoporosis (wormian born, fracture)

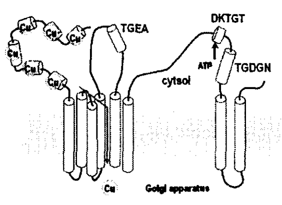


Fig.3 Structure of ATP7A/B

Table 1 Disorders of Cu metabolism in humans and animals

Humans	Responsible gene
Menkes disease	*ATP7A*
Occipital horn syndrome	*ATP7A*
Wilson disease	*ATP7B*
Indian children cirrhosis	?
Endemic Tyrolean infantile cirhosis	?
Idiopathic copper toxicosis	?
Animals	
Canine copper toxicosis	*COMMD1*
Mottled mouse	*Atp7a*
Brindled mouse	*Atp7a*
LEC rat	*Atp7b*
Toxic milk mouse	*Atp7b*
North Ronaldsay sheep	?

Bie P et al. J Heredity 96: 803, 2005

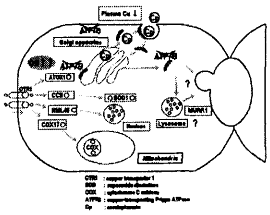


Fig. 5 Disruption of Cu homeostasis in the hepatocytes of Wilson's disease

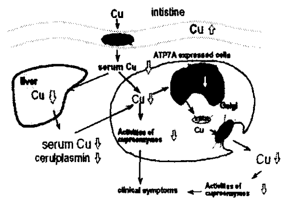
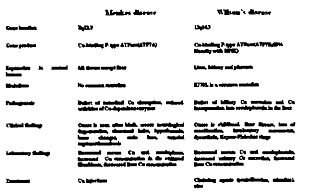


Fig. 4 Copper metabolism in patients with Menkes disease



	Menkes disease	Wilson's disease
	Cu-binding P-type ATPase(ATP7A)	
	All tissues except liver	Liver, kidney and placenta
	No common mutation	
	Cu injections	

Table 2 Characteristics of Menkes and Wilson's diseases

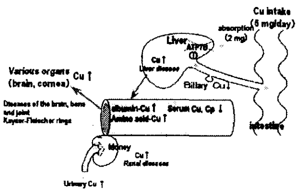


Fig. 6 Disruption of Cu homeostasis in Wilson's disease

銅の体内動態と疾病

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The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Abstracts

Focus Lectures

Focus Lecture I Asbestos-related carcinogenesis and immunological effects

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1. Introduction[1-7)]

Although many advanced nations, with a few exceptions such as Canada and Russia, have been banned the industrial usage of all kinds of asbestos, its increased usage has been reported from developing countries, particularly those in the Asian region. In Japan, most asbestos has been legally banned since October 2004. However, asbestos has been in the public eye due to the fact that some residents near factories, wives of workers and many workers in the factories using asbestos have died from asbestos-related diseases. These issues have been both social and a public problem from the medical, industrial and political viewpoints.

Asbestos causes asbestosis/pulmonary fibrosis and various malignancies such as lung cancer and malignant mesothelioma, but, the mechanisms of its role in these disease shave not yet been clearly defined. Investigations have clearly shown that asbestos causes DNA damage and apoptosis via iron-derived reactive oxygen species (ROS) and reactive nitorogen species (RNS), alteration of mitochondrial function, and activation of the death receptor pathway of apoptosis. In addition, our laboratory has been focusing on the effects of asbestos on the human immune system.

In this abstract for the FOCUS lecture at the 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements, we will review the features of asbestos, the toxicological mechanisms causing pulmonary toxicity due to asbestos exposure, and the immunological effects of asbestos.

2. Mineralogy of Asbestos[8-12)]

Asbestos is a naturally occurring mineral, which is divided into two groups; *amphibole* group including crocidolite (blue asbestos), amosite (brown asbestos), tremolite, anthophylite, and actinite, and the *serpentine* group, in which crysotile (white asbestos) is the sole variety. Among the *amphibole* asbestos, only crosidolite and amosite have been used widely on a commercial basis. However, non-commercial amphiboles are also important since they may be contaminated into other mineral, such as chrysotile, vermiculite, and talc.

The chemical composition of asbestos is very important in considering its biological effects on human. Chrysotile is a hydrated magnesium silicate with the chemical composition indicated in Table 1. Individual fibers of chrysotile have a diameter of 20 to 40 nm, These fibers vary in length, but may exceed 100 μm. Typically, chrysotile fibers have a curved, curly and wavy morphology.

The amphiboles are a group of hydrated silicates with a wide range of cation substitutes, as shown in Table 1. To consider the biological effects, these cations, particularly the iron, should be gained recognition because it produce the -OH by the fenton-catalyzed, Harber-Weiss reaction as summarized below.

$$Fe(II) + H_2O_2 \rightarrow OH^- + \cdot OH + Fe(III)$$
$$O_2^- + H_2O_2 \rightarrow Iron \rightarrow HO^- + \cdot OH + O_2$$
$$O_2 + Fe(II) \rightarrow O_2 + Fe(III)$$

Table 1. Chemical structure of various forms of asbestos	
Chrysotile	$Mg_3Si_2O_5(OH)_4$
Crocidelite	$Na_2Fe_3^{++}Fe_2^{+++}Si_8O_{22}(OH)_2$
Amosite	$(Fe\text{-}Mg)_7Si_8O_{22}(OH)_2$
Tremolite	$Ca_2Mg_5Si_8O_{22}(OH)_2$
Anthophyllite	$(Mg\text{-}Fe)_7Si_8O_{22}(OH)_2$
Actinolite	$Ca_2MgFe_5Si_8O_{22}(OH)_2$

3. The mechanisms of asbestos-induced genotoxicity[1-7]

Usually, a carcinogen, such as asbestos, that is capable of causing genotoxity, is considered to produce a variety of lesions in cellular DNA, such as single- or double-strand breaks, intra- and interstrand cross-linking, and base damage. Although repair of these lesions will restore the physiological DNA structure, abnormal DNA repair may occur resulting in gene mutations, chromosomal aberrations, and cell transformation. In addition, apoptosis is an important mechanism by which cells with DNA damage are eliminated without inciting an inflammatory response. Considering the cellular DNA damages and oncogenesis induced by asbestos exposure, these mechanisms should be investigated in detail.

DNA- strand break formation is on of the earliest abnormalities occurring in cells exposed to oxidant stress, including that from asbestos. Asbestos-induced DNA strand breaks were studied in rat and human alveolar epithelial cells and rat pleural mesothelial cells, which are the target cells for asbestos-induced caricinogenesis as reported from various investigators. Several investigations have demonstrated that asbestos induces apoptosis in relevant lung target cells, such as mesothelial and lung epithelial cells.

The molecular mechanisms involved in asbestos-induced DNA damage and apoptosis have been investigated strenuously. First, the production of ROS by asbestos should be mentioned, since iron is the core sturucture of a surface contaminant and it can catalyze the formation of -OH, as mentioned above. The role of ROS in asbestos-induced pulmonary toxicity including DNA damage and apoptosis has been demonstrated using alveolar epithelial cells and mesothelial cells. In addition, the mitochondorial apoptotic pathway has been implicated as a central regulator of apoptosis induced by asbestos.

Figure 1 shows a schematic presentation of the molecular mechanisms involved in asbestos-induced DNA damage and apoptosis modified from the review of Upadhyay and Kamp [6].

4. Immunological effects of silica and silicates/asbestos[13,14]

Our laboratory has been investigating for the last 15 years the mechanisms involved in the immunological effects of silica or silicates including asbestos.

Silicosis patients suffer not only from respiratory disorders but also from autoimmune diseases. To clarify the mechanisms involved in the dysregulation of autoimmunity found in silicosis patients, we have been focusing on Fas and Fas-related molecules in the Fas-mediated apoptotic pathway, since Fas is one of the most important molecules regulating autoimmunity in T cells. Our findings have shown that silicosis patients exhibit elevated serum soluble Fas levels, an increased relative expression of the soluble fas and dcr3 genes in peripheral blood mononuclear cells, other highly detectable variant messages of the fas transcript, a relatively decreased expression of several physiological inhibitors (survivin and toso), and a dominancy of lower membrane Fas expressers in lymphocytes, which transcribe soluble fas dominantly, when compared with healthy donors. These findings are consistent with immunological factors such as serum immunogulobulin G levels and the titer of anti-nuclear autoantibodies. In addition, anti-caspase 8 autoantibody and anti-Fas autoantibody have been detected serum from silicosis patients, and a functional assay showed that anti-Fas antibody stimulated Fas-mediated apoptosis. We hypothesize that there are two subpopulations of silicosis lymphocytes. One is a long-term survival fraction including a self-recognizing fraction showing lower levels of membrane Fas and inhibition of Fas/Fas ligand binding in the extracellular spaces. The other is a fraction exhibiting apoptosis caused by silica/silicates, recruited from bone marrow, showing higher levels of membrane Fas, and sensitive to anti-Fas autoantibody, as shown in Figure 2. Further investigations should be performed to confirm the effects of silica/silicates on the human immune system.

On the other hand, asbestos (e.g. chrysotile, crocidolite, and amosite) is known to cause malignant lung cancer or mesothelioma. The International Agency for Research on Cancer (IARC) categorizes both asbestos and

crystalline silica as group I carcinogens. According to the IARC classification, asbestos affects alveolar epithelial and mesothelial cells. There have been many studies concerning asbestos-induced apoptosis in these cells. In experiments, these cells underwent apoptosis on relatively high-level, short-term exposure to asbestos via production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) with activation of the mitochondrial apoptotic pathway. Thus, it has been considered that during low-level, long-term exposure to asbestos in the human body, alveolar epithelial and mesothelial cells escape from this apoptotic pathway due to genetic changes and undergo malignant transformation. We also found that asbestos polyclonally activated CD4-positive T cells and caused activation-induced cell death. In addition, peripheral blood mononuclear cells (PBMCs) from healthy individuals exposed to asbestos in culture underwent apoptosis. However, many patients with asbestosis have had chronic, occupational and recurrent exposure to silicates. Therefore, there seems to be a need to develop an in vitro experimental model of chronic exposure to analyze the immunobiological effects of silicates during long-term exposure.

To investigate the effects of asbestos on human immune system, we tried to establish a chrysotile-induced apoptosis-resistant human HTLV-1 immortalized polyclonal T cell line, an MT-2 subline (MT-2Rst). To establish an *in vitro* model of long-term, low-level recurrent exposure to asbestos, MT-2 cells were continuously cultured in an RPMI 1640 medium containing 10 μg/ml of chrysotile for more than eight months. Assays were conducted monthly using the TUNEL method to determine whether or not the apoptotic fraction had changed. Before each monthly assay, chrysotile was removed from the culture using the Ficoll-Hypaque method. Then, the MT-2 cells were re-started without chrysotile for seven days. Thereafter, the TUNEL assay was performed after exposure to 0, 10, 25, and 50 μg/ml of chrysotile for 48 hours. The percentage of TUNEL-positive cells was very low among the cells continuously exposed to 10 μg/ml of chrysotile compared with the original (MT-2Org) cells, which were never exposed to chrysotile. Reflecting to the appearance of TUNEL-positive fraction in MT-2Org cells, the growth of the MT-2 cells continuously exposed to 10 μg/ml of chrysotile was not affected by re-exposure to chrysotile, whereas the MT-2Org cells exposed to 10, 25 and 50 μg/ml of chrysotile revealed a marked reduction in growth as analyzed by the WST-1 assay. These results suggest that the MT-2 cells continuously exposed to 10 μg/ml of chrysotile, gained resistance to chrysotile -induced apoptosis over the course of more than eight months. This subline was designated MT-2Rst.

Using the MT-2 original line and MT-2Rst line, we have been investigating the mechnisms for obtaining the resistance against asbestos-induced apoptosis, and differences between these two lines in cytokine production and the T cell receptor Vβ repertoire as shown in Figure 2.

5. Conclusion

With the increasing detection of patients with malignant mesotheliomas among residents living near factories and facilities handling asbestos in the past, asbestos-related diseases have becomea big medical, social and political issue in Japan. Therefore, we should seriously consider what we, as researchers into the biological effects of asbestos in the field of preventive medicine, can do to clarify the problem and which type of investigations may eliminate the anxiety of Japanese people. One answer is to establish biomarkers for asbestos exposure, since most people do not know for sure whether or not they were exposed to asbestos 30 to 40 years ago. In addition, we should develop markers to detect asbestos-induced malignancies such as lung cancer and malignant mesothelioma. Moreover, the establishment of the markers for molecular prevention of asbestos-induced carcinogenesis would be much appreciated news to persons feeling anxiety about the uncertainty of past exposure to asbestos.

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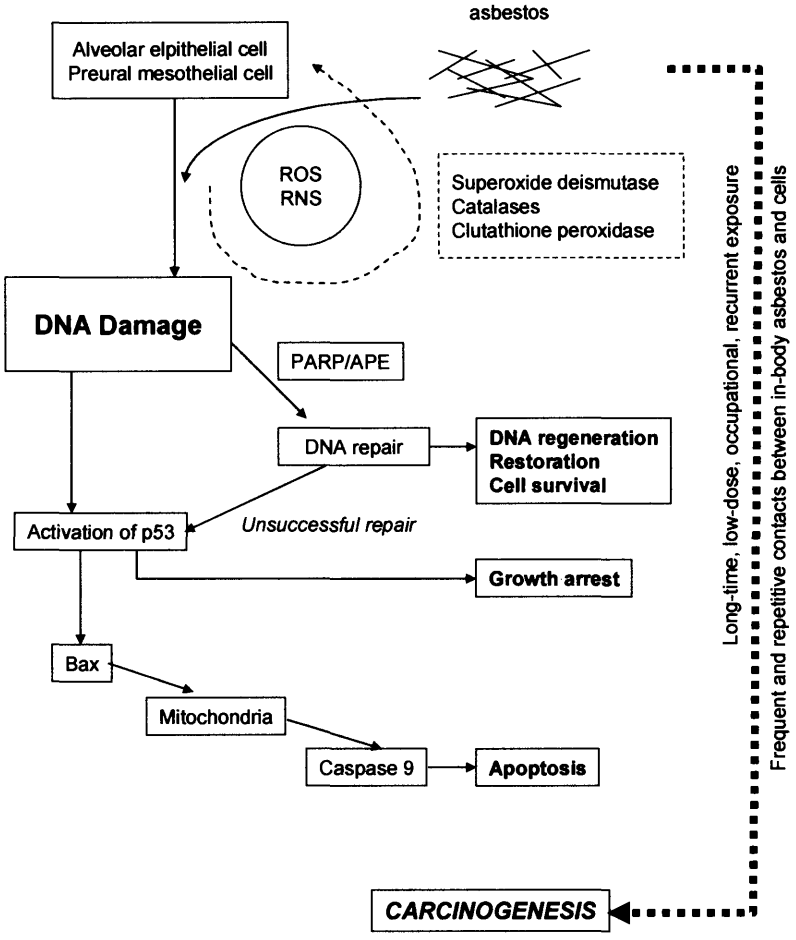

Figure 1 The molecular mechanisms involved in asbestos-induced DNA damage and apoptosis. (modified from Upadhyay & Kamp, Exp Biol Med 228: 650-659, 2003)

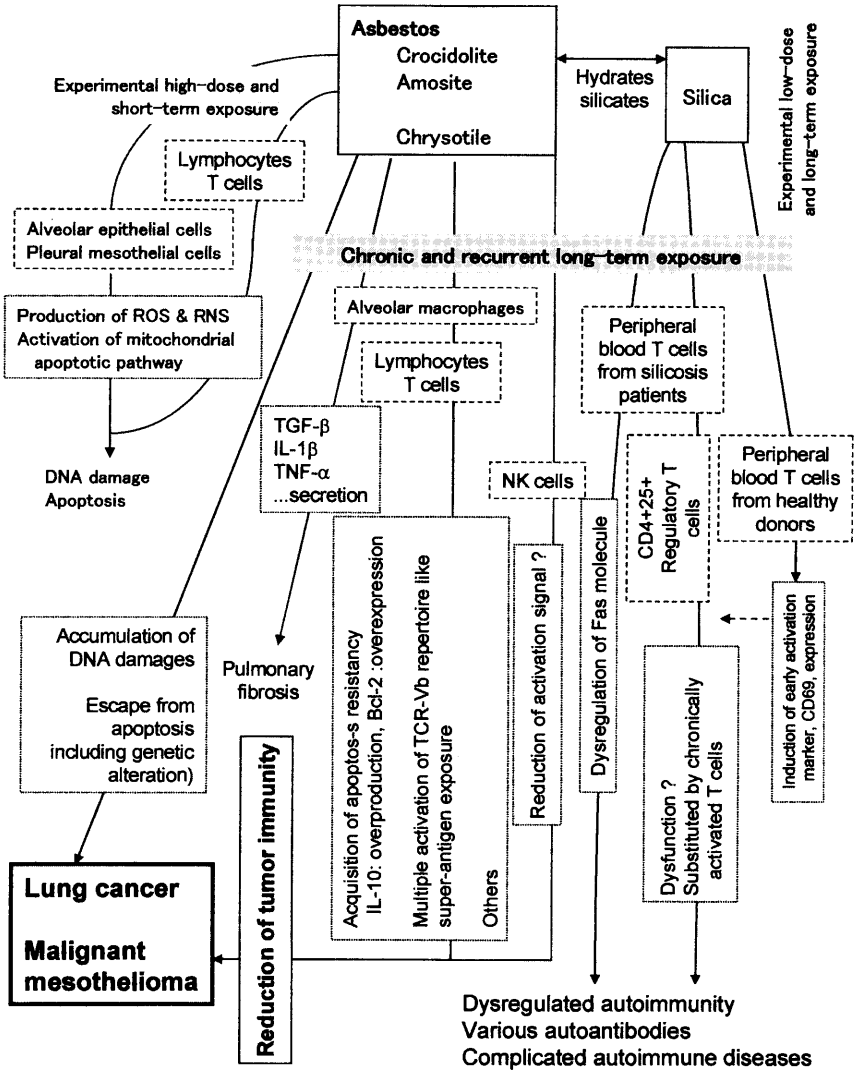

Figure 2 Schematic presentation of effects of asbestos/silica on human immunocompetent cells

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アスベスト関連発癌と免疫影響

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Focus Lecture II Malignant Mesothelioma : Incidence and Clinical Approach

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Abstract

Malignant mesothelioma is an insidious neoplasm which has a strong etiological relationship with asbestos exposure. The incidence is rising worldwide, but is expected to start dropping in Sweden, New Zealand, and US, because of prohibitions on the use of asbestos starting in the early 1970s. In June 2005, several patients with mesothelioma in Amagasaki, Hyogo prefecture, were in the news, who were residents in the neighborhood one kilometer away from the asbestos factory and had no identifiable occupational exposure. The risk of neighborhood-exposed mesothelioma in the general population is debatable. Mesothelioma has such social impact that governments are forced to consider not only the banning of the environmental carcinogen asbestos but also a reappraisal of the whole tort system for compensation to asbestos-victims. The proliferation of mesothelioma-specific treatment strategy has led to an increase in the number of symposiums devoted to mesothelioma. In the meeting, mesothelioma and its relationship with asbestos and clinical aspects will be discussed.

Incidence in Japan

Malignant mesothelioma is no longer considered a rare neoplasm, because of rapid increase in incidence. In 1995, the 10th Revision of the International Classification of Diseases (ICD-10) was adopted in Japan for classifying the causes of death. In this document, the annual numbers of deaths from mesothelioma in 1995, 1996, 1997, 1998, 2000, 2001, 2002, 2003 and 2004 were 500, 576, 597, 570, 647, 710, 772, 810, 878 and 953, respectively (Fig. 1). According to 'Vital Statistics in Japan' using the 9th Revision of ICD, the annual number of deaths from malignant neoplasms of pleura and pleural mesothelioma was about 100 in the early 1980s, and 250 in the late 1980s. These mortality data suggest that the incidence of mesothelioma is rapidly increasing in Japan. Specialized registry-based incidence and mortality data for mesothelioma in the whole of Japan are not available. The male:female ratio is approximately 3:1. The rate of 500-1000 cases per year seems to be a fairly small number, compared to that in other industrialized countries. About 2000-3000 new cases of mesothelioma per year is recorded in the US each year, where the peak at 3060 cases in 2002 and will begin to decrease in incidence. The estimates for mesothelioma mortality in UK are similar, with a peak in about the year 2020 of 2700 to 3300 deaths. The reason for the low incidence of malignant mesothelioma in Japan, in spite of increasing tendency, is found in the history of the asbestos industry, as described below.

Asbestos and mesothelioma in Japan

Asbestos is divided into two main groups: amphiboles (straight fibers), of which the most important are crocidolite (blue asbestos), amosite (brown asbestos), tremolite and anthophylite, and serpentine (curly fibers), of which there is only one type, chrysotile (white asbestos). Chrysotile accounts for 95% of the asbestos used commercially. Crocidolite is the most dangerous asbestos fiber and is not mined hereafter, which is the main cause of malignant mesothelioma and lung cancer. Anthophylite has been mined in Finland and Japan, which is not used industrially. A very small amount of asbestos was produced in the Matsubase district on Kyushu island (Anthophylite) and the Furano district on Hokkaido island(Chrysotile). Therefore, the amount of asbestos imported is nearly equal to the total used in Japan. In the history of the Japanese asbestos industry, 30,000 - 40,000 tons was imported per year before World War II. Thereafter, imports of asbestos ceased between 1942-1948 because of the economic blockade against Japan during World War II and the complete destruction of industry as a result of the war. The latent period between initial exposure to asbestos and the onset of malignant mesothelioma is extremely long, nearly 40 years. The Australian Mesothelioma Surveillance of 1979-85 has demonstrated that more than three-quarters of the 450 exposed cases were first exposed to asbestos during the time of its extensive use between 1930-1959. The very low level of asbestos use in the period during and after the war could account for the low incidence of mesothelioma in the late 1990s in Japan, because of its long latent

period. The recovery of Japanese industry has been accelerating since the Korean War(1950-53), and the import of asbestos vastly increased in the late 1960s. In contrast to the situation in Japan, the production and use of asbestos in the United States and other industrialized countries increased rapidly during and soon after World War II at its peak from the 1950s through 1970. In Japan, more than 300,000 tons of asbestos were imported between 1973 and 1977. After asbestos spraying was prohibited in 1974, asbestos consumption began to decrease in Japan. But, from the mid-1980s, an economic policy to increase domestic consumption so that Japan's trade surplus would decrease, markedly increased demand for building construction. The import of asbestos, therefore, rose again to 320,000 tons in 1988, but recently dropped again to 176,000 tons in 1997, because of the serious recession in the Japanese economy. Japan is one of the greatest consumers of chrysotile asbestos and one of the largest producers of asbestos-contain products. The highest incidences of mesothelioma are in those countries where there is or was a large production of crocidolite. In Japan, however, only very small amounts of chrysotile and anthophyllite were produced. Considering the long latent period of about 40 years and the more than 300,000 tons of asbestos used between 1973 and 1977 in Japan, the incidence of malignant mesothelioma can be expected to increase dramatically hereafter, maybe from 2010.

Clinical features

Malignant mesothelioma originates most frequently from the lining cells of the pleural (80%) and peritoneal cavities (20%) as well as the pericardium and the tunica vaginalis testis. The most common initial presentation of pleural origin is a pleural effusion, symptoms are dominated by dyspnea or chest pain. Direct invasion of adjacent structures and metastasis along needle biopsy tracks or surgical incisions are typical features of mesothelioma, and uncommon with other types of tumor. Conversely, the finding of malignant seeding favors a diagnosis of mesothelioma. Radiographic examination may show any of several patterns. Effusion is almost invariable, approximately in 80% of patients, and its absence makes the diagnosis much less likely. When the effusion is drained, the pleura may appear diffusely thickened or nodular, and tumor often encases the underlying lung. Thickened of the entire circumferential pleural and involvement of the fissure can be seen on the thoracic CT (Fig. 2). FDG-PET promises to aid the detection of mesothelioma (Fig. 3), and the uptake of FDG has been shown to correlate with prognosis; the higher the uptake the shorter the survival. Calcified or noncalcified plaques may also be identified. A prospective series followed more than 1500 Swedish men with pleural plaques for 16,000 plus person-years and observed a risk of mesothelioma of 1 in 1700 per year.

Diagnostic Serum Markers

Mesothelin is a cell surface protein on normal mesothelial cells, which is highly expressed in several cancers, including mesothelioma, ovarian and pancreatic cancers. Elevated levels of soluble mesothelin in serum have been reported in 84% patients with malignant mesothelioma, and in only 2% of patients with other cancers. Serum CEA is usually within normal limits and is an important marker to distinguish adenocarcinoma from mesothelioma. Recently, it has been demonstrated that serum osteopontin levels can be used to distinguish persons with exposure to asbestos who do not have cancer from those with exposure to asbestos who have pleural mesothelioma.

Therapeutic methods

Most patients with malignant mesothelioma are candidates for systemic chemotherapy during the course of their disease. Over the past two decades numerous single-drug and combination regimens have been examined, but no standard treatment with chemotherapy alone exists for mesothelioma.. Although the true impact of chemotherapy in mesothelioma remains to be determined, agents with consistent antitumor activity include the platinum agents, gemcitabine, irinotecan and the antifolates. Phase II studies of combination chemotherapy are associated with higher response rates but not necessarily longer median survivals. Recent data from a large randomized phase III international clinical trial established the superiority of cisplatin/pemetrexed compared to cisplatin alone. The response rate was twice as high with the combination treatment (41% versus 17%). Extrapleural pneumonectomy and pleurectomy/decortication are attempted with curative intent in patients with IMIG stage I disease and selected patients with stage II disease. Surgery alone, however, did not yield satisfactory results. Therefore, combined modality therapy, including surgery, radiation and chemotherapy, has been performed to be of some therapeutic value in the management of early-stage pleural mesothelioma. The poor prognostic factors identified in the Cancer and Leukemia Group B and the European Organization for Research and Treatment of Cancer are as follows: non-epithelioid histology, poor performance score, chest pain, age older than 75 years, male gender, white blood cell count 8300/mm3 or greater, platelets greater than 400,000,

and LDH greater than 500 IU/L.

Conclusion

A major breakthrough in mesothelioma treatment is not to come yet. Novel molecular targets and their respective targeted therapies in mesothelioma will be important for further advances in the treatment.

Fig.1 Annual numbers of malignant mesothelioma in Japan
Fig.2 Competed tomography scan of chest of patient with malignant pleural mesothelioma.
Fig.3 FDG-PET in patient with malignant pleural mesothelioma

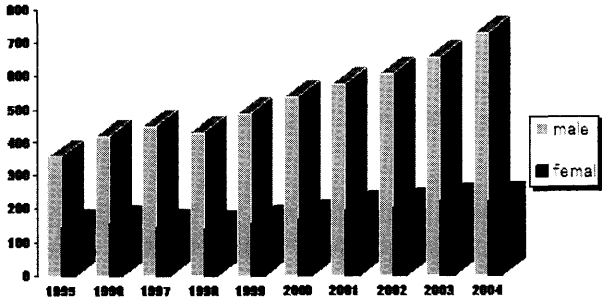

Fig. 1 Annual numbers of malignant mesothelioma in Japan

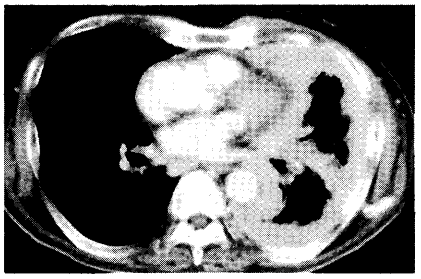

Fig. 3. FDG-PET in patient with malignant pleural mesothelioma

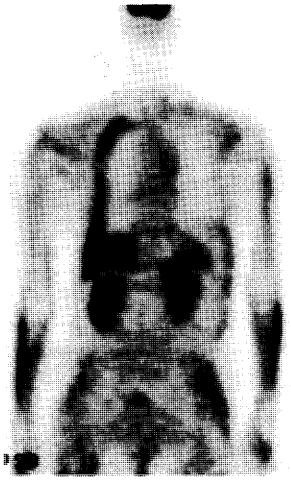

Fig.2 Competed tomography scan of chest of patient with malignant pleural mesothelioma.

悪性中皮腫：頻度と臨床的アプローチ

○中野 孝司
兵庫医科大学 呼吸器内科

The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Abstracts

Topic Lectures

Topic Lecture I Molecular Mechanism of Cadmium Transport in Mammalian Cells

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Metallothionein (MT) plays important roles in resistance to metal toxicity in mammalian cells. Especially, MT plays a critical role in the protection against cadmium (Cd) toxicity. When cells are exposed to Cd, MT synthesis is induced, and the increased MT serves as a scavenger for intracellular Cd ions. MT-null cells derived from MT-null mice are highly sensitive to Cd toxicity. Most Cd-resistant cell lines thus far established have shown enhanced production of MT. However, the presence of high concentrations of MT, which trap cellular Cd ions efficiently, has hindered precise understanding of Cd influx and efflux. Non-MT factors responsible for Cd tolerance and transport have been poorly elucidated. Thus the utilization of MT-null cells can give new insights into MT-independent Cd-resistance factors. To understand MT-independent mechanisms for Cd resistance in mammalian cells, we have established Cd-resistant cells from MT-null mouse fibroblast cells of MT-I and -II knockout mice and demonstrated that Cd resistance of these cells is conferred by markedly reduced accumulation of Cd. Further studies revealed that both uptake and efflux of Cd were changed in these Cd-resistant cells.[1)] However, the cause for the change in Cd accumulation has not been clarified.

To identify genes responsible for Cd resistance as well as Cd transport, we carried out several DNA microarray analyses using cDNAs obtained from two clones of Cd-resistant cells and parental cells. A competitive hybridization of Cy3- and Cy5-probed cDNAs on a DNA chip was carried out with dye-swapping. After a careful examination of the reproducibility and reliability of the data obtained using five different chips, it was found that the expression of 78 genes was enhanced and that of 48 genes was reduced in Cd-resistant cells compared with those in parental cells. Several candidate genes of ATP-binding cassette transporters and solute carrier family transporters were identified as enhanced or suppressed genes. The examination of mRNA levels using quantitative real-time PCR revealed that the expression of Slc39a14 encoding ZIP14, a member of the zinc transporter ZIP family, was markedly down-regulated in both clones of Cd-resistant cells.[2)] Although it is not yet clear whether ZIP14 has the ability to transport Cd, these results suggest that the lowered expression of ZIP14 may be involved in Cd resistance in MT-null cells. Further study is needed to clarify whether ZIP14 can permeate Cd in addition to Zn or not.

As one of the ZIP family genes was selected by DNA microarray, we investigated the gene expression of all ZIP family members between Cd-resistant and parental cells using Real-time PCR analyses. As a result, we found that mRNA levels of ZIP8 in Cd-resistant cells were also markedly lower (ratio, 0.05 in A7 and 0.03 in B5) than those in parental cells. Recently, it was reported that ZIP8 is involved in Cd transport in vascular endothelial cells of testis, and is a determinant of sensitivity to Cd-induced testicular haemorrhage. Therefore we focused on ZIP8 and transfected the plasmid which expresses siRNAs for ZIP8 gene into parental cells, and found that Cd accumulation in ZIP8 siRNA-transfected cells were lower than that in vector-transfected cells.

Thus, ZIP8 might be a candidate for the Cd transporter that was suppressed in Cd-resistant cells. Further characterization of these cells shall clarify the roles of ZIP8 and ZIP14 in transport and tolerance of Cd.

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2) H. Fujishiro, S. Okugaki, S. Nagao, M. Satoh, S. Himeno, Characterization of Gene Expression Profiles of Metallothionein-Null Cadmium-Resistant Cells. *J. Health Sci.*, 2006, 52(3), *in press.*

動物細胞におけるカドミウム輸送の分子メカニズム

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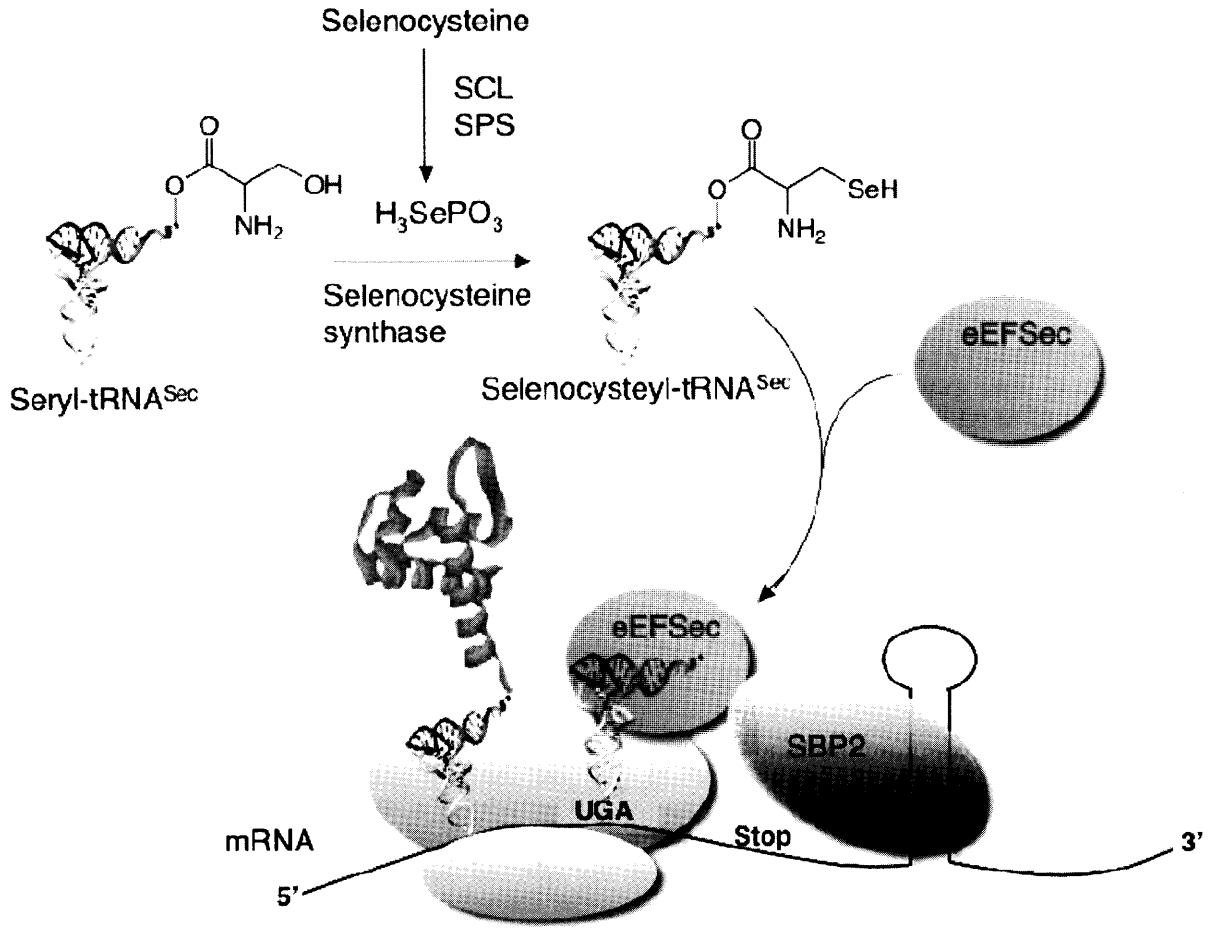
Topic Lecture II Selenoprotein Biosynthesis and Selenium-Specific Enzymes

○ Hisaaki MIHARA[1] , Suguru KUROKAWA[1] , Rie OMI[1] , Tatsuo KURIHARA[1] , Ken HIROTSU[2] , Nobuyoshi ESAKI[1]

[1]Institute for Chemical Research, Kyoto University, Kyoto, Japan , [2]Graduate School of Science, Osaka City University, Osaka, Japan

Selenium is an essential micronutrient for humans and other mammals. It occurs in proteins in the form of the 21st naturally occurring amino acid, selenocysteine (Sec). Sec is co-translationally incorporated into prokaryotic and eukaryotic selenoproteins at UGA codons. Decoding of UGA as Sec, rather than termination, requires a specific stem-loop structure in selenoprotein mRNAs, known as a Sec insertion sequence (SECIS) element, several trans-acting factors, and a unique tRNA ($tRNA^{[Ser]Sec}$) with an anticodon complementary to UGA. $tRNA^{[Ser]Sec}$ is aminoacylated initially with serine by seryl-tRNA synthetase and further converted to Sec-$tRNA^{[Ser]Sec}$ by selenocysteine synthase. In addition, there is a specific SECIS-binding protein, SBP2, and a specialized elongation factor, EFsec, required for Sec insertion into a protein. Although the mechanism of eukaryotic Sec-$tRNA^{[Ser]Sec}$ biosynthesis is not well characterized, it is postulated to be similar to that in *Escherichia coli* but with some distinct differences. The in vitro synthesis of Sec-$tRNA^{[Ser]Sec}$ has been established with partially purified selenocysteine synthase from mammalian tissues. The active selenium donor species in this reaction is selenophosphate, which is proposed to be synthesized from selenide and ATP by selenophosphate synthetase (SPS). Human and mouse carry a pair of SPS, designated SPS1 and SPS2. SPS2 contains a Sec residue (Sec-60 and Sec-69 in the human and mouse enzymes, respectively) at the site corresponding to the active Cys-17 residue in E. coli SPS, termed SelD. In contrast, SPS1 has a Thr residue at the corresponding site. The physiological roles and reaction mechanism of SPS1 and SPS2 remain incompletely characterized. However, previous studies have suggested that selenocysteine lyase (SCL), NifS/IscS-type cysteine desulfurase, or other proteins may bind selenium and deliver an active form of selenium to SPS in the biosynthesis of selenophosphate. SCL was discovered during studies of the enzymatic synthesis of L-selenocysteine in rat liver and identified in various mammalian tissues as the first proven enzyme that specifically acts on selenium compounds. SCL catalyzes the cleavage of L-selenocysteine to L-alanine and selenium in a pyridoxal 5'-phosphate-dependent manner and predominantly exists in the cytosol of liver, kidney, testis, spleen, brain, heart, lung, and stomach, in this order. However, the physiological relevance of the selenium-specific action of the enzyme has not been addressed. cDNA cloning of mouse SCL revealed moderate sequence identity (23%) to mouse cysteine desulfurase Nfs1, which is believed to catalyze the desulfurization of L-cysteine to provide sulfur for the de novo formation of Fe-S clusters. Nfs1 is located predominantly in mitochondria, yet small amounts are present in the cytosol and the nucleus. Since high concentrations of free sulfide and free iron are toxic to cells, Nfs1 is proposed to deliver the sulfur atom for the Fe-S cluster assembly via a protein-bound cysteine persulfide intermediate. Although the catalytic activity of mammalian Nfs1 has not been experimentally characterized, biochemical studies of its close homologs from yeast and bacteria suggest that Nfs1 probably acts on L-selenocysteine in addition to its proposed natural substrate, L-cysteine. Because the chemical properties of selenium and sulfur are similar, most enzymes cannot distinguish selenium compounds from their sulfur analogs when they are present at similar levels. However, the intracellular concentration of selenium is >1000-fold lower than that of sulfur, and free selenide is far more toxic than sulfide. Therefore, the metabolic discrimination between selenium compounds and the corresponding sulfur compounds is very important in order for cells to metabolize selenium compounds without disturbing the sulfur metabolism and vice versa. However, molecular mechanism of discrimination between Se and sulfur remains unclear. In the present work, we have addressed the question of whether SCL is involved in the biosynthesis of selenoprotein in mammalian cultured cells. First, we investigated the cellular roles of SCL by RNA interference (RNAi). Second, we overproduced SCL in HeLa cells and determined its effects on selenoenzymes. Finally, we examined the cellular roles of Nfs1 by RNAi and discussed the implications for the functional distinction between SCL and Nfs1 in mammalian cells. We also investigated the Se recognition mechanism of SCL from rat liver.

Mutation of Cys375 to alanine resulted in inactive SCL. The measurement of UV-vis spectroscopic studies suggested that L-cysteine is bound to the enzyme as a PLP-cysteine adduct. Electrospray ionization mass spectrometry was used to analyze SCL incubated with L-selenocysteine or L-cysteine. The mass measurement of wild-type SCL incubated with L-selenocysteine showed a monomer molecular mass with +84, probably corresponding to a covalently attached Se. On the other hand, the wild-type enzyme incubated with L-cysteine did not produce any modified molecule. The mutant enzyme with L-selenocysteine or L-cysteine did not form a Se-attached enzyme. The results indicate that SCL catalyzes the conversion of L-selenocysteine to L-alanine and Se via the formation of a perselenide intermediate on a conserved Cys375.



セレンタンパク質の生合成とセレン特異的酵素

○三原 久明[1]、黒川 優[1]、近江 理恵[1]、栗原 達夫[1]、広津 建[2]、江崎 信芳[1]
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Topic Lecture III α-Glucosidase inhibitory effect and action mechanism of anti-diabetic zinc and vanadium compounds

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Introduction

Diabetes mellitus (DM) is a lifestyle-related disease. Since 2002, in Japan, over 16 million people (including pre-diagnosed) have been suffered from DM.[1] We have reported that zinc(II) and vanadium(IV) compounds exhibit insulinomimetic activity and blood glucose lowering effects.[2-3] Recently, we have proposed the insulinomimetic mechanism in rat adipocytes.[4-6] The alpha-glucosidase inhibitors, a type of oral anti-diabetic medicine, are used as not only medicines but also dietary supplements.[7] In addition, the amount of zinc in type 2 diabetic patients treated with insulin has been shown to decrease when compared with that of healthy people.[8] Therefore, it might be important to enhance the zinc uptake. There are several reports on the relationships between metals and enzymes,[9-10] however, studies on the relationship between metals and alpha-glucosidase are quite limited.[11] Thus, we examined the alpha-glucosidase inhibitory effect of zinc(II) and vanadium(IV) compounds.

Methods

Zinc(II) and vanadium(IV) complexes with 6-methyl picolinic acid were prepaerd, and their alpha-glucosidase inhibitory effects were evaluated by the Dahlqvist method with modification.[12] In brief, 0.1 ml of the test compounds in 0.15 M HEPES buffer (pH 6.8) and 0.1 ml of alpha-glucosidase (*Saccharomyces sp.*) 5 units/ml in 0.015 M HEPES buffer were added to 0.1ml of the substrates (0.1 M sucrose in 0.15 M HEPES buffer), and then incubated at 37℃ for 60 min. After incubation, all reactions were stopped in a dry bath incubator and glucose concentration was determined by a glucose CII-test Wako kit. For a positive control, acarbose was added instead of the test compounds. The alpha-glucosidase inhibitory effect was calculated by using the following formula:

Alpha-glucosidase inhibitory effect (%) = [(Ac-As)/Ac]×100

Ac: glucose concentration in the control (alpha-glucosidase and substrate)

As: glucose concentration in the test compounds (alpha-glucosidase, substrate, and test compounds)

On active zinc(II) compounds, we performed the oral carbohydrate tolerance test in ddY mice.

Results and discussion

The alpha-glucosidase inhibitory effect of acarbose (positive control), zinc(II), and vanadium(IV) compounds were dose-dependent. From the curves, the apparent IC_{50} values, a 50% inhibitory concentration of the compounds on alpha-glucosidase activity were estimated as shown in Table 1.

Then we examined the in vivo alpha-glucosidase inhibitory effect of zinc(II) compounds ($ZnSO_4$ and $Zn(6mpa)_2$), which shows high effectivity in in vitro experiment. The elevation of the blood glucose level in ddY mice administered a disaccharide (sucrose) in normal ddY mice was significantly suppressed after single oral administration of zinc(II) compounds as compared with the control mice administered the vehicle. On the other hand, in the oral monosaccharide (glucose) tolerance test, the suppression of the elevated blood glucose levels in mice was not observed by the zinc(II) compounds and the vehicle. These results indicated that zinc(II) compounds inhibited alpha-glucosidase action in the epithelium of the small intestine and reduced the disaccharide digestion, while the intestinal absorption of monosaccharide was suppressed or delayed.

It has been reported that the active site of alpha-glucosidase was closely related to the imidazole and carboxy groups.[13] Generally, zinc(II) and vanadium(IV) compounds have high affinity to electron donors. Zinc(II) and vanadium(IV) compounds may bind with electron donors such as imidazole or carboxy groups in alpha-glucosidase and inhibits the function. Further investigations are required to understand the reaction mechanism.

Conclusion

We observed the alpha-glucosidase inhibitory effect of insulinomimetic zinc(II) and vanadium(IV) compounds in type 1 diabetic STZ mice and type 2 diabetic KK-A^ymice. In the *in vitro* experiments, two zinc(II) compounds

exhibited strong alpha-glucosidase inhibitory effects those were approximately 100 times greater than that of acarbose. Two vanadium(IV) compounds showed alpha-glucosidase inhibitory effect around 2 times greater than that of acarbose. We then evaluated the in vivo alpha-glucosidase inhibitory effect in normal ddY mice. The result indicated that zinc(II) compounds had a high alpha-glucosidase inhibitory effects.

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Table 1. Estimated IC_{50} values of acarbose, $ZnSO_4$, $Zn(6mpa)_2$, $VOSO_4$, and $VO(6mpa)_2$

	IC_{50} (μM)		IC_{50} (μM)
$ZnSO_4$	$4.7 \pm 0.06^{\dagger}$	$VOSO_4$	$201 \pm 5.4^{**}$
$Zn(6mpa)_2$	$8.9 \pm 0.16^{\dagger}$	$VO(6mpa)_2$	$448 \pm 5.1^{*}$
acarbose	747 ± 7.4		

† Significance; $p<0.001$ *vs.* acarbose, **Significance; $p<0.01$ *vs.* acarbose, * Significance; $p<0.05$ *vs.* Acarbose

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抗糖尿病作用を有する亜鉛およびバナジウム化合物によるα－グルコシダーゼの阻害と作用機構

○吉川　豊、平田 良子、桜井　弘
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Topic Lecture IV Metabolic pathway of arsenic based on analytical toxicology

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Introduction

Inorganic arsenicals such as arsenite (iAs^{III}) and arsenate (iAs^{V}) are well-known human carcinogens. The metabolism of arsenic is generally accepted by repetitive reduction and oxidative methylation, the former is mediated by arsenic reductase and glutathione (GSH), reducing agent, and the latter is mediated by arsenic methyltransferase (Cyt19) and*S*-adenosyl-L-methionine (SAM), methyl group donor, respectively (Fig. 1). Previously, it was generally accepted that the methylation of arsenic is detoxification process, since methylated arsenicals such as monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}), major metabolites in human urine, are less toxic than inorganic arsenicals (Yamauchi and Fowler, 1994). Recently, however, it has been reported that monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) were founds in human urine (Aposhian et al., 2000; Mandal et al., 2001), and these trivalent arsenic metabolites are more toxic than inorganic arsenicals (Styblo et al., 2000; Hirano et al., 2003, 2004). These results suggest that the methylation of arsenic is bioactivation rather than a detoxification process. It has been shown that iAs^{III} administered intravenously or orally is excreted into bile as arsenic-glutathione (As-GSH) complexes such as arsenic triglutathione [ATG; $As(GS)_3$] and methylarsenic diglutathone [MADG; $CH_3As(GS)_2$] (Kala et al., 2000; Suzuki et al., 2001; Cui et al., 2004; Kobayashi et al., 2005). Further, it has been reported that ATG and MADG were found in urine of mice deficient in γ-glutamyl transpeptidase that are unable to metabolize GSH (Kala et al., 2004). Recently, we proposed a new metabolic pathway of iAs^{III} in which As-GSH complexes are substrates for Cyt19 (Fig. 2) (Hayakawa et al., 2005). After reduction of iAs^{V} to iAs^{III}, iAs^{III} forms a complex with GSH producing ATG, and ATG is methylated to MADG by Cyt19 in the presence of SAM. MADG and MMA^{III} are in equilibrium depending on the GSH concentration. Recently, we showed that both ATG and MADG were unstable in both bile and phosphate buffer and were hydrolyzed to iAs^{III} and MMA^{III}. Further, MMA^{III} appeared to be oxidized to MMA^{V} only in bile (Kobayashi et al., 2005). Aposhian et al. (2003) suggested that the production of H_2O_2 by xanthine oxidase might be an important route for decreasing the toxicity of trivalent arsenicals by oxidizing them to their less toxic pentavalent analogues. From these results, we presume that the oxidation of trivalent arsenicals plays an important role in the detoxification of arsenicals.

Material and Methods

Cytotoxicity of arsenicals in endothelial cells. We investigated acute toxicity of arsenicals in rat heart microvessel endothelial (RHMVE) cells. The cell monolayer was trypsinized and suspended in the growth medium. A sample of 100 μl cell suspension was aliquotted into a 96-well culture dish (Costar, Cambridge, MA, USA). After 3 days of culture the conditioned medium was replaced with fresh basal medium containing various concentrations of iAs^{III}, iAs^{V}, MMA^{V}, DMA^{V}, or MADG, and the cells were cultured further for another 24 h. The cell monolayer was washed three times with phenol red-free minimum essential medium. Cell viability was measured using a modified 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay kit according to the manufacture's manual (WST-8; Dojindo, Kumamato, Japan). To investigate effects of buthionine sulfoximine (BSO) on viability, cells were exposed to various concentrations of iAs^{III}, iAs^{V}, MMA^{V}, or DMA^{V} for 24 h in the presence or absence of 0.5 mM BSO, and the viability was assayed with WST-8. Arsenic concentrations in the cell monolayer and culture medium were measured by ICP MS after digestion of those samples with nitric acid-H_2O_2.*Arsenic speciation in bile following oral exposure to iAs^{V} in rats.* The orally exposed six-week old male Sprague-Dawley (SD) rats were allowed free access to distilled water containing 0, 1, 10, and 100 μg As/ml as iAs^{V}. The bile fluid was collected into a 1.5-ml microcentrifuge tube on ice for 15 min. The speciation of arsenic in bile was carried out by high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP MS). Arsenicals were separated in bile by HPLC on a reversed phase column (ODS-3, GL Science, Tokyo, Japan) at a flow rate of 1.0 ml/min at 50°C. The pH of the mobile phase

containing 5 mM TBAH, 3 mM malonic acid, and 5% (v/v) methanol was approximately 5.6.*Stability of arsenic metabolites, ATG and MADG, in rat bile.* We synthesized ATG and MADG in order to study their stability in rat bile. Each As-GSH complex was added to bile at final concentrations of 1, 10 and 100 μg As/ml and then incubated at 37°C for 10 min. Their stability was also studied in 150 mM phosphate buffer (pH 8.0). The samples were diluted to 10 ng As/ml with mobile phase and analyzed by HPLC-ICP MS. Concentration of arsenicals in bile was calculated from the corresponding standard with plasma chromatographic software (Yokogawa Analytical Systems). Separation of arsenicals was performed by HPLC on an anion-exchange column (Shodex RSpak JJ50-4D, Showa Denko, Japan) at a flow rate of 0.6 ml/min. The pH of the mobile phase containing 20 mM oxalic acid was adjusted with ammonia to 2.3.*In vitro methylation of arsenicals by human recombinant Cyt19.* The reaction mixture (100 μl), containing 0.2 μM iAs^{III}, 1 mM SAM, 0-5 mM GSH, and 50 μg human recombinant Cyt19 in 25 mM phosphate buffer (pH 7.0), was incubated at 37°C for 3 h. We next examined the conversion of MADG to DMA^{V} in the presence or absence of 5 mM GSH. MADG (0.2 μM) was reacted with 1 mM SAM and Cyt19 (25 mg/ml) in 25 mM phosphate buffer at 37°C for 3 h. Further, we examined whether MMA^{V} is a precursor of MMA^{III} or DMA^{V} in the metabolic pathway as shown in Fig. 1. The reaction mixture (100 μl), containing either 0.2 μM MMA^{V} or iAs^{III}, 1 mM SAM, 5 mM GSH, and 25 μg human recombinant Cyt19 in 25 mM phosphate buffer (pH 7.0), was incubated at 37°C for 3 h. The reaction was quenched by boiling the samples for 5 min and the samples were treated with H_2O_2. The speciation of arsenic in reaction mixture was carried out by HPLC-ICP MS. Separation of arsenicals was performed by HPLC on a reversed phase column (ODS-3, GL Science, Tokyo, Japan) at a flow rate of 1.0 ml/min at 50°C. The pH of the mobile phase containing 5 mM TBAH, 3 mM malonic acid, and 5% (v/v) methanol was approximately 5.6.*Determination of total arsenic and H_2O_2 in liver and body fluids, and analysis of arsenic metabolites in bile.* Eight-week old male SD rats were injected intravenously with saline or iAs^{III} at a dose of 0.2 mg or 2.0 mg As/kg body weight, and the bile fluid was collected into a 1.5-ml microcentrifuge tube on ice for 30 min. Urine samples were taken from the bladder by needle puncture. H_2O_2 in each sample was assayed with hydrogen peroxide/peroxidase detection kit (Cell Technology, Inc, CA, USA). Concentrations of total arsenic in bile, liver, serum and urine were determined by ICP MS. The speciation of arsenic and sulfur in bile was carried out by HPLC-ICP atomic emission spectrometry (AES), and concentrations of As-GSH complexes and glutathione were calculated by HPLC-ICP AES using standard ATG and MADG, and GSH and GSSG solutions, respectively. Separation of arsenicals was performed by HPLC on an anion-exchange column (Shodex RSpak JJ50-4D, Showa Denko, Japan) at a flow rate of 0.6 ml/min. The pH of the mobile phase containing 0.2% formic acid was approximately 2.56.

Results

Accumulation and toxicity of arsenicals in RHMVE cells. LC_{50} values for iAs^{III}, and iAs^{V}, MMA^{V}, DMA^{V}, MADG were calculated to be 0.036, 0.22, 36.6, 2.54, 0.0041 mM, respectively. The accumulation rate for those arsenical were 0.225, 0.030, 0.013, 0.0084, and 9.3 % of dose. BSO, a cellular GSH depleting agent, enhanced iAs^{III}-, iAs^{V}-, and MMA^{V}-induced cytotoxicity. However, BSO reduced, rather than enhanced, the cytotoxicity of DMA^{V}. *Stability of arsenic metabolites, ATG and MADG, in rat bile.* Both ATG and MADG were unstable in bile and phosphate buffer. ATG was changed to iAs^{III} by hydrolysis except at 100 μg As/ml, where some remained in the solution. Similarly, MADG was changed to MMA^{III} in both bile and phosphate buffer. However, MMA^{III} appeared to be oxidized to MMA^{V} only in bile. ATG appeared to be stable in the presence of 10 mM GSH. Exogenously added GSH also stabilized MADG in bile at concentrations of 5 mM or higher. *Arsenic speciation in bile following intravenous or oral exposure to inorganic arsenicals in rats.* Animals were injected intravenously with iAs^{III} at a dose of 0 (control), 0.2 (lower dose) or 2.0 mg As/kg body weight (higher dose), respectively. Control rats were injected with saline instead of iAs^{III}. The concentrations of total arsenic in all samples were increased with dose. The speciation of bilialy metabolites was carried by HPLC-ICP AES. We calculated the amount of ATG, MADG, GSH, and GSSG in the bile using each applicable standard. We detected MADG, but not ATG, and found that it accounted for 70.6% of arsenic in bile at lower dose. ATG was major metabolite in bile at higher dose. The recovery of ATG and MADG were 89.9% and 7.93%, respectively. The concentrations of GSH and GSSG in bile were 2.2±0.56 and 0.22±0.18 mM in control bile, 2.7±1.1 and 0.30±0.053 mM after injection of lower dose, and 10±0.51 and 2.2±0.55 mM after injection of higher dose. Because human exposure to inorganic arsenicals occurs largely via drinking water, we administered iAs^{V}-containing water (1, 10, 100 μg As/ml) for one week to rats, and collected bile for analysis of arsenic speciation by HPLC-ICP MS. At the lower dose (1 μg As/ml) of iAs^{V} that are relevant to human exposure, we were unable to detect DMA^{V} excreted into the bile. However, in the 10 and 100 μg As/ml exposure group, we

detected DMA^V in bile. In contrast, DMA^V was unable to detect in the bile of rats intravenously injected with 0.25 mg iAs^V/kg b.w.*A new metabolic pathway of arsenic.* We investigate whether these As-GSH complexes are substrates for arsenic methyltransferase by using human recombinant Cyt19. First, we examined whether As-GSH complexes were produced in vitro in the presence of a physiological concentration of GSH. iAs^{III} (0.2 μM) was incubated with 0-5 mM GSH for 3 h at 37°C. The generation of ATG increased with the concentration of GSH, and the conversion of iAs^{III} to ATG appeared to reach a plateau at 4 mM GSH. Second, under similar conditions as those for ATG formation (0-5 mM GSH), human recombinant Cyt19 and SAM were added to the reaction mixture for in vitro arsenic methylation experiments. Cyt19 catalyzed transfer of a methyl group from SAM to arsenic and produced monomethyl and dimethyl arsenicals. The methylation of arsenic was catalyzed by Cyt19 only when ATG was present in the reaction mixture. When MADG was dissolved in 25 mM phosphate buffer (pH 7.0) in the absence of GSH, MADG was promptly hydrolyzed to MMA^{III}. On the other hand, in the presence of 5 mM GSH, the chemical form of MADG remained unchanged. A hydrolysis product of MADG, MMA^{III}, was not methylated to dimethyl arsenical by Cyt19. Further methylation of arsenic was catalyzed by Cyt19 only when MADG was present in the reaction mixture. These results suggest that As-GSH complexes such as ATG and MADG were converted by Cyt19 to MADG and DMAG, respectively. In the classical metabolic pathway (Fig. 1), iAs^{III} and MMA^V (or MMA^{III}) are precursor of MMA^V and DMA^V, respectively. We tested either iAs^{III} or MMA^V as initial substrate under in vitro arsenic methylation to examine whether iAs^{III} could be converted to DMA^V via MMA^V. When iAs^{III} was reacted with Cyt19, a large amount of dimethyl arsenical was produced. However, when MMA^V was used, only a small amount of dimethyl arsenical was produced. A small amount of MADG was produced by incubating MMA^V with 5 mM GSH for 3 h. These results suggest that second methylation was produced from MADG rather than from oxidative methylation of MMA^{III}.*Determination of H_2O_2 and arsenic metabolites in rat bile.* Concentrations of H_2O_2 in bile, liver, serum and urine were measured after intravenously injection with iAs^{III} at a dose of 0 (control), 0.2 (lower dose) or 2.0 mg As/kg body weight (higher dose), respectively. Control rats were injected with saline instead of iAs^{III}. Although biliary H_2O_2 concentration of the lower dose group was not changed significantly, that of the higher dose group was increased 12.6 times. Whereas, although H_2O_2 concentration in serum of the higher dose group was not changed significantly, that of the lower dose group was increased 2 times as compared with control group. The concentration of H_2O_2 in liver and urine of control rats was slightly higher than that of As-treated rats.

Discussion

We demonstrated that As-GSH complexes such as ATG and MADG were substrate for human arsenic methyltransferase Cyt19 (Hayakawa et al., 2005). It has been reported that ATG and MADG were major metabolites in the bile of rats intravenously with iAs^{III} (Kala et al., 2000; Suzuki et al., 2001; Cui et al., 2004; Kobayashi et al., 2005). Our in vitro study revealed good correlation between the production of ATG and methylated arsenic metabolites. Similarly, monomethyl arsenicals were further methylated to dimethyl arsenicals by Cyt19 only in the presence of MADG. In the classical pathway, arsenic is thought to be metabolized by repetitive reduction and oxidative methylation (Fig. 1). However, a large amount of DMA^V was produced from iAs^{III} than from MMA^V, suggesting that iAs^{III} is a better substrate for Cyt19 than MMA^V in the catalysis of dimethyl arsenical production. These results contradict the metabolic pathway shown Fig. 1 in which MMA^V is depicted as a metabolite of iAs^{III}. Thus, it is unlikely that iAs^{III} is metabolized to dimethyl arsenicals through MMA^V. Arsenite is metabolized and converted to DMA^V by Cyt19 via formation of As-GSH complexes rather than via repetitive reduction and oxidation methylation. After reduction of iAs^V to iAs^{III}, iAs^{III} forms a complex with GSH producing ATG, and ATG is methylated to MADG by Cyt19 in the presence of SAM. MADG and MMA^{III} are in equilibrium depending on the GSH concentration. We reported that MADG and/or DMA^V were found in the bile of rats that were orally exposed (Cui et al., 2004), but that MADG and ATG are the main forms excreted into bile both in intravenously iAs^{III}- and iAs^V-injected rats (Cui et al., 2004; Kobayashi et al., 2005). We speculated that after hepato-enteric circulation a part of the MADG is further metabolized to DMAG and finally excreted into bile and urine in DMA^V form. These results suggest that MADG is further methylated to DMAG by Cyt19. However, DMAG was not detected in the reaction mixture and in the bile by HPLC-ICP MS, probably because DMAG was not stable and decomposed easily in solution and body fluids. Since DMA^{III} is very easily oxidized to DMA^V (Kato et al., 2003), it is plausible that DMAG was immediately converted to DMA^V in solution and body fluids. ATG and MADG were unstable in bile and were hydrolyzed to iAs^{III} and MMA^{III} in the absence of GSH. Exogenously added GSH stabilized ATG and MADG in bile at the concentration of 10 mM and 5 mM (or higher), respectively (Kobayashi et al., 2005). It has been suggested that trivalent arsenicals are more toxic than corresponding pentavalent ones. LC_{50} values for iAs^{III}, and iAs^V,

MMA^V, MADG were calculated to be 0.036, 0.22, 36.6, 0.0041 mM, respectively (Hirano et al., 2003, 2004). MADG is highly toxic compared to other arsenicals because of faster accumulation of this species by cell. However, exogenous GSH prevents cellular uptake and cytolethality of MADG in TRL 1215 cells (Kojima et al., 2005). It was generally accepted that GSH complexes were not able to be taken up into cells. MADG was probably unstable in medium and was hydrolyzed to MMA^{III} in the absence of GSH. MADG and MMA^{III} are in equilibrium depending on the GSH concentration in medium, and MMA^{III}, a hydrolysis product of MADG, is highly toxic than other arsenicals. These results suggest that GSH plays an important role in preventing hydrolysis of As-GSH complexes and the generation of well-known toxic trivalent arsenicals. MADG was changed to MMA^{III} in both bile and phosphate buffer, however, MMA^{III} appeared to be oxidized to MMA^V only in bile (Kobayashi et al., 2005). Recently, Aposhian et al. (2003) suggested that the production of H_2O_2 by xanthine oxidase might be an important route for decreasing the toxicity of trivalent arsenicals by oxidizing them to their less toxic pentavalent analogues. We examined the concentration of H_2O_2 and in rat bile after intravenously injection with iAs^{III} at a dose of 0 (control), 0.2 or 2.0 mg As/kg body weight, respectively. Compared with control rats, biliary H_2O_2 concentration of the higher dose group (2.0 mg As/kg body weight) was increased 12.6 times. The GSH concentration of the higher group was 4.6 times higher than that of the control group (Kobayashi et al., 2005). Even thought H_2O_2 was excreted into bile, the simultaneously excreted GSH stabilizes As-GSH complexes. The As-GSH complexes are decomposed by intestinal γ-glutamyl transpeptidase. These results suggest that GSH play an important role in preventing hydrolysis of As-GSH complexes and the generation of well-known toxic trivalent arsenicals, and H_2O_2 play an important role in actively oxidizing of trivalent arsenicals and the generation of low toxic pentavalent arsenicals. From the results of our experiment, we conclude that metabolism of iAs^{III} to methylated arsenicals by Cyt19 was via As-GSH complexes (Fig. 2) rather than by oxidative methylation (Fig. 1). It has been reported that the concentration of nonprotein sulfhydryl level in whole blood decreased among Chinese in an endemic area of chronic arsenic poisoning in inner Mongolia (Pi et al., 2000). The toxicity and detoxification of the arsenic depends on redox state of arsenic in body, and it seems that clinical symptom of the chronic arsenic poisoning happened as a result of disintegration of redox state balance of arsenic in body.

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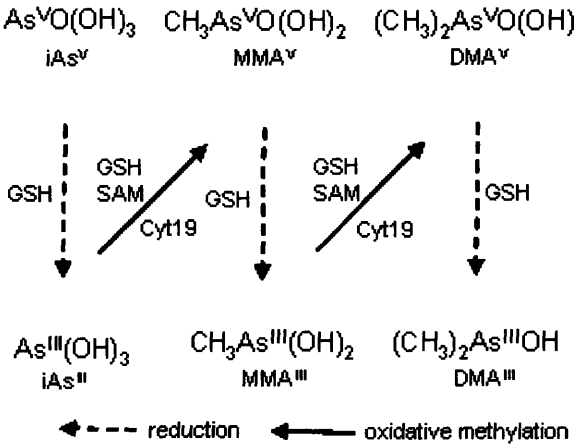

Fig. 1. The classical metabolic pathway of inorganic arsenic in mammals.

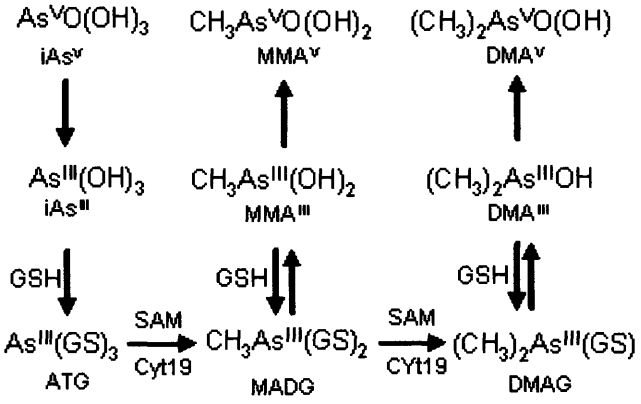

Fig. 2. A new metabolic pathway of inorganic arsenic via arsenic-GSH complexes.

分析毒性学からみたヒ素の代謝機構

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Topic Lecture V Potential Toxicity of Organoltin Compounds Functioning as Nuclear Receptor Ligands

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Organotin compounds are widely used as antifoulants on ship and fishnets, wood preservatives, and biocides for cooling system. Therefore, they have been shown to be ubiquitous in the aquatic environment. In animal experiments, exposure to organotin compounds caused thymus atrophy with peripheral T cell depletion and suppression of T cell-mediated immue responses. In aquatic invertebrates, particularly marine gastropods, organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), induce an irreversible sexual abnormality of females which is termed "imposex". In human, occupational exposure of workers to organotins results in skin and eye irradiation, together with inflammation of the airways. Although, there are many reports described about potential toxicity of organotins, the critical target molecules for the toxicity of organotin compounds remains unclear. In gastropods experiment, these compounds may act as potential competitive inhibitors of aromatase, which converts androgen to estrogen, although effective concentrations are high. TBT and TPT may therefore increase levels of unconverted androgens in both invertebrates and vertebrates. However, they are generally toxic to cells at effective concentrations for aromatase inhibition. In our experiments, organotin compounds markedly enhance aromatase activity, 17β-hydroxysteroid dehydrogenase activity, and human chorionic gonadotropin (hCG) production, along with their mRNA expression, at very low concentrations in human choriocarcinoma cells (J. Clin Endocrinol Metab 2002, Mol Endocrinol 2005, Biochem. Phamcol. 2006). In addition, other investigators reported that, in human ovarian granulosa cells, these compounds suppress aromatase gene expression at the same concentrations. Therefore it is suspected that these organotin compounds would affect target molecules which regulate the gene expression of aromatase, 17β-HSD, and hCG, rather than functioning as aromatase inhibitors. Recently, we demonstrated that TBT and TPT directly bind to retinoid X receptor (RXR) and peroxisome proliferator-activated receptor (PPAR)γ with high affinity and function as transcriptional activators (Mol Endocrinol 2005, Mol Pharmcol 2005). These compounds promote adipocyte differentiation, which is triggered by the PPARγ / RXR receptor signaling pathway (Mol Pharmcol 2005). They may therefore exert their toxic effects through activation of these receptor pathways. Here, I will review the potential toxicity of organotin compounds via these nuclear receptors.

核内受容体リガンドとしての有機スズ化合物とその毒性

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The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Abstracts

Symposium I Lectures

Symposium Zinc physiology

Ⅰ-1

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In recent years, the number of patients visiting outpatient clinics with complaints of abnormal sense of taste and/or olfaction has been on an increase. It has been estimated that about 30% of these patients may have dietary zinc deficiency. This deficiency is more likely to develop among children whose daily requirements of zinc are greater, among elderly people whose dietary consumption of nutrients is poor, and among young women who are often on diets for weight reduction. Zinc deficiency may be associated with features such as hypogeusia, hyposmia, growth retardation, dermatitis, alopecia, compromised gonadal function, susceptibility to infections, and delayed wound healing. In this symposium, I talk about zinc physiology (distribution in the body, absorption, transport, metabolism and excretion, factors causing changes in serum levels, major functions, and safety of replacement therapy etc) for better understanding of the clinical features of zinc deficiency.

亜鉛の生理

○柳澤 裕之

埼玉医科大学 医学部 基礎医学部門衛生学

Symposium I -2 The role of zinc in taste disorders

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Abstracts : There are numerous causes of taste disorders, and, accordingly, their clinical manifestations vary. Of these disorders, those found frequently are secondary taste disorders that occur as a result of zinc deficiency or medication used to treat other diseases. The mechanism of occurrence of taste disorders has been studied in association with zinc, a trace element. In this article, the causes of taste disorders are described mainly in connection with zinc. In addition, zinc therapy, which has been examined relatively extensively heretofore, is evaluated. Key words: taste disorder, zinc, zinc deficiency, zinc therapy.

I. Introduction

According to a survey that took place in 2003 [1], the number of patients presenting with taste disorders was 240,000 annually, indicating that taste disorders are prevalent. The number of patients increased 1.8-fold from 140,000 in 1990. One cause of this marked increase is the extreme increase in the number of the aged during the last decade in Japan. In 1990, there were about fifteen million persons aged 65 years or more whereas there were about twenty-two million in 2000, representing a 1.5-fold increase. Since this trend is expected to escalate in the future, increases in the number of patients with taste disorders are also expected to continue. The causes of taste disorders are various [2], and the mechanisms whereby they occur are also diverse. The mechanism of the occurrence of taste disorders has been studied mainly in connection with zinc, an essential trace element. This report concerns zinc, which seems to be involved in the mechanism of occurrence of taste disorders in association with several causes, and then examines zinc therapy for taste disorders.

II. Causes and mechanisms of taste disorders

1. Taste disorders resulting from zinc deficiency

Symptoms of zinc deficiency are diverse. The taste disorder is one of important impairments of sensory organs as olfactory and visual disorders. The criteria for hypozincemia is a serum zinc concentration below 69 ug/dl. Since decreases in blood zinc are frequently found in patients with systemic diseases accompanied by taste disorders and in patients with drug-induced taste disorders, it is conceivable that zinc deficiency is causally involved in taste disorders. Zinc is distributed at high concentrations in epithelial tissue, such as the skin, hair, and nails. Particularly, the basal part of filiform papillae and the epithelium, including taste buds at the tip of vallate papillae, contain large amounts of zinc. In addition, numerous zinc enzymes, which are conceivably involved in the taste sensation, are in taste buds, particularly in tissue surrounding gustatory pores. Taste disorders are observed at a high rate in rats raised on fodder deficient in zinc [3.4]. In such rats, morphological changes such as decreased density of gustatory papillae on the surface of the soft palate, flattening of papillae, and disappearance of microvilli on the top of taste cells were observed by scanning electron microscope [5]. The following morphological changes were also observed by transmission electron microscopy: a decrease and disruption of microvilli at the top of taste cells, disappearance of an electron dense substance in gustatory pores, decrease in dark granules derived from the Golgi apparatus and distributed in taste cells, and vacuolization of taste cells [6]. These morphological changes observed in rats were similarly observed in taste buds of patients with a taste disorder due to zinc deficiency [7]. Zinc has a direct modulating action on DNA-polymerase activity and thereby influences DNA synthesis. It also modifies RNA-polymerase activity. Thus, zinc regulates cell division and protein synthesis and plays important roles in growth and protein metabolism in individuals. Taste cells of the normal rat are produced incessantly from epithelial cells and continue to renew themselves, and the interval is as brief as about 250 hours [8]. In zinc-deficient rats with taste disorders, this turnover time increases by 100 hours. The delay in replenishment of taste cells makes it difficult to maintain the normal taste reception mechanism. The disturbance in turnover of taste cells is therefore considered to be one of the mechanisms whereby zinc deficiency brings about taste disorders.

2. Idiopathic taste disorders

In some patients with a taste disorder the serum concentration of zinc is in the normal range, and no causes or precipitating factors can be assumed. However, symptoms in these patients are ameliorated after treatment with oral zinc preparations [9,10]. It is conceivable that a subclinical zinc deficiency is present in these patients. The amount of zinc in the human body ranges between 1.4 and 2.3 g, and that in blood accounts for only 0.3% of the total. However, serum zinc only represents 10-20% of zinc in the blood. Serum is consequently not ideal material with which to estimate the amount of zinc in the body. Therefore, we consider that a patient may be zinc-deficient even if serum zinc concentrations are in the normal range.

3. Drug-induced taste disorders

Taste disorders can be induced by ingestion of medicines for treatment of various diseases [2]. The incidence of a taste disorder caused by medication is particularly high in the aged. In 1970, Henkin [11] noted a taste disorder occurring after administration of D-penicillamine as an example of a drug-induced taste disorder and considered the zinc chelating action of the sulfhydryl group of D-penicillamine as the cause. The occurrence of taste disorders cannot completely be explained by chelation of zinc. However, some medicines associated with the occurrence of taste disorders have been shown to have chelating actions on zinc in in vitro experiments [12]. Since the chelating action of pharmacological agents on zinc are influenced directly or indirectly by conditions in the reaction environment, results of in vitro experiments under simplified circumstance do not necessary mimic those of in vivo experiments. Therefore, in vivo studies should be conducted on the influence of specific medications on zinc metabolism and whether they actually cause a taste disorder. Tetracycline (TC) is known to cause a taste disorder and to have a chelating action on zinc. Yoshikawa [13] studied the occurrence of taste disorders after TC administration using rats. A taste disorder occurred in 10 (26.3%) of 38 rats after intraabdominal injection of TC everyday for 4 weeks. However, the taste sensation recovered in all 10 rats 2 weeks after withdrawal of TC and thus was reversible. In the rats that developed the taste disorder, significant decreases in the serum concentration of zinc as well as in the serum concentration of protein occurred simultaneously. It seems possible, therefore, that not only a decrease in the serum concentration of zinc but also a decrease in the amount of zinc in tissue due to a decrease of zinc-binding proteins in serum was involved in the occurrence of the taste disorder after TC administration. In a similar study performed in rats using imipramine, a medication also known to induce a taste disorder, 28.6% of the study rats developed such a disorder. In this study, the serum concentration of zinc alone decreased with no changes in the serum concentration of zinc-binding proteins. It is conceivable therefore that only the decrease in serum concentration of zinc is involved in the occurrence of the taste disorder induced by imipramine [13]. Thus, these results indicate that the administration of certain medicines can induce a taste disorder as well as influence zinc metabolism in the body. Different medicines probably influence zinc metabolism through different mechanisms. Some directly cause decreases in the serum concentration of zinc, while others cause changes in serum proteins and thereby indirectly influence zinc metabolism.

4. Taste disorders resulting from systemic diseases

Taste disorders occur in patients with renal failure, hepatic failure, and diseases of digestive organs (including disorders after surgery), with about 40% of these patients having hypozincemia. The possibility that renal failure influences the nutritional condition of zinc was examined as the mechanism for taste disorders in patients with renal failure [14]. Proteinuria causes increases in excretion of zinc into urea. Mahajan et al. [15] reported that oral zinc was effective in ameliorating taste disorders in patients with uremia and low blood levels of zinc. Acute and chronic hepatic impairment is accompanied by taste disorders [16]. The serum concentration of zinc decreases in patients with severe acute hepatitis or chronic liver diseases. This probably results from plural factors, such as increases in zinc excreted into urine, decreases in the amount of zinc absorbed in digestive organs, and decreases in serum albumin concentration. A taste disorder can result from such zinc deficiency. In patients with very severe diabetes, larger amounts of zinc are excreted in urine and the serum concentrations of zinc are lower than in normal humans [17]. This abnormality of zinc metabolism appears to be the cause of taste disorders in diabetic patients.

III. Zinc therapy for taste disorders

When zinc deficiency is suspected to be involved in the occurrence of a taste disorder, oral zinc preparations are administered. Taste disorders that exhibit a markedly beneficial response to zinc therapy are those that result from zinc deficiency or are idiopathic [9,10].

1. Therapy with zinc sulfate

The first treatment of a taste disorder with zinc sulfate was reported by Henkin et al. [17] in 1970. The taste disorder occurred after D-penicillamine administration and recovery was noted after administration of oral zinc sulfate. Since then other reports of the beneficial effects of zinc sulfate on taste disorders have appeared [18,19]. Henkin et al. [20] examined the effect of zinc sulfate on taste disorders in a double-blind study and failed to show its effectiveness. Study subjects were those whose taste disorders resulted from various causes related and unrelated to zinc deficiency. It is not surprising, therefore, that no significant effects of zinc sulfate were revealed. Mahajan et al. [21] studied the effect of zinc sulfate in a double-blind manner with 22 chronic dialysis patients with taste disorders and low serum concentrations of zinc, and reported beneficial effects of therapy with zinc sulfate. Zinc sulfate was also found to be effective for taste disorders after radiation therapy [22]. Tomita et al. reported the outcome of therapy with zinc sulfate in 1975 for the first time in Japan. They reported that oral zinc sulfate alleviated the taste disorder of patients with enteropathic acrodermatitis who had congenital inability of zinc absorption. In addition, they examined the efficacy of zinc sulfate therapy separately for patient groups with different causes of taste disorders. Efficacy was 73.7% in the zinc-deficient group and 75.8% in the idiopathic taste disorder group.

2. Therapy with zinc gluconate and zinc picorinate

In Japan, double-blind studies on the outcome of zinc therapy were performed using zinc gluconate [9] and zinc piconate [10] instead of zinc sulfate. Zinc gluconate causes disorders of the digestive organs less frequently than does zinc sulfate. At first, the outcome of therapy with zinc gluconate was examined in a double-blind manner on subjects with taste disorders resulting from various causes [9]. No significant therapeutic effects of zinc gluconate were revealed as in the study of Henkin et al. with zinc sulfate [20]. A subsequent analysis was made with a subject group consisting of only zinc-deficient patients and those with idiopathic taste disorders and the efficacy of zinc gluconate therapy was 82%. A significant difference was found between subjects treated with zinc gluconate and those treated with placebo. In a study of zinc picolinate therapy using a double-blind protocol in a patient group composed of only zinc-deficient patients and patients with an idiopathic taste disorder [10] efficacy was 76%. A significant difference was found between subjects treated with zinc picolinate and those treated with a placebo. Results of these examinations indicate that we can expect beneficial effects from zinc therapy for a taste disorder due to zinc deficiency alone but not generally for all taste disorders.

3. Therapy with polaprezinc

Polaprezinc is a zinc-containing oral medicine developed in Japan and extensively administered to patients with gastric ulcer. It is available as white, odorless granules that contain 75 mg of polaprezinc, i.e. about 17 mg of zinc in 0.5 g. Since patients usually take 0.5 g of the granules twice a day, about 34 mg of zinc are administered to each patient daily. Since polaprezinc is commercially available, it can be prescribed in any pharmacy and is widely accepted by clinicians. According to results of a questionnaire completed by ear, nose and throat specialists, 70.4% used this medicine for patients with taste disorders. Of these physicians, 77.5% felt that the medication was effective for this purpose [1]. We divided 242 patients with taste disorders into groups according to causes of the disorder and studied the therapeutic effects of polaprezinc therapy for the individual groups. Complete and partial recovery rates were 46% and 24%, respectively, in the whole patient group: In total, polaprezinc therapy was effective in 69% of subjects. Efficacy of polaprezinc ranged between 66% and 71% for drug-induced and idiopathic taste disorders, those resulting from systemic diseases, and those from zinc deficiency. The effect of aging on efficacy of polaprezine therapy was also examined and found to be 71% in patients under the age of 49, 63% in those between the ages of 50 and 64, and 73% in those aged 65 years or older. Since there were no significant differences in efficacy among these groups, zinc therapy with polaprezinc seems to be as effective in the aged as in young patients. The beneficial effect of polaprezinc for taste disorders is now being evaluated objectively in a prospective controlled study. According to results of the study partially made open, a therapeutic effect was demonstrated. Significantly high efficacy was found in a dose-dependent manner in patients placed on oral polaprezinc. Complete results will be announced in the near future.

IV. Conclusions

Various causes of taste disorders and the mechanisms whereby they occur have been described in connection with zinc. The treatment accepted most extensively is zinc therapy and is the most effective therapy, particularly for disorders related to zinc deficiency. The beneficial effect of oral polaprezinc for taste disorders is now being investigated in a double-blind manner. At the present stage of the investigation, significantly high efficacy has been found in a dose-dependent manner in patients placed on oral polaprezinc.

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味覚障害と亜鉛

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Symposium I -3 Induction of heat shock proteins and mucosal protection by zinc derivatives in the gastrointestinal mucosa

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Abstract

IntroductionN-(3-aminopropionyl)-L-histidinato zinc (polaprezinc), a chelate compound consisting of zinc ion and L-carnosine, is an antiulcer drug developed in Japan. Polaprezinc has been shown to be effective in preventing gastric lesions in a wide variety of experimental models in vivo and in vitro without affecting gastric acid secretion and endogenous prostaglandins [1-3]. The mechanisms of antiulcer effects of polaprezinc have been thought of its membrane stabilizing, antioxidative function, and induction of insulin-like growth factor [4-6]. Also, it was reported that polaprezinc has the beneficial effects on experimental colitis induced by trinitrobenzenesulforic acid [7]. Many studies showed that various stresses, such as heat, toxins, and some heavy metals induce heat shock proteins (HSPs). HSPs are known to be highly conserved and ubiquitous proteins expressed by eukaryotic and prokaryotic cells. It is thought that HSPs have essential functions for survival of cells and developmental process [8-10]. Previously, it has been reported that HSP70 has cytoprotective functions in gastric mucosa [11-14]. We have previously reported that in the colonic mucosa 72-kDa heat shock protein (HSP72; stress inductive HSP70) and HSP90 were induced by hyperthermia and pre-induction of HSP72 prevented the acetic acid-induced mucosal damage mediated by their function a molecular chaperon [15]. Whereas we have reported that HSP60 had no cytoprotective function in small intestinal and colonic mucosa in vivo [16, 17]. On the other hand, it has been reported that zinc induces HSP70 in cultured rat hepatocytes and in rat small intestinal mucosa [18-23]. In this study, we investigated the influence of polaprezinc on expression of HSPs in rat colonic mucosa and the effect of pre-induction of HSPs on acetic acid-induced colitis.

Methods

Animals

Fifteen to 20-week old male Sprague-Dawley rats (weight: 250-300 g) were fed on standard laboratory diet and water ad libitum, and kept in cages in temperature (22 ± 2°C) and humidity (55 ± 5%) controlled room with a 12 hr dark-light cycle before and during experiments.

Expression of HSPs after polaprezinc administration

Rats were intrarectal administrated with 30 mg/kg of polaprezinc (Zeria Pharmaceutical Co. Ltd., Tokyo, Japan). Each rat was sacrificed by stunning and cervical dislocation before and every 3 hr after polaprezinc administration until 12 hr (n = 4 at each time point), and the colonic mucosa was removed. Also in order to examine the dose-relation of polaprezinc to the expression of HSP72, rats were administered with 0 (control), 10, 20, 30, or 40 mg/kg of zinc L-carnosine (n = 4 in each dose), and sacrificed 6 hr after administration. In order to compare the effects of polaprezinc and its components alone, zinc and L-carnosine, rats were given intrarectal administration of 30 mg/kg polaprezinc, 29.6 mg/kg $ZnSO_4$-$7H_2O$, 23.8 mg/kg L-carnosine, 29.6 mg/kg $ZnSO_4$-$7H_2O$ and 23.8 mg/kg L-carnosine, or vehicle and sacrificed 6 hr later. The doses of L-carnosine and zinc sulfate used were equivalent to the amounts present in polaprezinc in the doses used. The colonic mucosa was quickly removed and was used immediately or frozen at - 80°C until use for Western blot analysis. Each colonic mucosa was homogenized with 5 volumes of ice-cold 25 mM Tris-Cl buffer (pH7.5). The homogenates were centrifuged at 18,000 x g for 20 min. The supernatants were collected and the protein concentrations were adjusted to 0.2 mg/ml. The protein concentration was measured by the method of Bradford [25]. Samples (20μg/lane) were analyzed by 9% polyacrylamide gel electrophoresis according to the method of Laemmli [26]. Gels were stained with 0.1% Coomasssie Brilliant Blue (R-250) in a mixture of 25% isopropyl alcohol-10% acetic acid and destained with 10% isopropyl alcohol-10% acetic acid. Expression of each HSP was evaluated by the method that we previously reported [27, 28]. Briefly, samples (20 μg/lane) were electrophoresed on 9% SDS-polyacrylamide gels, transferred electrophoretically to PVDF membrane (Nihon Millipore Kogyo, Tokyo, Japan), and processed as described by Towbin et al [29]. The membrane was incubated with anti-HSP60, anti-HSP72 (STRESSGEN, Victoria, BC, Canada) or anti-HSP90 [30] antibody and treated with horseradish peroxidase-conjugated anti-rabbit

IgG (1:1500) (Bio-Rad, Richmond, CA, USA). The peroxidase substrate was 3, 3-diaminobenzidine tetrahydrochloride. The density of the immunologically stained band was analyzed by scanning densitometer using NIH image program. The relative density of the stained band was calculated using following formula: relative density (%) = density (after zinc L-carnosine administration) / density (before polaprezinc administration) x 100. Sections of the colonic mucosa before and 6 hr after polaprezinc administration were stained with hematoxyline and eosin for light microscopy to evaluate the histopathologic alteration.

Effect of preinduction of HSPs on acetic acid-induced colonic lesion

In an experiment to determine the effect of preinduction of HSPs by polaprezinc on acetic acid-induced colonic lesion [31], twenty rats were divided into two groups. In group A, rats received intrarectal infusion of 1.0 ml of 5% acetic acid and placed into a cage without pretreatment with polaprezinc administration. In group B, rats received intrarectal infusion of 1.0 ml of 5% acetic acid 3, 6, 9, or 12 hr (n = 4 at each point) after polaprezinc administration and placed into a cage. To compare the effects of polaprezinc and its components alone, zinc and L-carnosine, rats were given intrarectal administration of 30 mg/kg polaprezinc, 29.6 mg/kg $ZnSO_4$-$7H_2O$, 23.8 mg/kg L-carnosine, 29.6 mg/kg $ZnSO_4$-$7H_2O$ and 23.8 mg/kg L-carnosine; 6 hr prior to intrarectal infusion of 1.0 ml of 5% acetic acid. Animals were not given food and water after the acetic acid infusion. Then rats were killed by stunning and cervical dislocation 24 hr after the acetic acid infusion. The colon without cecum was removed, opened along the mesenteric border, and rinsed with ice-cold saline. Macroscopically, the damage area of the colonic mucosa was measured by using a CCD video camera connected to personal computer with the NIH image program. The lesion score was expressed as total damage area of the colonic surface area (square millimeters per rat).

Results

Expression of HSPs after polaprezinc administrationThe relative densities of HSP72 compared with the density at 0 hr were 185.5 ± 25.9% (3 hr; $P < 0.05$), 247.5 ± 20.1% (6 hr; $P < 0.001$), 188 ± 21.4% (9 hr; $P < 0.01$), 156.5 ± 35.0% (12 hr; N.S.). The relative densities of HSP60 were 194.7 ± 20.4% (3 hr; $P < 0.01$), 238.2 ± 18.9% (6 hr; $P < 0.001$), 186 ± 28.2% (9 hr; $P < 0.05$), 137.5 ± 18.9 % (12 hr; N.S.)(means ± SE). No significant increase was observed in HSP90.

Dose-relation of polaprezinc to expression of HSP72 and HSP60 in the colonic mucosa

The synthesis of HSP72 and HSP60 were significantly increased in rat colonic mucosa after polaprezinc administration in a dose-dependent manner at doses ranging from 10 to 40 mg/kg (n = 4 in each dose). The relative densities of HSP72 evaluated by densitometer were 108.6 ± 15.9% (10 mg/kg), 146.0 ± 16.2% (20 mg/kg), 213.0 ± 20.1% (30 mg/kg), 290.2 ± 29.6% (40 mg/kg) compared with the density before polaprezinc administration ($r = 0.872$, $P < 0.001$). The relative densities of HSP60 evaluated by densitometer were 155.2 ± 31.8% (10 mg/kg), 177.0 ± 15.9% (20 mg/kg), 237.2 ± 24.3% (30 mg/kg), 286.0 ± 24.6% (40 mg/kg) compared with the density before polaprezinc administration ($r = 0.800$, $P < 0.001$). No significant increase was observed in HSP90.

Comparison of effects of polaprezinc, zinc, and L-carnosine on expression HSPs

The relative densities of HSP72 evaluated by densitometer were 115.7 ± 11.0% (23.8 mg/kg of L-carnosine), 145.5 ± 8.4% (29.6 mg/kg of $ZnSO_4$-$7H_2O$), 159.5 ± 13.5% (29.6 mg/kg of $ZnSO_4$-$7H_2O$ + 23.8 mg/kg of L-carnosine), 218.7 ± 10.7% (30 mg/kg of polaprezinc) compared with the density of vehicle administration. The relative densities of HSP60 evaluated by densitometer were 108.7 ± 10.0% (23.8 mg/kg of L-carnosine), 150.5 ± 10.4% (29.6 mg/kg of $ZnSO_4$-$7H_2O$), 155.5 ± 10.5% (29.6 mg/kg of $ZnSO_4$-$7H_2O$ + 23.8 mg/kg of L-carnosine), 226.7 ± 13.6% (30 mg/kg of polaprezinc) compared with the density of vehicle administration.

Effect of polaprezinc on acetic acid-induced colonic lesion

Macroscopic findings of acetic acid-induced colitis with or without pretreatment with polaprezinc administration are shown. The lesion scores were significantly lower in Group B, which received the acetic acid infusion 3 hr (202.6 ± 31.5 mm^2/rat, $P < 0.05$), 6 hr (109.7 ± 14.0 mm^2/rat, $P < 0.01$), 9 hr (204.7±12.0 mm^2/rat, $P < 0.05$), 12 hr (314.6 ± 28.3 mm^2/rat, N.S.) after polaprezinc administration compared with those of group A (357.7 ± 19.1 mm^2/rat). The strongest preventive effect on acetic acid-induced colitis was found in rats received acetic acid infusion 6 hr after administration of polaprezinc.

Discussion

When gastric mucosa was pre-exposed to mild irritant or stress, development of mucosal lesion caused by cytotoxic agents could be suppressed, and this phenomenon is called as "adaptive cytoprotection"[32, 33].Originally, this term is applied for gastric mucosal ability to produce prostaglandins to reduce gastric mucosal damage [32-34]. In recent studies, it has been suggested that many endogenous and exogenous factors, such as prostanoids, nitric oxide and endothelin, associate with adaptive cytoprotection [35-39]. We have reported that preinduction of HSP72 in rat gastric mucosa by water-immersion stress prevented HCl-induced mucosal lesions [12, 13]. Also we have reported that gastric mucosal adaptation was accompanied by an increase in HSP72 level after long-term administration of low dose of aspirin which decreases mucosal prostaglandins [14]. Further our recent study showed that hyperthermia increased expression of HSPs in rat colonic mucosa and the severity of acetic acid-induced colonic mucosal damage was significantly reduced [15]. On the other hand, we have reported that functional diarrhea caused by exposure to water-immersion stress, 5-hydroxytriptamine (5-HT), and thyrotropin-releasing hormone (TRH) administration specifically induced the synthesis of HSP60 but not HSP72 without histopathologic alteration in rat colonic mucosa. In other words, we claimed that the synthesis of HSP60 was associated with colonic motility. Although TRH administration specifically induced HSP60, preinduction of HSP60 did not reduce the severity of acetic acid-induced colonic mucosal damage [17]. These findings could suggest that physiological meaning of HSP60 expression might be distinct from those of HSP72 in the colonic mucosa and HSP72 might play important roles for cytoprotection in the gastrointestinal mucosa in vivo. And it could be possible that each HSPs plays different functions in each organ in vivo. Polaprezinc, a chelate compound consisting of zinc ion and L-carnosine, is an antiulcer drug developed in Japan. Dupuy and Szabo have reported that various divalent metals such as zinc, cadmium, and copper protect against ethanol-induced gastric injury and speculated that endogenous sulfhydryls might mediate the cytoprotective effects of metals [41]. Cho et al. showed that the protective effects of zinc sulfate on gastric lesions induced by ethanol without affecting mucosal prostaglandins [42]. And also, it has been reported that polaprezinc has protective effects against gastric lesions induced by restraint, pylorus ligation, reserpine, ethanol, and aspirin without affecting mucosal prostaglandins and gastric acid secretion [1-3]. Yoshikawa et al. has demonstrated that an experimental model of inflammatory bowel disease, trinitrobenzenesulforic acid induced colitis of model of human Crohn's disease, was inhibited by administration of polaprezinc [7]. The mechanisms of cytoprotection have been considered to its membrane stabilizing, antioxidative actions [4, 5]. On the other hand, it is well known that upon exposure to zinc and other heavy metals, metal binding proteins, called as metallothioneins, are induced in all eukaryotes [43, 44]. It has been demonstrated that metallothioneins could detoxify heavy metals and transfer zinc and copper ions to metalloenzymes in mammalian cells [45-47]. Induction of metallothioneins is mediated by activation of metal responsive elements, which is present in the promoter regions of metallothionein gene [48, 49]. It has been also demonstrated that zinc and other heavy metals could activate metal responsive elements [50-52]. And the metal responsive elements are present in the HSP70 promoter regions [53]. Thus, it has been thought that zinc and heavy metals might induce heat shock protein synthesis by activation of heat shock gene transcription. It was reported that low concentration of zinc sulfate could induce the expression of HSP70 in HeLa cells and rat hepatocytes without any cytotoxic effects [18-20]. Tons et al. reported that expression of HSP70 was increased in small bowel by zinc injection and preinduction of HSP70 protected the small bowel lesions induced by ischemia in vivo [22]. Klosterhalfen et al. showed that intraperitoneal administration of zinc induced expression of HSP70 in liver, lung and kidney, and preinduction of HSP70 by zinc prevented the endotoxemia and apoptosis induced by lipopolysaccharide [21]. In the present study, polaprezinc induced HSP72 and HSP60 in colonic mucosa without any pathologic change in a dose-dependent manner. Mucosal HSP72 and HSP60 expression peaked 6 hr and significant increment was observed from 3 to 9 hr after polaprezinc administration whereas HSP90 did not increase. Preinduction of HSP72 and HSP60 by exposure to polaprezinc prevented acetic acid-induced colonic lesion significantly. Also, the effects of polaprezinc, zinc sulfate, and L-carnosine on induction of HSPs in rat colonic mucosa polaprezinc was more effective than zinc sulfate compared with equivalent moles of zinc, although L-carnosine did not induce the expression of HSP72 and HSP60. And only by administration of polaprezinc, the mucosal damage caused by acetic acid was clearly prevented. It was reported that zinc content after intragastric administration of polaprezinc was higher than that of zinc sulfate [54]. Therefore, it was speculated that zinc itself might induce the expression of HSP72 and HSP60, and L-carnosine might enhance adhesive and/or permeable action of zinc on colonic mucosa. We think that colonic HSP72 has crucial cytoprotective function against acetic acid-induced mucosal damage. In spite of preinduction of HSP72 (about 50% increment in protein level) by exposure to zinc sulfate administration, there was no significant difference in mucosal lesion compared with control group. Although preventive effects on acetic acid-induced colonic lesion were observed by about 90% increment of HSP72 at 3 and 9 hours after polaprezinc administration,

about 50% increment of HSP72 after 12 hours polaprezinc administration did not prevent the lesion. These results could indicate that more than 90% increment of HSP72 is required to suppress mucosal lesion induced by acetic acid and 50% increment of HSP72 by zinc sulfate is not enough to prevent it. As colonic HSP60 has no cytoprotective function, HSP60 might play some roles in membrane transportation, transportation or rearrangement of other proteins, which is also as important chaperon function [55-57].

Conclusion

Expression of HSP72 was significantly induced by zinc derivatives. Same phenomenon was observed also in the gastric mucosa. Therefore, zinc derivatives could be a potent chaperon inducer in gastrointestinal mucosa to protect gastrointestinal mucosa.

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消化管疾患と亜鉛 －亜鉛による大腸粘膜、胃粘膜におけるストレス蛋白質誘導作用と粘膜保護作用－

○大高 道郎、渡辺 純夫
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Symposium I -4 Significance of evaluation of serum zinc concentrations in C-viral chronic liver disease

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Abstract
We evaluated zinc concentrations in patients with hepatitis C virus (HCV)-positive chronic liver disease and correlated them with the clinical profiles of the patients. 11 healthy subjects also were included in this study. Serum zinc concentrations were evaluated using conventional atomic absorption spectrometry. The median concentration of zinc in patients with CH was statistically lower than that in healthy control subjects. The median zinc concentrations of the LC and HCC groups were significantly lower than that of the CH group. A significant correlation was observed between the zinc concentrations and the platelet counts and albumin concentrations. The zinc concentrations did not correlate with tumor size and number and decreased with the development of Child-Pugh stage. The zinc concentrations did not correlate with tumor size and number and decreased with the development of Child-Pugh stage. The cumulative survival rate after therapy for HCC nodules in the low zinc concentration group was significantly lower than in the high group.We concluded the serum concentration of zinc influenced the clinical profiles in patients with C-viral chronic liver disease.

Key words; Zinc concentration, Zinc supplementation, chronic hepatitis C

Zinc is a trace element and around 2 g may be found in the adult human body. It contributes to the activity of about 300 metal enzymes and metal-activated enzymes and is indispensable for the metabolism of nucleic acids and proteins. Thus, zinc plays an important role as a component of numerous enzymes and its deficiency results in various disorders. Patients with chronic hepatitis C (CH), particularly those with liver cirrhosis (LC) and liver failure, have low serum zinc concentrations. In this study, we evaluated serum concentrations of zinc in patients with HCV antibody-positive (C-viral) CH, LC and HCC, and examined the relationship between clinical profiles of C-viral chronic liver disease and the serum concentrations of zinc.

PATIENTS AND METHODS

Subjects.

A total of 100 patients with CH, 29 patients with LC and 6 patients who were asymptomatic HCV carriers (ASC) who were examined at Nihon University Itabashi Hospital from January through June 2004, gave informed consent for their participation in this study. All of the patients were positive for serum HCV RNA (AMPLICOR HCV MONITOR; Roche Diagnostic K.K., Tokyo, Japan). In addition, 118 patients who were found to have space-occupying lesions (SOLs) on abdominal ultrasonography and computed tomography (CT) examination, and were diagnosed as having HCC by abdominal angiography from November 1999 through July 2004, were included in this study. All of the HCC patients were positive for serum HCV RNA (AMPLICOR HCV MONITOR; Roche Diagnostic K.K., Tokyo, Japan). No heavy drinkers (more than 30 g ethanol intake per day) were included in the study.

Measurement of serum zinc concentrations.

Zinc concentrations were evaluated using conventional atomic absorption spectrophotometry using Z-6100 polarized zeeman Atomic Absorption Spectrophotometer (HITACHI, Tokyo, Japan).

Hematological and biochemical examinations.

Serum levels of aspartate aminotransferase (AST), ALT, total bilirubin, alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), total protein (TP), albumin (alb), serum ammonia (NH3), branched-chain amino acids (BCAA) and tyrosine molar ratio (BTR; Daiyacolor BTR Enzymatic Methods, Ono Pharmaceutical Co., Ltd., Osaka, Japan), white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), platelet counts, Hepaplastintest (HPT) and prothrombin time (PT) were determined. In addition, serum levels of alpha-fetoprotein (AFP; EIA) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) as a tumor marker were determined by enzyme immunoassay. Serum hyaluronic acid was evaluated by Latex

Immunoturbidmetric method (Mitsubishi Kagaku Iatron, Inc, Tokyo, Japan). Serum type IV collagen 7S was evaluated by Radioimmunoassay (Mitsubishi Kagaku Iatron, Inc). Serum procollagen III peptide (P-III-P) was evaluated by Immunoradiometric assay). The correlations between serum zinc concentrations and values of the above variables were examined.

Long-term outcome in patients with HCC after initial therapy for HCC nodule.

We compared the long-term outcome of patients with HCC in terms of the cumulative probability of survival time after initial therapy for a HCC nodule according to whether they had high ($\geqq$54 μg/ml) or low (<54 μg/ml) concentrations of zinc.

Statistical analysis.

The serum concentrations of zinc were compared using the chi-square test for independence. The differences between groups were assessed using the log-rank test. The remaining parameters were compared using analysis of variance and Fisher's Protected Least Significant difference post hoc test with Statview 4.5 software (Abacus Concepts, Berkeley, CA, USA). A p value of less than 0.05 was considered significant.

RESULTS

Evaluation of zinc concentrations in chronic hepatitis C.

The median concentration of zinc in the 11 healthy control subjects was 86 μg/ml (range: 69-94). In patients with chronic hepatitis C, the median zinc concentration was 52.5 μg/ml (48-65) in the ASC group, 65.5 μg/ml (45-109) in the CH group, 50 μg/ml (27-69) in the LC group, 50 μg/ml (26-89) in the HCC with LC group and 58 μg/ml (35-80) in the HCC without LC group. The median concentrations of zinc in patients with ASC, CH, LC and HCC with LC or HCC without LC were statistically lower than that in healthy control subjects. The median zinc concentration in the LC group was significantly lower than those in the CH and ASC groups. Furthermore, the median zinc concentration in the HCC with LC group was significantly lower than those in the ASC, CH and HCC without LC groups. The median zinc concentration in the HCC without LC group was significantly lower than those in the ASC and CH group. The median zinc concentration in the HCC without LC group was significantly higher than those in the HCC with LC and LC groups. Furthermore, the median zinc concentration in patients with LC according to Child-Pugh classification, A was 55.5 μg/ml (38-69), B was 52.0 μg/ml and C was 32.5 μg/ml. The median zinc concentration in the Child-Pugh C group was significantly lower than those in the A ($P>0.0001$) and B ($P=0.0033$) groups.

Comparison of zinc concentrations among patients with different platelet counts.

The median zinc concentration was 75.0 μg/ml (55-109) in patients in the CH and LC without HCC groups with platelet counts of more than 200,000/mm^3, 65.0 μg/ml (43-97) in the patient groups with platelet counts from 150,000 to less than 200,000/mm^3, 59.0 μg/ml (41-80) in the patient groups with platelet counts from 100,000 to less than 150,000/mm^3, and 50.0 μg/ml (27-69) in the patient groups with platelet counts less than 100,000/mm^3. The median concentration of zinc in the patient group with platelet counts less than 100,000/mm^3 was significantly lower than those from other groups.

Comparison with clinical background factors and biochemical variables.

The median zinc concentration did not differ between males and females according to disease grouping. However, the median zinc concentration in the less than 60 yrs old group was significantly higher than that of the more than 60 yrs old group ($P<0.0001$).The zinc concentration was significantly correlated with indicators of blood and biochemical examinations, such as the RBC counts, Hb concentration, platelet counts, PT, HPT, total bilirubin level and albumin concentration. Furthermore, the median zinc concentration did not differ between groups with serum HCV RNA levels more than 100 kiu/ml and less than 100 kiu/ml or among HCV genotypes in patients with C-viral CH or LC. We compared serum NH3 concentrations or BTR and serum zinc concentrations in patients with LC. In this comparison, serum zinc concentrations tended to be low in those with high concentrations of serum NH3. When serum zinc concentrations were compared to the BCAA concentrations and tyrosine concentrations, serum zinc concentrations and BCAA concentrations were not correlated. However, a significant correlation was observed between serum zinc concentrations and tyrosine concentrations. Serum zinc concentrations were significantly correlated with hyaluronic acid ($R=0.425$, $P<0.0001$).On the other hand, although serum zinc concentrations and serum P-III-P concentrations or serum Type IV collagen concentrations were not correlated, serum zinc concentrations tended to be low in those with high concentrations of these markers of fibrosis in the liver. The relationships between the zinc concentrations and the serum AFP and PIVKA-II concentrations in patients with HCC with LC or with HCC without LC were examined. No significant correlation was observed between the serum zinc concentrations and the serum AFP concentrations ($r=0.059$, $P=0.555$), or between the serum zinc concentrations and the PIVKA-II concentrations ($r=-0.063$, $P=0.581$).

The relationships among tumor morphology, classification and zinc concentrations in patients with HCC.
The median zinc concentrations were not statistically significant according to the development of clinical stage of HCC (stage 1, 56.0 ng/ml; stage 2, 52.0 ng/ml; stage 3, 54.0 ng/ml; stage 4, 54.0 ng/ml). Furthermore, the median serum zinc concentrations were not statistically significant according to the development of size of tumor nodule in patients with a single nodule. When the serum zinc concentrations were compared to the status of the HCC nodules, ranked according to size and numbers, the serum zinc concentrations did not rise with increases in the size of spaces occupied by tumors or the numbers of tumors in the livers.
Long-term outcome in patients with HCC after initial therapy for an HCC nodule.
The cumulative incidence of survival time of HCC patients was compared between low zinc group (<54 μg/ml) and high zinc group (≧54 μg/ml) according to their serum zinc concentrations at the initial therapy for HCC nodule. Death occurred in 20 patients (31.3%) in the low zinc group but in only 10 patients (18.5%) in the high zinc group. The cumulative incidence of survival rate for patients with HCC for three years was 29.8% in the low zinc group and 67.1% in the high zinc group. The difference between the two groups was statistically significant ($P = 0.011$).

CONCLUSIONS

The serum concentration of zinc decreased significantly with development of C-viral chronic liver disease, and reflected the degree of liver damage. The zinc concentrations did not correlate with tumor size and number and decreased with the development of Child-Pugh stage. It was suggested that zinc was not correlated with the presence and growth of cancerous nodules. The cumulative survival rate after therapy for HCC nodules in the low zinc concentration group was significantly lower than in the high group. Because the serum zinc concentration was low in patients with LC and HCC, supplementary administration of zinc to such patients was thought to be necessary. It was suggested that monitoring of the serum zinc concentration was necessary, particularly in patients treated with special amino acid preparations. We concluded the serum concentration of zinc influenced the clinical profiles in patients with C-viral chronic liver disease.

肝疾患と亜鉛

○森山 光彦、荒川 泰行
日本大学 医学部 内科学講座消化器肝臓内科部門

Symposium Zinc and skin diseases I -5

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Acrodermatitis enteropathica is well known as a zinc deficiency skin disease. Recently, we had a patient with systemic scleroderma. He had dysgeusia and weight loss by lack of zinc. With oral polaprezinc (Promac®) of antigastric ulcer drug, his symptoms were improved and he had a normal serum level. Also, we examined the serum zinc level in another patient with systemic sclerosis.
Skin eruptions are very important in diagnosis of several zinc deficiency diseases.

1. Acrodermatitis enteropathica

1) Hereditary (Congenital) Acrodermatitis enteropathica
Hereditary Acrodermatitis enteropathica is an autosomal recessive hereditary disease. This disease often occurrs 8 to 9 months after birth. Skin eruptions, severe alopecia of scalp, and diarrhea are three major symptoms. Pathogenesis of Acrodermatitis enteropathica is a hereditary deficit of absorption disorder of zinc and zinc-combined protein in the intestinal tract.
Skin eruptions of this disease show erythema with small blisters and pustules on extremities periocular, perioral, genital, perianal region, and axillar and inguinal area. These eruptions resemble contagious impetigo. Alopecia occurs in all cases. Diarrhea, anorexia, nausea, and vomiting are found as intestinal symptoms.
Serum zinc level showed very low (8~20μg/dl). Zinc level in urine is also low. Serum alkali phosphatase relates to serum zinc value.
For the treatment of Hereditary Acrodermatitis enteropathica, 200mg-400 mg/day of oral zinc sulfate is usual. Symptoms are improved within 1 week, but the patient must take this drug throughout his life.

2) Aquired Acrodermatitis enteropathica
Aquired Acrodermatitis enteropathica is found in patient with high calorie infusion, postoperation of gastrointestinal diseases such as Crohn disease, and tube feeding. Compared to Hereditary Acrodermatitis enteropathica, skin eruptions of Aquired Acrodermatitis enteropathica usually show only slight erythema on perioral and nasal region and vulva, without hair loss and diarrhea. Several cases are reported due to low zinc in breast-feeding. Aquired Acrodermatitis enteropathica showed slighty lower levels of serum zinc, so a small dosage is enough compared to Hereditary Acrodermatitis enteropathica.

2. Systemic scleroderma
Systemic scleroderma is classified into two types. One is a diffuse type (severe disease) and the other is a limited type (light disease). We measured serum zinc level in 55 patients (20 diffuse types, 35 limited types) of Systemic scleroderma. A lower serum zinc level was found in 18 patients (10 diffuse types, 8 limited types, all women). Those who had lower serum zinc levels showed clinical symptoms such as dysgeusia in 6 patients (6 diffuse types, 2 limited types) and weight loss in 2 patients (2 diffuse types).
When we treated these patients with oral polaprezinc, their conditons (dysgeushia, weight-loss, serum zinc level) were improved. We presumed that low serum levels in systemic scleroderma patients was due to absorption disturbance of intestinal disorder. Some patients informed us that oral zinc sulfate had a bad taste, so we treated these patients with oral polaprezinc. If serum zinc levels are not normal, patients should take this drug 3 times per day.

3. Bedsore
In an aging society, bedsore patients are expected. In addition, most of bedsore patients have some basic diseases such as cerebrovascular disease, myelopathy, malignant tumors, bacterial infectious disease and also their serum zinc levels are low. We should examine serum zinc levels as well as albumin, hemoglobin, serum iron in bedsore patients.

4. Alopecia areata
Dr. Hanada and his colleagues reported that they administered zinc sulfates (200mg/day) to a total of alopecia patients. After 6 to 8 weeks, it was effective in 5 patients.

5. Other skin diseases
Zinc treatment is effective in acne pustulosa, psoriasis vulgaris, arteriosclerosis obliterans, and lichenoid amyloidosis are repored. In any case, zinc treatment should be performed in the patients with lower serum zinc levels.

皮膚疾患と亜鉛

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獨協医科大学 皮膚科学

The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Abstracts

Symposium II Lectures

Symposium Ⅱ-1 Blood lead levels in Japanese children: Effects of passive smoking.

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[Introduction] Lead (Pb) is one of major environmental pollutants, and is highly toxic to human body. Since children are much more vulnerable to lead toxicity than adults, pediatric lead poisoning has been a public health problem not only in the developing countries, but also in the developed countries. Therefore, many studies have been conducted to investigate blood lead level (BLL) of children of those countries. There have been few data, however, on the BLL of Japanese children. On the other hand, it has been reported that children of smoking parents have a higher BLL than those of non-smoking parents [1)2)]. At first in 1993, and again in 2004-2005, we measured BLL of Japanese children and studied the effects of passive smoking on their BLL.

[Materials and Methods] # Study in 1993 [3)]: Venous blood samples were obtained from 188 children (106 boys, 82 girls) aged 1 to 15 years, who visited the pediatric clinic of Shizuoka General Hospital in 1993. We asked their parents with questionnaire about their smoking style in the home. Whole blood lead was measured using a graphite furnace atomic absorption spectrophotometer. The subjects were divided into three groups according to the parent's smoking style as follows; Group A: Children of smoking parents who usually smoke in the same room with their children. Group B: Children of smoking parents who usually take care not to smoke in the same room with their children. Group C: Children of non-smoking parents. They were classified further into preschool children and school children. As a result, there were 6 subgroups in all. Statistical analysis of the data was performed by one-way analysis of variance (Bonferroni's test). # Study in 2004-2005: Venous blood samples were obtained from 282 children (140 boys, 142 girls) aged 3 months to 15 years, who visited Shizuoka Children's Hospital in 2004-2005. We asked their parents orally about their smoking style in the home. Whole blood lead was measured using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). The subjects were divided into 6 subgroups and statistical analysis of the data was performed as mentioned above.

[Results] # Study in 1993 [3)]: The BLL (mean ± standard deviation) of all the subjects was 3.16±1.50μg/dl, and ranged between 0.80 and 9.51μg/dl. The BLL of boys was 3.17±1.34μg/dl and that of girls was 3.14±1.69μg/dl. There was no significant difference between them. The BLLs of groups A, B and C in preschool children were 4.15±1.56μg/dl (n=28), 3.22±1.46μg/dl (n=32) and 3.06±1.31μg/dl (n=31), respectively. The BLLs of groups A, B and C in school children were 2.97±1.50μg/dl (n=34), 3.24±1.66μg/dl (n=26) and 2.56±1.19μg/dl (n=37), respectively. The mean BLL of group A preschool children was significantly higher than those of the other five subgroups, and there were no significant differences among the other five subgroups. # Study in 2004-2005: The BLL of all the subjects was 1.55±0.85μg/dl, and ranged between 0.19 and 7.68μg/dl. The BLL of boys was 1.60±0.81μg/dl and that of girls was 1.49±0.88μg/dl. There was no significant difference between them. The BLLs of groups A, B and C in preschool children were 1.90±0.92μg/dl (n=20), 1.82±1.27μg/dl (n=61) and 1.41±0.60μg/dl (n=60), respectively. The BLLs of groups A, B and C in school children were 1.53±0.65μg/dl (n=36), 1.55±0.75μg/dl (n=44) and 1.30±0.52μg/dl (n=61), respectively. The mean BLLs of group A and B preschool children were significantly higher than those of group C preschool children and group C school children. There were no significant differences among the others.

[Discussion] There have been few data on the BLL of Japanese children. We presented here recent data on the BLL of pediatric patients seen in outpatient clinics. Since the patients selected had common diseases and all patients with metabolic disorders or substance poisoning were excluded, the BLL was unlikely to be influenced by their diseases. Reviewing several data on the children's BLL from various regions of the world around 1990, Hayes et al. reported that the mean BLL of children aged 6 months to 5 years (mean: 2.7 years) was 12μg/dl in Chicago in 1988 [4)]. Sherlock et al. measured the BLL of Caucasian and Asian children (age range 2.5 to 5 years, age and sex-matched) living in London, and reported that the mean value was 9.7μg/dl in Caucasian children and 8.1μg/dl in Asian children in 1985 [5)]. Jin et al. reported that the mean BLL was 6.0μg/dl (range: 1.24 to 17.6μg/dl)

in children aged 24 to 36 months in Vancouver in 1995 [6]. Cambra et al. reported that the mean BLL of children aged 2 to 3 years in Basque county in Spain was 5.7μg/dl and that 14% of the children had levels exceeding 10μg/dl in 1995 [7]. Andren et al. reported that the mean BLL of Swedish children (8 to 13 years of age) of non-smoking parents was 2.95μg/dl in 1988 [1]. Comparing to these reported values, the mean BLL of Japanese children, according to our study in 1993, was among the lowest levels in the industrialized world in the early 1990's. Fortunately the BLLs of children and adults have been decreasing steadily in many countries during these two decades, of which the primary reason is prohibition of leaded gasoline use and subsequent reduction of air pollution with lead. In Switzerland, for example, leaded gasoline was predominantly used formerly, but unleaded gasoline use was encouraged since the late 1980's. Then the mean BLL of adults in Switzerland decreased remarkably in only nine years; from 12.2 to 6.8μg/dl in men and from 8.5 to 5.2μg/dl in women [8]. Decreases of BLL of children have been also observed in many industrialized countries. In Swedish children, for example, dramatic decrease of BLL was found during the period 1978-2001, from about 6 to 2μg/dl, which was considered to reflect a beneficial effect of gradual banning of leaded gasoline [9]. Reviewing the latest data on the children's BLL, Zhang et al. reported that the mean BLL of Chinese children aged 0 to 6 years was 5.95μg/dl in 2005 [10]. Kirel et al. reported that the mean BLL of Turkish children was 3.56μg/dl in 2005 [11]. Friedman et al reported that the mean BLL of 3-year-old Ukrainian children was 3.15μg/dl in 2005 [12]. Gulson et al. reported that the mean BLL of Australian children aged 0.29 to 3.9 years was 2.6μg/dl in 2006 [13]. Comparing to these values, the mean BLL of Japanese children according to our latest study is very low, as it was in the 1990's. The reasons proposed are that leaded gasoline use was prohibited in Japan more than three decades ago, among the earliest in the world, and Japanese people generally do not often do house-painting, which has been considered to be a major cause of lead poisoning in children in the United States and European countries. It had long been considered that blood lead of less than 20μg/dl was almost harmless to human body, because no clinical or biochemical effects had been recognized in such condition until about two decades ago. Since then, however, many studies have revealed that much lower levels of blood lead could adversely affects human health, especially to childhood growth and development. There have been several studies investigating the relationship between BLL and growth of children. Schwartz et al. examined about 2,700 children aged 7 years and younger in the Second National Health and Nutrition Examination Survey (NHANES II) in the United States, and found the inverse correlation between BLL in the range of 5 to 35μg/dl and body height. They concluded that low-level lead exposure could impair the somatic growth of children [14]. Kafourou et al. also revealed negative relationships between BLL and growth parameters in Greek children aged 6-9 years, an increase in BLL of 10μg/dl being associated with a decrease of 0.86cm in height, 0.33cm in head circumference and 0.40cm in chest circumference [15]. The adverse effects of low-level lead exposure on children's intellectual and academic performance at school are more serious problems. Bellinger et al. evaluated the relationship between BLL and neuropsychological test score in middle-class and upper-middle-class children, and found that over the range of approximately 0 to 25μg/dl, a 10μg/dl increase of BLL at 24 months was associated with a 5.8-point decline in WISC-R (Wechsler Intelligence Scale for Children-Revised) Full Scale IQ and an 8.9-point decline in K-TEA (Kaufman Test of Educational Achievement) Battery Composite score at age 10 years. These data indicated that slightly elevated BLL around the age of 2 years is associated with intellectual and academic performance deficits at age 10 years [16]. Scientific understanding of the health effects of lead has flourished over the past two decades. Advances in this area of research have spawned a series of efforts by governmental agencies to enhance the protection of public health from the adverse effects of lead. The United States Centers for Disease Control and Prevention and the World Health Organization defined a BLL of 10μg/dl as the threshold of concern in young children. Therefore, one of main goal of those efforts was to reduce numbers of children who had BLL exceeding 10μg/dl during these two decades. Blood lead of less than 10μg/dl was considered to be almost harmless, because any derangement of biochemical indices were not observed originated from lead of the lower level. Most recently, however, it has been considered that there does not exist the safe level of blood lead, and lead exposure is still a serious health problem for children. Many studies have demonstrated that even very low-level lead exposure causes intellectual and behavioral impairment in children. Lanphear et al. reviewed recent international population-based longitudinal cohort studies investigating the relationship between low-level lead exposure and intellectual deficits in children, and found an inverse relationship between BLL and IQ score even in the range of low BLL. They reported that using a log-linear model, a 6.9 IQ point decrement was found associated with an increase in concurrent BLLs from 2.4 to 30μg/dl. The estimated IQ point decrements associated with an increase in blood lead from 2.4 to 10μg/dl, 10 to 20μg/dl, and 20 to 30μg/dl were 3.9, 1.9, and 1.1, respectively. For a given increase in blood lead, the lead-associated intellectual decrement for children with a maximal BLL less than 7.5μg/dl was significantly greater than that observed for those with a maximal BLL exceeding 7.5μg/dl. They concluded that environmental lead exposure in children who had maximal BLL less

than 7.5μg/dl was associated with intellectual deficits [17]. Moreover, several recent studies indicated the adverse effects of low-level lead exposure on the sexual maturation in adolescents. Wu et al. assessed measures of puberty in girls in relation to BLL to determine whether sexual maturation might be affected by current environmental lead exposure, using data from the Third National Health and Nutrition Examination Survey (NHANES III) in the United States. They showed negative relationship between BLL and attainment of menarche or stage 2 pubic hair, which remained significant in logistic regression even after adjustment for race, age, family size, residence in metropolitan area, poverty income ratio, and body mass index. They concluded that higher BLL was significantly associated with delayed attainment of menarche and pubic hair among U.S. girls [18]. Selevan et al. analyzed the relations between BLL and pubertal development among girls aged 8-18 years, including three ethnic groups; non-Hispanic white, non-Hispanic African-American and Mexican American, also using data from NHANES III. They reported that BLL of 3μg/dl was associated with significant delays in breast and pubic hair development in African-American and Mexican American girls. They suggested that environmental exposure to lead might delay growth and pubertal development in girls, although confirmation should be warranted in prospective studies [19]. It has been suggested that children who are exposed to cigarette smoke have higher BLL than children who are not [1)2)]. We measured BLL of Japanese children and evaluated the effects of passive smoking on the BLL, and found that passive smoking increased the BLL of preschool children in the study in 1993. The mean BLL of preschool children who were exposed to cigarette smoke in their home was 4.15μg/dl, and those whose family never smoked was 3.06μg/dl, the difference significant. We also found that passive smoking did not increase the BLL of school children. About the reasons of the difference of the effects of passive smoking on the two groups of children, we speculate that preschool children might spend more time with their parents and might have more contact with cigarette smoke than school children, and additionally, young infants have limited ability to excrete lead from the body because of the immaturity of renal function. Ballew et al. investigated the BLL of a total of 4,391 non-Hispanic white, non-Hispanic black, and Mexican-American children of the United States aged 1 to 7 years, using data from NHANES III. They reported that the mean BLL of the children who had smoking family was 4.36μg/dl and that of the children who had not was 3.29μg/dl [20], very similar to the values reported in our study. Stromberg et al. also reported a significant effect of parental smoking habits on the BLL of Swedish children, 18% increase on average [9]. We measured BLL of Japanese children and evaluated the effects of passive smoking on the BLL also in the study in 2004-2005, and found that the BLLs of preschool children who had smoking parents (both Group A and B) were higher than that of preschool children who had non-smoking parents (Group C). In this study, the BLL of Group B preschool children was as high as that of Group A, for which reason we speculate insufficiency of protection of children against cigarette smoke in their home, in spite of the Group B parents' answer that they usually take care not to smoke in the same room with their children. It is evident that passive smoking is now a major cause of lead exposure for children. Children should be protected from cigarette smoke for the purpose of avoiding the risk of increased BLL which might adversely affect their intellectual development and physical growth.

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わが国の小児の血中鉛濃度 －受動喫煙の影響－

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Symposium Ⅱ-2 Study on detoxification and utilization of selenomethionine: a role of cystathionine γ-lyase in selenomethionine metabolism

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Selenium (Se) is an essential trace element for mammals. However, it is also known to be highly toxic, and the range of Se concentrations between physiological requirement and toxic appearance is narrow. In human, Se is supplied by various kinds of food, and populations inhabiting areas with a low or high concentration of it in soil are risk as to a deficiency or excess. Additionally, Se is becoming popular as a supplement for nutritional demand and also for preventing cancer. Both inorganic and organic forms of Se can be used as nutritional sources.
Selenomethionine (SeMet), the Se analog of methionine (Met), was first suspected in the mid 1930s to be one of the toxic principles of seleniferous plants. Chromatographic evidence for presence of SeMet in seleniferous wheat protein extracts was obtained in 1949. SeMet was also identified in plant protein in the 1950s-1960s. Concurrent studies established that SeMet was synthesized by certain marine algae, *Escherichia coli*, *Candida albicans* and rumen bacteria when these were grown in media containing Se. At the same time, the discovery of the nutritional essentiality of Se created the need for an appropriate supplemental form of Se to prevent Se deficiency diseases in farm animals. In the 1970s, metabolic studies indicated that SeMet is well absorbed and retained, suggesting its use for nutritional Se supplementation. To provide an economical food source of SeMet for use in human nutrition, methods for the industrial production of Se-enriched yeast were developed. By mid 1980s, synthetic SeMet was also beginning to be produced at a cost comparable to that of Se-enriched yeast. Numerous experimental studies have shown that SeMet and Se-enriched yeast are suitable for nutritional Se-supplementation. However, concerns have also been raised that SeMet might, under some conditions, accumulate in body to toxic levels. Therefore, it is important to elucidate the metabolic pathway of SeMet in mammals.
The metabolism of SeMet taken through diet or supplement has been proposed as fellows: it is activated initially by adenosylation, demethylated, and converted to selenocysteine (SeCyH) via selenohomocysteine and selenocystathionine in analogy to the Met pathway without SeMet-specific enzyme. SeCyH formed could be degraded further by SeCyH β-lyase to hydrogen selenide (H_2Se) and might then be incorporated into functional selenoproteins as SeCyH residues via gene products though selenocysteinyl-tRNA. H_2Se is classified as a more toxic compound than other Se compound, so an excess amount of it is immediately converted to methylated form of Se. One of monomethylated Se compounds, 1β methylseleno-*N*-acetyl-D-galactosamine, is excreted as the major form into urine at nutritional dose or low-toxic dose. Meanwhile, trimethylselenonium ion, $(CH_3)_3Se^+$, is known to be excreted into urine at high-toxic doses. Our recent work indicates that SeMet is enzymatically converted to CH_3SeH, α-ketobutyrate, and ammonia by mouse hepatic enzyme. We termed the novel enzyme SeMet α,γ-elimination enzyme and purified it to homogeneity for the first time. We introduce new metabolic pathway of SeMet from the point of view of both Se detoxification metabolism and selenoprotein biosynthesis.

Purification and characterization of mouse hepatic SeMet α,γ-elimination enzyme.
The enzyme that catalyzes SeMet to transform CH_3SeH was ubiquitous in tissues from ICR mice and the activity was relatively high in liver. Aging and sex of the mice did not have any significant influence on the activity in the liver. The enzyme was purified from mouse liver by ammonium sulfate precipitation and four kinds of column chromatography (**Table 1**). The specific activity of final preparation was approx 1000-fold higher than that of the cytosolic fraction. Overall recovery was approx 8 %. It was found that the pure enzyme could be stored at 4°C for 3 wk without loss of activity. The enzyme had highest activity at pH 8.0 and 50°C. The molecular mass of purified enzyme was approx 160 kDa, with four identical subunits. The enzyme catalyzed both α,γ-elimination and α,β-elimination reaction: as well as L-SeMet, the analogs D,L-homocysteine, D,L-homoserine,

Se-methylseleno-L-cysteine, and L-cysteine were effective substrates. However, L-methionine, D-methionine, D,L-ethionine, and L-serine were inert. The structural characteristic of these substrates for the specificity of this enzyme was not recognized. The purified enzyme catalyzed mainly L-SeMet rather than D,L-SeMet, indicating that the enzyme might only weakly catalyze the α,γ-cleavage reaction of D-amino acid. We speculated that the L-configuration of the α-carbon might be essential for binding of substrate to the active site of this enzyme. The Michaelis content (*K*m) of the enzyme for the catalysis of L-SeMet, estimated by the Lineweaver-Burk plot, was 15.5 mM, and the maximum rate of metabolism for enzyme reaction (*V*max) was 0.29 units/mg protein. Pyridoxal 5'-phosphate (pyridoxal-P) was required as a cofactor because the holoenzyme could be resolved to the apoenzyme by incubation with hydroxylamine and reconstituted by addition of pyridoxal-P.

Identification of mouse SeMet α,γ-elimination enzyme.

SeMet α,γ-elimination enzyme separated by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was subjected to in-gel trypsin digestion followed by matrix-assisted laser desorption/ionization-time-of-flight mass (*o*MALDI-Qq-TOF MS) spectrometric analysis. Mass spectrometric data are shown in **Fig. 1**. In the peptide mass fingerprinting (PMF) search, the mass numbers of 14 peptides produced by tryptic digestion of the enzyme were consistent with the theoretical mass numbers calculated from amino acid sequence of mouse cystathionine γ-lyase (EC 4.4.1.1). Because this search is not method of structural elucidation, there is a possible that different sequences of peptides might have the same mass number and modified peptides might have unexpected mass number. Therefore, the structural information by a peptide sequence tag (PST) search based on the fragmentation data of tryptic peptides is indispensable. As a result of PST search, the amino acid sequences of five peptides were unique to tryptic digests of mouse cystathionine γ-lyase (**Fig. 2**). From these results, it became clear that the purified mouse enzyme was consistent with cystathionine γ-lyase. SeMet is transformed to SeCyH by the several enzymes contributing to Met metabolism, and cystathionine γ-lyase catalyses the conversion of selenocystathionine in addition to cystathionine. SeCyH produced from SeMet through this pathway is converted to H_2Se by SeCyH β-lyase. H_2Se may be used for either selenoproteins biosynthesis or sequential methylation. We revealed that cystathionine γ-lyase also catalyzes the α,γ-elimination reaction of SeMet and directly converts to CH_3SeH, a well-known methylated intermediate for excretion. Therefore, we proposed that cystathionine γ-lyase might be a key enzyme for detoxification and physiological utilization of SeMet in mammals.

Contribution of cystathionine γ-lyase to SeMet detoxification.

When Se-deficient mice received a single or repeated oral administration of SeMet at toxic dose, the cystathionine γ-lyase activity for SeMet in liver was invariable, suggesting that this enzyme may be valuable for the detoxification. An excess amount of SeMet is known to be excreted in the urine in the form of $(CH_3)_3Se^+$, which is generated from H_2Se and CH_3SeH by *S*-adenosylmethionine (SAM)-dependent methyltransferases. H_2Se and CH_3SeH are nucleophilic agents and form the acid-volatile Se with various proteins containing SH group when their amount exceeds the capacity for methylation. Actually, the contents of $(CH_3)_3Se^+$ and acid-volatile Se in liver rapidly increased after oral administration of SeMet at 10 mg/kg. Periodate-oxidized adenosine (PAD) is an inhibitor of *S*-adenosylhomocysteinase (EC 3.3.1.1) and blocks both the conversion of SeMet to SeCyH and the conversion of H_2Se to $(CH_3)_3Se^+$. Propargylglycine (PPG), an effective inhibitor of cystathionine γ-lyase, inhibits both the direct conversion from SeMet to CH_3SeH and the transformation from selenocystathionine to SeCyH. In the PPG-pretreated mice after oral administration of SeMet at 10 mg/kg, the production of acid-volatile Se derived from either H_2Se or CH_3SeH in the liver was strongly suppressed. However, the acid-volatile Se content of PAD-pretreated mice liver was increased 1 h after the administration of SeMet as well as control mice. We speculated that this increase of acid-volatile Se in the PAD-pretreated mice is mainly the result of CH_3SeH produced from SeMet by cystathionine γ-lyase because free H_2Se are hardly produced via SeCyH. Meanwhile, there was no production of $(CH_3)_3Se^+$ because SAM-dependent methylation was blocked by PAD simultaneously. We presumed that CH_3SeH generated from SeMet by cystathionine γ-lyase would be transformed to $(CH_3)_3Se^+$ unless the methylation process is inhibited. By the way, Met adenosyltransferase was strongly inhibited by selenide. Met adenosyltransferase is related to the Met metabolism; it converts Met to SAM and converts SeMet to *Se*-adenosylselenomethionine. SAM produced from Met is necessary for Se methylation as a methyl donor. Therefore, there is a possibility that H_2Se produced from SeMet via SeCyH delays the final urinary excretion and enhances the toxicity. In contrast, the metabolic pathway that directly transforms SeMet to CH_3SeH is likely more significant in the detoxification when SeMet is administered at toxic doses, because CH_3SeH itself is not an inhibitor of Met adenosyltransferase. Additionally, based on the *K*m value for SeMet, we suggested that SeMet

might be metabolized slowly by cystathionine γ-lyase *in vivo*. The low turnover seems to be reasonable for the regulation of CH_3SeH content that exists in tissues, because an excess amount of CH_3SeH might cause serious damage by the formation of acid-volatile Se. These findings suggest that the direct conversion of SeMet to CH_3SeH, intermediate of methylation from H_2Se to $(CH_3)_3Se^+$, by cystathionine γ-lyase plays an important role in the quick excretion of SeMet detoxification.

Contribution of CH_3SeH to cellular glutathione peroxidase (cGPx) biosynthesis.

The biological action of SeMet in animals is dependent on its metabolites. It is believed that the production of H_2Se via SeCyH is essential for achieving physiological function as selenoproteins. H_2Se forms selenophosphate with ATP, and then the selenol group is transferred to selenoproteins via gene products through selenocysteinyl-tRNA. If CH_3SeH is demethylated to H_2Se in mammalian tissues, cystathionine γ-lyase that directly converts SeMet to CH_3SeH is a notable enzyme contributing to the selenoprotein synthesis. We focused on the alteration of cGPx mRNA level and cGPx protein level in the liver of the Se-deficient mice treated with SeMet at nutritional doses (5, 50, 100 μg Se/kg/d). The activity of hepatic cystathionine γ-lyase for SeMet was not significantly changed by the Se deficiency resulting from dietary Se depletion for 8 wk. It was recognized that the Se deficiency causes a decrease in both levels of cGPx mRNA and the protein as well as its activity in mouse liver. After SeMet was administered orally at 5 μg Se/kg/d, the diminished cGPx mRNA and cGPx protein levels were quickly restored compared with the enzyme activity. Although the metabolism of SeMet via SeCyH was inhibited by PAD, levels of both cGPx mRNA and cGPx protein in Se-deficient mice were significantly restored 1 d after the administration of SeMet. In the case of PPG-pretreated mice, these recoveries were strongly restrained because the metabolism of SeMet to generate H_2Se or CH_3SeH was inhibited. These results suggested that CH_3SeH generated from SeMet by cystathionine γ-lyase is utilized for cGPx biosynthesis. Because the physiological concentration of SeMet in the tissues is lower than the *K*m value, there was some doubt as to whether the enzyme catalyzes a small amount of SeMet. The present results showed that the total activity of the enzyme in the liver is most likely sufficient to metabolize SeMet administered at a nutritional level. We also speculated that CH_3SeH produced from SeMet by cystathionine γ-lyase is converted to selenide that is utilized for cGPx biosynthesis. However, there is no report that free H_2Se was actually detected in the living body. It seems that the existence of free H_2Se in tissues is impossible, because it has the active selenol group. A trace amount of H_2Se must be immediately converted to stable forms. Although it might be one of the stable forms, acid-volatile Se was not detected in Se-deficient mice after administration at a nutritional dose. In our previous study, the content of reduced glutathione (GSH) in the liver was decreased by the administration of SeMet. A trace amount of H_2Se generated from CH_3SeH might immediately react with GSH and then might form glutathione selenopersulfide (GSSeH). Because this compound is readily converted to H_2Se by glutathione reductase, GSSeH is a more reasonable and effective form that provide selenide for cGPx biosynthesis. Cystathionine γ-lyase, which plays an important role in not only the SeMet detoxification but also the selenoproteins biosynthesis, is a notable enzyme in SeMet metabolism in mammals (**Fig. 3**).

Table 1 Purification of SeMet α,γ-elimination enzyme from mouse liver

Purification step	Total protein (mg)	Total activity (Units)	Specific activity (Units/mg)	Purification factor (fold)	Yield (%)
Cytosolic fraction	3,447	0.603	0.00017	1.0	100
Ammonium sulfate fractionation	1,581	0.745	0.00048	2.8	124
Phenyl Sepharose 6 Fast Flow (high sub) hydrophobic-interaction chromatography	142	0.452	0.0032	18.8	75.0
Sephacryl S-200 gel-filtration chromatography	40.3	0.292	0.0072	42.4	48.4
Hydroxyapatite chromatography	5.21	0.185	0.036	212	30.7
Phenyl Sepharose High Performance hydrophobic-interaction chromatography	0.29	0.049	0.17	1000	0.1

One unit of enzyme was defined as 1 pmol of α-ketobutyrate generated per minute.

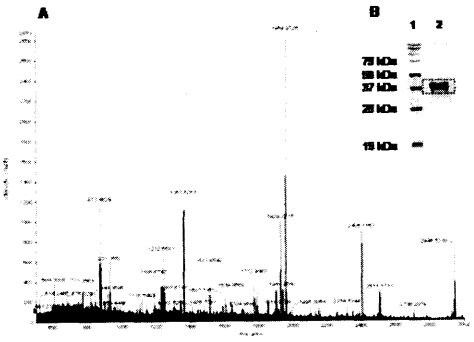

Fig. 1 MALDI-TOF MS spectrum of the tryptic peptide mixture of SeMet α,γ-elimination enzyme applied to a one-dimensional polyacrylamide gel
A, Mass spectrum obtained by MALDI-TOF MS from the protein band shown in B.
B, Scanned picture of the silver-stained gel with the enzyme loaded. Lines: 1, molecular mass marker proteins; 2, purified enzyme. Approximate molecular masses (in kDa) are indicated on the left.

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  1 MQKDASLSGF LPSFQRFATQ AIRVGQEPEQ WHSRAVVLPI SLATTFKQDF PGQSSGFKYS
 61 RSGRPTRRCL EKAVAALDGA KRSLAFASGL AATITITRLL KAGDEIICMD EVYGGTRRYF
121 RRVASEFGLK ISFVDCSRTK LLEAAITPQT KLVWIETFTN PTLRLADIGA CAQIVRRGD
181 IILVVDRTFM SAYFQRPLAL QADICMCSAT KYMRGRSDVV MGLVSVRSDD LRSRLRFLQR
241 SLGAVPSPFD CYLCCRGLKT LQVRMKKRFK RGMAVAKFLE TRPRMRRVVY PQLPSRFQRE
301 LRRRQCSGCP GMVSFYIRGA LQRAKAFLRR LKLFTLAESL GGYESLAELP AIMTRASVPE
361 KDRATLGIRD TLIRLSVGLE RRQDLLRDLD RALKAARP
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Fig. 2 Amino acid sequences of the tryptic peptides of SeMet α,γ-elimination enzyme determined by PST search
Peptide sequences were significantly matched with cystathionine γ-lyase and 17 % sequence coverage as underlined.

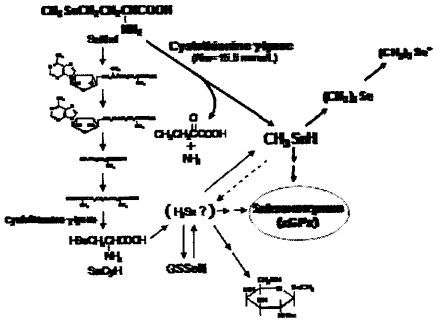

Fig. 3 Metabolic pathway of SeMet in mouse liver

セレノメチオニンの解毒代謝ならびに生理学的利用

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Symposium II-3 Hippocampal Cell Death by Synaptic Zinc

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1. Zinc as a negative-feedback factor

Zinc has three functions in zinc metalloproteins, i.e., catalytic, coactive (or cocatalytic), and structural. Zinc is necessary for DNA replication and transcription, and protein synthesis. Numerous zinc enzymes and proteins are associated with the metabolism of proteins, nucleic acids, carbohydrates, and lipids. In the brain, zinc turnover is strictly regulated via the brain-barrier system. Intracellular zinc concentration in the brain is estimated to be approximately 150 μM, judging from zinc concentration in the total brain, while extracellular zinc concentration in the brain is estimated to be approximately 0.15-1 μM from zinc concentration in the cerebrospinal fluid and extracellular zinc concentration measured by in vivo microdialysis. Zinc concentration in the hippocampus is relatively high in the brain and zinc action is closely linked to functions and pathological processes in the hippocampus[1]. Zinc serves not only as an intracellular signal factor via the interaction with many proteins but also as an extracellular signal factor in synaptic neurotransmission. There is a large number of evidence on zinc-containing glutamatergic neurons that sequester zinc in the presynaptic vesicles and release it in a calcium- and impulse-dependent manner. Zinc in the presynaptic vesicles is histochemically reactive and stained by Timm's sulfide-silver method. Zinc concentration is high in the giant boutons of hippocampal mossy fibers and all giant boutons of mossy fibers contain zinc in the presynapttic vesicles, while approximately 45% of Schaffer collateral boutons are zinc-positive. Numerous reports have demonstrated that zinc is released with glutamate; zinc is released by tetanic stimulation (10-100 Hz), which induces the mossy fiber long-term potentiation (LTP). When mossy fiber terminals were preferentially double-stained with zinc and calcium indicators and tetanic stimuli (100 Hz, 1 s) were delivered to the dentate granule cell layer, the increase in calcium orange signal during the stimulation was enhanced in mossy fiber terminals by addition of 1 mM CaEDTA, a membrane-impermeable zinc chelator, and was suppressed by addition of 0.1 mM zinc[2]. The decrease in FM4-64 signal (vesicular exocytosis) during tetanic stimulation (10 Hz, 180 s), which induced mossy fiber LTP, was also enhanced in mossy fiber terminals by addition of CaEDTA and was suppressed by addition of zinc. Zinc released from mossy fibers may negatively modulate exocytosis during tetanic stimulation and be a negative-feedback factor against presynaptic activity.

2. Zinc action in excessive excitation

To understand the action of extracellular zinc during excessive excitation, extracellular zinc and glutamate concentrations during stimulation with 100 mM KCl were compared between the hippocampal CA3 and CA1 by the in vivo microdialysis. Extracellular zinc concentration was immediately increased in the CA3 after stimulation with high K^+, while its increase was delayed in the CA1. The total amount of extracellular zinc during stimulation with high K^+ was more in the CA3 than in the CA1. However, the total amounts of extracellular glutamate and aspartate during stimulation with high K^+ were more in the CA1 than in the CA3. It is likely that zinc release form mossy fibers are more than that from Schaffer collaterals during stimulation with high K^+ and that more increase in extracellular zinc is linked with less increase in extracellular glutamate in the CA3. To see zinc action in mossy fiber synapses during excessive excitation, 1 mM glutamate was regionally delivered to the stratum lucidum, in which mossy fiber synapses exist, in hippocampal slices double-stained with zinc and calcium indicators. Extracelluar zinc signal was markedly increased in the stratum lucidum and intracellular calcium signal was increased in the CA3 pyramidal cell layer. These results suggest that the excessive delivery of glutamate leads to the release of zinc and glutamate from mossy fibers and excites mossy fiber synapses. The persistent increase in calcium signal in the CA3 pyramidal cell layer during stimulation with glutamate was significantly attenuated in the presence of 0.1 mM zinc, while significantly enhanced in the presence of 1 mM CaEDTA. Zinc released from

mossy fibers may attenuate the increase in intracellular calcium signal in mossy fiber synapses and postsynaptic CA3 neurons after excessive inputs to dentate granular cells. The zinc may negatively modulate the activity of mossy fiber synapses even under the condition of excessive excitation. However, it is possible that the zinc becomes neurotoxic because of the translocation of the zinc to CA3 neurons. Thus, excessive zinc release from mossy fibers seems to be linked with both functional and toxic aspects.

3. Zinc neurotoxicity in epileptic seizures

Zinc concentration in the brain may be decreased by epileptic seizures. Kainate is an agonist of glutamate receptor subtypes and kainate-challenged mice and rats are an experimental model of human temporal lobe epilepsy. They have been used to understand the relationship between zinc movement in the brain and epileptic seizures. Zinc concentration in the hippocampus is decreased in kainate-challenged mice[3]. A selective loss of Timm's stain is observed in the hippocampal mossy fibers after electrical stimulation of the perforant path, which evokes hippocampal granule spikes and epileptiform discharges. Timm's stain was also attenuated in the hippocampus and other brain regions after challenge with kainate[3]. In the hippocampus of young rats challenged with kainate, extracellular concentrations of zinc and glutamate were markedly increased. Thus, the attenuation of Timm's stain is linked with excessive excitation of zinc-containing glutamatergic neurons and implies the translocation of zinc to postsynaptic neurons. When kainate-induced hippocampal cell death was analyzed by cresyl violet staining, neuronal loss was observed in the CA1, CA2 and CA3 pyramidal cell layers after challenge with kainate[4]. Hippocampal cell death seems to be due the translocation of zinc to postsynaptic neurons through calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate and NMDA receptors.

4. Enhanced glutamate excitotoxicity by zinc deficiency

Susceptibility to kainate-induced seizures was enhanced in young mice and rats fed a zinc-deficient diet for 4 weeks, in which Timm's stain was attenuated in the brain[5]. When the release of zinc and neurotransmitters in the hippocampus of kainate-challenged zinc-deficient rats was studied using in vivo microdialysis, extracellular zinc concentration in the hippocampus was less than 50% of that of the control rats and increased levels of extracellular zinc by challenge with kainate were lower than the basal level in control rats. Extracellular glutamate concentration in the hippocampus of the zinc-deficient rats were more increased than in the control rats, whereas extracellular γ-amino butyric acid (GABA) concentration in the hippocampus were not at all increased in the zinc-deficient rats unlike in the control rats. These results demonstrate an enhanced release of glutamate associated with a decrease in GABA concentrations as a possible mechanism for the increased seizure susceptibility in zinc deficiency.

Neuronal loss, which was analyzed by cresyl violet staining, was more observed in the CA1, CA2 and CA3 pyramidal cell layers of zinc-deficient group than those of the control group after challenge with kainate[4]. TUNEL-positive cells were significantly more detected in the CA1 and CA3 pyramidal cell layers of zinc-deficient group. Kainate-induced hippocampal cell death may occur more easily in zinc deficiency. When intracellular calcium levels were checked in hippocampal slices with calcium indicators, the basal calcium signal in the hippocampus was increased by zinc deficiency[6]. Furthermore, intracellular calcium signal was more increased in the hippocampal CA3 areas of zinc-deficient group after delivery of kainate to dentate granule cells. It is likely that alteration of calcium signaling is linked to the increase in kainate-induced hippocampal cell death in zinc deficiency[7]. Glutamate excitotocity seems to be enhanced by zinc deficiency. Glutamate exitotoxicity is a final common pathway for numerous pathological processes such as Alzheimer's disease and amyotrophic lateral sclerosis, in addition to stroke/ischemia, temporal lobe epilepsy. It is estimated that pathological processes associated with glutamate excitotoxicity are aggravated by zinc deficiency. Therefore, adequate zinc supply to the brain is important for brain function and prevention of neurological diseases.

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シナプス小胞亜鉛による海馬神経細胞死

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Symposium II-4 Analysis of induction mechanism of imposex caused by tributyltin and triphenyltin in gastropod molluscs

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Abstract

Imposex, the superimposition of male type genital organs (penis and vas deferens) on female gastropods, is cause-specific and occurs at low concentrations of certain organotins such as tributyltin (TBT) and triphenyltin (TPT) from antifouling paints. Reproductive failure may be involved at severely affected stages, resulting in population decline or mass extinction. Fundamental knowledge of endocrinology in gastropod molluscs is briefly described. Four hypotheses, such as aromatase-inhibition, about the induction mechanism of imposex caused by organotins in gastropods are reviewed. Finally, our new hypothesis that RXR plays an important role in inducing the development of imposex, the differentiation and growth of male type genital organs in female gastropods, is reviewed and discussed toward elucidation of the entire mode of action of TBT or TPT on the development of imposex in gastropods.

Certain environmental chemicals could cause feminization of males and/or masculinization of females in organisms, and such phenomena are generally called endocrine disruption. Here, the authors will focus on reviewing masculinization of female gastropod molluscs, imposex, in terms of the basic biology and induction mechanism of imposex, and then discussing our new hypothesis on the imposex induction mechanism in gastropods.

The first report on the masculinized female gastropod molluscs was made by Blaber (1970), showing a penis-like outgrowth behind the right tentacle in spent females of the dog-whelk, *Nucella lapillus* around Plymouth, U.K. The term *imposex*, however, was coined by Smith (1971) to describe the syndrome of a superimposition of male type genital organs, such as penis and vas deferens, on female gastropods. Imposex is thought to be irreversible. Reproductive failure may occur in females with severe imposex, resulting in population decline or even mass extinction. In some species, imposex is typically induced by tributyltin (TBT) and triphenyltin (TPT), chemicals released from antifouling paints used on ships and fishing nets.

As of 2004, approximately 150 gastropod species have been reported to be affected by imposex worldwide. Regarding Japanese gastropods, at least 39 species (7 mesogastropods and 32 neogastropods) have been found to be affected by imposex among 69 species examined.

Due to insufficiency in basic biology of molluscs, the knowledge of reproductive physiology and/or endocrinology in gastropods has been limitedly obtained. The knowledge has been mainly accumulated in certain species in Opisthobranchia (e.g., *Aplysia californica*) and Pulmonata (e.g., *Lymnaea stagnalis*): Several neuropeptides released from visceral ganglia, cerebral ganglia, or the prostate gland of gastropods (e.g., *A. californica* and *L.stagnalis*) are egg-laying, ovulation, or egg-releasing hormones. Less knowledge of reproductive physiology and/or endocrinology in Prosobranchia (including Archaeo-, Meso- and Neogastropoda), however, has been accumulated.

Because sex steroid hormones, such as testosterone and 17β-estradiol, play physiologically important roles in the development of sex organs and maturation of gonads (i.e., oogenesis and spermatogenesis) in vertebrates, similar sex steroid hormones might also regulate the reproduction of invertebrates, such as gastropods. Several vertebrate-type sex steroids (androsterone, estrone, 17β-estradiol and testosterone) as well as the synthetic estrogen (ethynylestradiol) were identified by high resolution gas chromatography with mass spectrometry (GC-MS) in gonads of *Thais clavigera* and *Babylonia japonica*. The identification of the synthetic estrogen, ethynylestradiol in the gonads indicated contamination of the habitat of *B. japonica* had occurred. Similarly, contamination by other vertebrate-type sex steroids of the habitat of *T. clavigera* and *B. japonica* may have occurred.

Evidence for steroid-producing cells as well as synthetic/metabolic enzymes for steroid biosynthesis must be completely obtained to clarify existence of vertebrate-type sex steroid hormones in gastropods. Although aromatase-like activity has been measured and reported in several gastropod species, measured aromatase-like activity does not necessarily mean the existence of vertebrate-type aromatase in gastropods. There has been no scientific report to elucidate the aromatase protein successfully isolated from invertebrates.

Based on a study of fully sequenced invertebrate genomes, homologues of estrogen receptor (ER) and androgen receptor (AR) have not been found in invertebrates. Thus, it is obscure whether gastropods have AR and ER. Although ER-like cDNA was isolated from *A. californica* (Gastropoda: Opisthobranchia), but it could not bind to estrogen and was a constitutively activated transcription factor. ER-like protein was also isolated from the rock shell (*T. clavigera*), but it could not bind to estrogen, either, and was a constitutively activated transcription factor, similarly to *A. californica* (Katsu, Iguchi and Horiguchi, unpublished data).Therefore, further studies are necessary to examine steroid receptors and the function of steroids in gastropods.

Regarding the induction mechanism of imposex, several hypotheses have been proposed and they can be summarized as 1) increased androgen levels, such as testosterone, due to aromatase inhibition by TBT, 2) inhibition by TBT of the excretion of sulfate conjugates of androgens, 3) disturbance by TBT of penis morphogenetic/retrogressive factor released from pedal/cerebropleural ganglia, and 4) increase in a neuropeptide, APGWamide level caused by TBT. Experimental evidence, however, is weak for these 4 hypotheses. Thus, at present, four hypotheses about the induction mechanism of imposex in gastropods cannot be fully supported.

There are several characteristics in the developing process of imposex caused by organotin compounds, such as TBT and TPT in gastropods: at the initial stage, the differentiation and growth of male type genital organs (penis and vas deferens) occur and lead to ovarian spermatogenesis at the severely affected stage, involving oviduct blockage due to proliferation of epidermal tissues surrounding vas deferens. Therefore, the authors consider that the true mode of action of TBT or TPT on the development of imposex in gastropods must be able to explain all of the characteristics mentioned above.

Nishikawa et al. (2004) proposed a unique mode of action of TBT or TPT on the development of imposex in gastropods, which was completely different from other hypotheses already proposed as the imposex induction mechanism. Nishikawa et al. (2004) elucidated that organotins (both TBT and TPT) bound to the human retinoid X receptors (hRXRs) with high affinity and that injection of 9-*cis* retinoic acid (9-*cis* RA), the natural ligand of hRXRs, into female rock shells (*T. clavigera*) induced the development of imposex. Cloning of the RXR homologue from *T. clavigera* revealed that the ligand-binding domain of rock shell RXR was very similar to vertebrate RXR and bound to both 9-*cis* RA and organotins. These findings suggest that RXR plays an important role in inducing the development of imposex, namely the differentiation and growth of male type genital organs in female gastropods.

Preliminary experimental results on RXR gene expression, RXR protein content, immunohistochemical

staining with anti-RXR antibody, and time-course RXR gene expression after exposure to organotins with the rock shell (*T. clavigera*) further support the hypothesis that RXR plays an important role in inducing the development of imposex caused by organotins in female gastropods (Nishikawa, Ohta and Horiguchi, in preparation). Further study with histological, immunohistochemical, biochemical and molecular biological techniques is needed to elucidate the entire mode of action of TBT or TPT on the development of imposex in gastropods.

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有機スズによる腹足類のインポセックス誘導機構の解析

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Symposium Ⅱ-5 Aluminum and human health: an old metal but new toxicity

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1. Introduction

Aluminum (Al) is the 3rd abundant element in the earth's crust and is wildly distributed in our environment. However, Al is highly reactive and does not exist as a free metal in nature but as compounds with other elements such as oxygen, or silicon. Therefore, Al first appeared as a silvery metal at 1825, and its commercial use was developed after 1886. Nowadays, Al metals and compounds are widely used in various important industrial applications or in consumer products such as antacids, food additives, and antiperspirants.

2. Characteristics of Al and its toxicity

Owing to the specific chemical characteristics of Al, Al is not an essential element and Al affects more than 200 biologically important reactions. Al favors negatively charged, oxygen-donor ligands Inorganic or organic phosphates, carboxylate, deprotonated hydroxy groups firmly bind to Al^{3+}. Thus, Al binds to the phosphate groups of DNA or RNA, influences DNA topology, and affects gene transcription. Al also binds to the phosphate groups of nucleoside di- and triphosphates, such as adenosine triphosphate (ATP), and affects energy metabolism. Al influences various functions of enzymes including protein kinases and phosphatases. Furthermore, the ligand-exchange rate for Al^{3+} is very low compared with those for other essential elements. Thus, biological processes involving a rapid Ca^{2+} or Mg^{2+} exchange could be inhibited by the substitution with the 10^8-fold slower Al^{3+} exchange. These properties enable Al to become useless in enzyme-requiring reactions, and to have the long half-life in the body.

Another crucial effect of Al is that Al binds to biologically important proteins and induces their conformational changes. Al has a property of firmly binding to various residues of proteins, such as Tyr, His, or phosphorylated amino acids, causes their aggregation or conformational changes, and inhibits the protein degradation by proteases. Recent lines of evidence suggest that the misfolding and aggregation of β-amyloid protein (AβP) plays important roles in the pathogenesis of Alzheimer's disease (AD). Numerous studies including our own results have revealed that Al enhances the aggregation and conformational changes of AβP.

Considering these properties of Al, Al is a widely recognized neurotoxin. A case of Al poisoning involves memory disorder was first reported in 1921. Al was revealed to cause epilepsy in experimental animals at 1951. Furthermore, Al has been associated with various diseases such as dialysis encephalopathy, β2-microglobulin amyloidosis, microcytic anemia, and osteomalacia (aluminum bone disease) in dialysis patients. Al has been linked to various neurodegenerative disorders including amyotrophic lateral sclerosis (ALS) and Parkinsonism dementia (PD) in the Kii Peninsula and Guam and AD.

Although there is still much debate about the link between Al and the etiology of AD, recent evidences support the hypothesis that Al may be one of a risk factor in AD. Numerous epidemiological studies revealed the positive relationship between Al in drinking water and AD. Al fed in experimental animals caused the accumulation of tau protein and AβP, which resembles the hallmarks of AD.

3. Intake of Al and its bioavailability

The amount of Al in foods varied in the wide range. Several plants or weeds originally hold Al in the body. The contamination from food additives, cooking utensils, or containers accounts for a large part of Al amount. Al is used as coagulants of water treatment process, and therefore, drinking water contains Al. Thus, our daily intake of Al is estimated to be 10-20 mg/day. Furthermore, much amount of Al is included in medicines such as antacids. However, Al intake does not correlate with the amount of Al in the body. The amounts of Al that are absorbed in the gastrointestinal tract, transported into the brain through blood brain barrier, and accumulated in the brain are crucial for our health.

Usually, small amount of Al (approximately less than 1%) is absorbed from food via the gastrointestinal pathway. However, the uptake of Al through gastrointestinal pathway is complex and is influenced by various factors

including age, pH, stomach contents, chemical speciation of Al, coexistence substances. In general, the intrinsic Al in food components such as tea exhibits relatively low bioavailability. The coexistence of organic ligands such as citric acid or maltol promote the absorption of Al, while silic acid prevents it. The rate of Al absorption is increased in aged people, in patients with Down's syndrome, patients with AD. The amounts of Al become high in bodies of patients with renal failure or kidney disease.

Most of absorbed Al binds to transferrin, an iron transporter, and carried in the plasma. Small, but considerable amount of Al can across the blood brain barrier maybe through transferring-receptor pathway, and enters into the brain. Once entered, Al retains in the brain and accumulated permanently over 30 days. The amount of Al in the brain is increased in age-dependent manner. Figure 1 depicts the intake of Al, its absorption, the transfer to the brain.

Meanwhile, Al in the medicinal usages is more crucial to our health. Huge amount of Al is included in antacids, and therefore, the continuous exposure of Al-containing antacids to patients with renal failure or kidney diseases may increase the risk of encephalopathy. Al is also contaminated in total parenteral nutrition (TPN) solutions. Considering that the absorption of Al from theses iatrogenic sources is high, it causes the severe health problem, in particular to infants. The Food and Drug Administration (FDA) proposed a rule to regulate the contamination of Al in TPN solutions.

4. Conclusion

It is widely accepted that Al is a neurotoxin, and could cause cognitive deficiency and dementia when Al enters the brain. In particular, Al can affect infants, elderly people, and patients with impaired renal functions, and can cause severe problem. Therefore, unnecessary exposure to Al should be avoided for such patients. Further detailed researches on the neurotoxic characteristics of Al, including its bioavailability, cellular effects, metabolisms, and in particular, metal-metal interactions are required.

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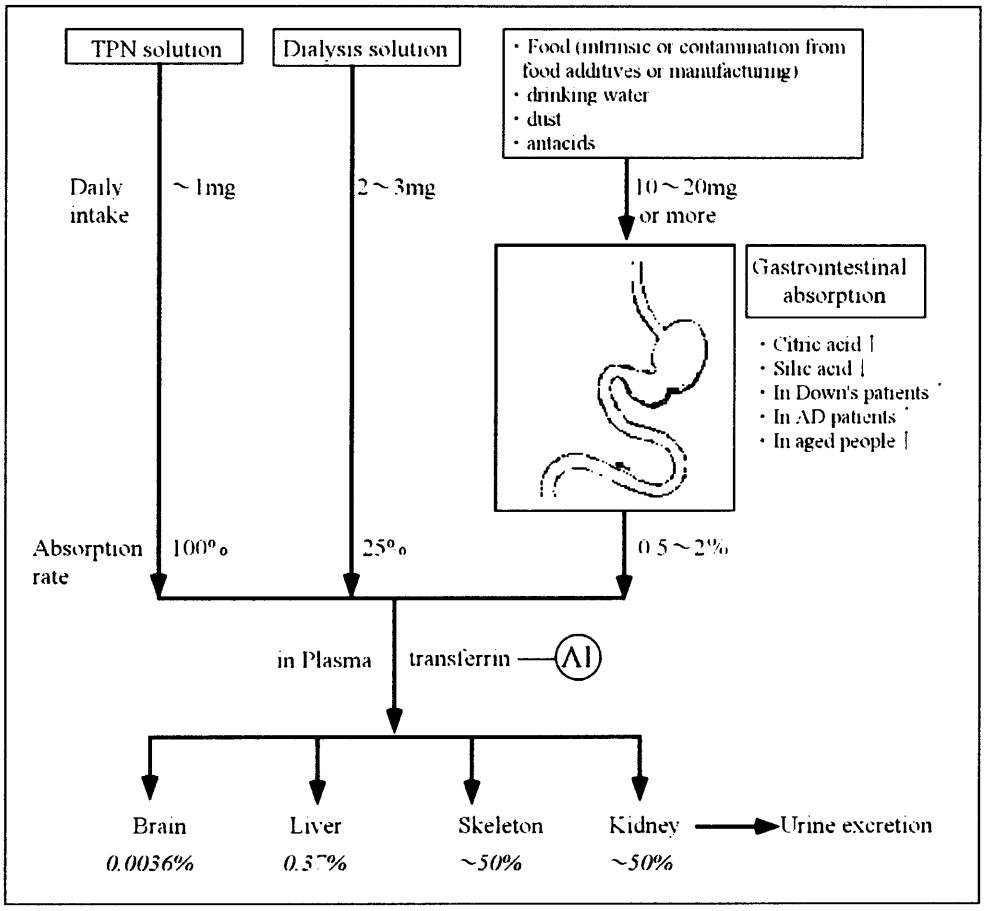

古くて新しい金属、アルミニウム：その体内動態および細胞死メカニズムと疾患との関連

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The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Abstracts

Award Lecture

Award Lecture I Chemical Biology of Arsenicals

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Arsenical is a metalloid element that is widely distributed in the environment as inorganic trivalent (As^{III}) or pentavalent (As^{V}) forms, and its toxicity has been well known. Chronic arsenic poisoning has occurred by the consumption of contaminated well water in the world. Inorganic As^{III} has also emerged as a potent chemotherapeutic agent with remarkable efficacy for human acute promyelocytic leukemia (APL). In humans, absorbed As^{V} is reduced to As^{III} and enzymatically methylated into pentavalent monomethylarsonic acid ($MMAs^{V}$) and dimethylarsinic acid ($DMAs^{V}$). The methylation of inorganic arsenicals was initially thought to be a detoxification process because the toxicity of $MMAs^{V}$ and $DMAs^{V}$ is substantially lower than that of inorganic arsenicals. However, some recent studies suggested that the methylation of inorganic arsenicals was not a universal detoxification mechanism. Trivalent methylated arsenicals such as monomethylarsonous acid ($MMAs^{III}$) and dimethylarsinous acid ($DMAs^{III}$) were found in urine collected from chronic arsenic poisoning patients, and synthetic trivalent methylated arsenicals were more cytotoxic *in vitro* than inorganic and pentavalent methylated arsenicals. In contrast, we recently demonstrated using chemical-biological technique that methyl arsenicals might be generated as trivalent arsenical-glutathione (GSH) conjugates, such as $MMAs^{III}$-diglutathione ($MMAs^{III}DG$) and $DMAs^{III}$-glutathione ($DMAs^{III}G$), in the human body. Trivalent arsenical-GSH conjugates were not cytotoxic because these large molecules could not be transported into cells. The conjugates showed strong cytolethality when they separated into trivalent methyl arsenicals and glutathione, however, various *in vivo* thiol agents and/or normal serum components greatly blocked the cytolethality of them by maintaining their conjugate forms. Arsenical-GSH conjugates might be rapidly excreted in the urine. Taken together, we suggested that the methylation of inorganic arsenicals is a positive and useful physiological response to excrete toxic arsenicals from human body.

On the other hand, it has been recently reported that peripheral blood arsenic concentrations in chronic arsenic poisoning patients and APL patients who were induced complete remission by intravenous As^{III} treatment were nM levels. Generally, arsenicals are not cytolethal in mammalian cells at nM concentrations. We examined the biological effects of arsenicals at nM concentrations using *in vitro* differentiation of human peripheral blood monocytes into macrophages with colony stimulating factors (CSFs). As a result, we found a very unique biological effect on As^{III} at nM concentrations. While As^{III} was found to induce cell death in a culture system containing macrophage CSF (M-CSF), macrophages induced by granulocyte-macrophage CSF (GM-CSF) survived the treatment, but were morphologically, phenotypically, and functionally altered. As^{III}-induced cells from human monocytes with GM-CSF were small, nonadhesive and dendritic. They expressed higher levels of the macrophage markers, such as a

major histocompatibility complex class II antigen, HLA-DR, and CD14. These data suggested that As^{III}-induced cells were abnormal macrophages (arsenic-induced macrophages; As-Mp). They were more effective at inducing allogeneic or autologous T cell responses and responded more strongly to bacterial lipopolysaccharide by inflammatory cytokine release as compared to cells induced by GM-CSF alone. As-Mp expressed lower levels of CD11b and CD54 and phagocytosed latex beads or zymosan particles less efficiently. We also demonstrated that the optimum amount of cellular reactive oxygen species induced by nM As^{III} might play an important role in this As-Mp differentiation. In contrast, the addition of other inorganic and organic arsenic compounds and other metallic compounds could not induce the same abnormal cell generation from monocytes with CSFs at any concentration and any additional time schedules; they showed only simple cytolethality in monocytes and macrophages at n-mM levels accompanied by cell death.

These studies may have implications in both chronic arsenic poisoning and in the chemotherapeutic use of this remarkable metalloid.

ヒ素の毒性および薬理作用の分子機構の解明・ヒ素のケミカルバイオロジー

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The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

Abstracts

Lectures

Lecture 01 Dietary intakes of copper, iron, manganese, and zinc for Ukrainians

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Global contamination by radioactive nuclides was caused by the Chernobyl accident in April 1986. Contamination in the environment has been studied during the years since the accident. We have made several studies in the Ukraine, from the viewpoints of radiation protection, nutrition and public health. In such studies, the relationship of radioactive and non-radioactive nuclides in food chains is important for understanding their environmental behaviors. Therefore, it is necessary to collect information on dietary mineral intake to quantify the background level in Ukrainian subjects. However, data on the dietary element intakes for Ukrainians are not well documented.

In the previous annual meeting, we presented dietary mineral intakes for Ukrainians living areas contaminated by the Chernobyl accident. A dietary intake of four trace elements (Cu, Fe, Mn and Zn) in whole Ukraine is reported in this report.

Approximately two hundreds ninety-diet samples were collected from twenty-five Oblasts (regions) of Ukraine by a duplicate portion method. At least six samples were collected from one Oblast. The diet sample was freeze-dried. A small portion of the sample was treated with a mixture of mineral acids by a microwave digestion system. Elements were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). A range, arithmetic mean and standard deviation, geometric mean and median of daily element intake in a Ukrainian were as follows: for copper, 0.084-4.12, 0.814±0.029, 0.708, and 0.695 mg ; for iron, 1.09-58.9, 9.46±0.41, 8.09, and 7.87 mg ; for manganese, 0.18-14.9, 2.67±0.12, 2.26, and 2.28 mg, and; for zinc, 0.43-22.8, 7.02±0.20, 6.29, and 6.57 mg, respectively. The intakes of copper, iron, manganese, and zinc in Ukrainian were lower than those of Japanese and worldwide reported values. The differences between the contaminated area and others will be discussed.

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ウクライナ国民の微量元素、Cu, Fe, Mn, Zn の摂取量推定

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Lecture 02 Element concentrations in blood and urine in relation to residents' drinking water in the Aral Sea region

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Purpose: Element concentrations in blood may reflect their excess or deficiency in the body. Elemental contents in urine often reflect the intake amounts of elements and renal function. General sources of elements are food, water, and ambient air. These are dependent on living area, natural environment, living custom, season and many other factors. In order to investigate the correlation of various element concentrations between blood or urine, and drinking water samples, several factors such as sources of drinking water, residents' living area near the Aral Sea region, seasonal variation were investigated.

Experiment: Urine samples were collected from 158 children living in 5 villages in summer 2002, and from 254 and 217 children living in 3 villages in summer 2004 and in winter 2005. Blood samples were collected from 161 children in summer 2002, 237 children in summer 2004, and 200 children in winter 2005, respectively. Drinking water of each home (n=155) in 2002, and water samples from eleven water sources in 2004 and in 2005 were collected. Blood plasma was obtained by centrifugation immediately after drawing blood. All samples were put in demineralized polypropylene tubes and carried to Japan in frozen state, then analyzed in our laboratory of Juntendo University. A portion of each sample was used for general clinical test and an aliquot of sample was used for element analyses after microwave digestion using HNO3. Concentrations of Na, K, P, Mg, Ca, Sr, Rb, Zn samples were determined using ICP-AES, and those of about thirty trace elements were determined using ICP-MS.

Results and Discussion: As the characteristic findings in the Aral Sea region Na, Cl^- and SO_4^{2+} concentrations were high in all water materials analyzed. Concentrations of typical toxic heavy metals such as Cd, Pb, and Cr were detectable, but not high, single ng/ml or less in all samples. Concentrations of several elements were different depending on the types of water sources; those of Ca, Mg, Sr, and U were higher in shallow well water than in deep well or piped water samples, and those of Na, Al, Cu, and Mo were higher in deep well water samples than shallow well or river water samples. Average concentrations of these elements in blood plasma and urine were different depending on living villages and seasons. Significant correlation of elements between plasma and urine were not observed. Nutritional situation and renal functions were important factors. Urine samples with higher concentrations of beta-2-microglobulin which is an indicator of renal tubule dysfunction showed higher concentrations of essential trace elements such as Zn, Cu, and Se in urine in cases of 2002 summer investigation. Most elements in urine did not correlate with those in their drinking water suggesting a little contribution of water as causes. It was found that the uranium concentrations in urine were higher in children living in villages using water containing higher uranium concentrations. This result indicated that the water was considerable source of uranium intake in this area. Factors affected on elements concentrations in water, plasma, and urine will be discussed further.

アラル海沿岸地域調査における血中および尿中元素濃度と飲料水中元素濃度の関係

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Lecture 03 Differences in the Serum Zinc Level of Rural and Urban Residents in Tomi City

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<ABSTRACT> Zinc deficiency is becoming a subject of greater clinical interest than ever before, especially in an aging society. In 2003, we studied serum zinc levels in the residents of Kitamimaki, a rural village in Nagano Prefecture. In April 2004, this village merged with Tobu Town, a larger agglomeration of 3 urban areas and 1 rural area, to form Tomi City. In 2005, during the annual mass health examination for residents of Tomi City, we examined the serum zinc level (Zn) of 1773 participating residents from each of its five areas, including Kitamimaki (KI). We sought to reveal whether any differences in Zn could be found in residents of the five areas, including two rural areas (NE, KI) and three urban areas (TA, KA, SH). Because individual levels of serum zinc vary throughout the day, we used Zn samples taken only from the 1017 subjects examined during morning hours, precluding the chance that daily Zn variances might distort the results. [Results] Zn of residents in one rural area (NE) was lower than that of residents in the other four areas. Zn in NE residents was almost the same as in KI residents in the 2003 Kitamimaki Study. Zn level shown in KI residents in the 2005 study, however, was significantly higher than that found in the 2003 study (See Table). [Conclusions] 1) Residents in rural areas tend to have lower Zn levels than those in urban areas. 2) Increased Zn levels in rural Kitamimaki found in the 2005 Tomi Study may suggest the need for educational approaches that change daily eating habits, improving health and promoting wellness through better nutrition.

Serum Zinc Levels of Residents in Each Area of Tomi City

(μg/dl, subjects' blood sampling done in the morning)

	Kitamimaki Study, 2003	Tomi Study, 2005 (n=1017)				
Area	KI (rural*)	KI (rural)	TA (urban**)	KA (urban)	SH (urban)	NE (rural)
mean Zn±SD***	75.8±10.3	78.0±10.3	78.3±10.2	77.8±9.8	76.6±10.0	75.6±10.9
significant difference (+: p<0.05, ++: p<0.01)	+	+				
		+				+
			++			++
				+		+
No. of subjects	308	198	276	199	165	179
mean age(year old)±SD	59.7±13.4	59.3±13.4	60.2±12.7	59.4±13.2	61.9±12.3	60.5±14.4

*: rural area **: urban area ***: standard deviation

長野県東御市住民の血清亜鉛濃度の実態～都市圏と農村圏での差異について～

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Lecture 04 Mathematical approach to estimate mineral requirements from population data

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Determination of mineral requirements is a crucial technique in developing dietary guidelines. The established methods for estimating mineral requirements are balance method, factorial method and saturation method. As an alternative, a mathematical approach for estimating mineral requirements from the population data of mineral status (distribution of dietary intake and the prevalence of inadequate status) is proposed here. The prevalence (D) of inadequate nutritional status is the proportion of individuals with inadequate nutritional status: D = (Sum of individuals whose dietary intake < requirement) / (total number of individuals). Assuming that the requirement and the dietary intake follow a certain distribution function such as bivariate normal distribution function, and the estimated average requirement (EAR) is obtained as the solution of the integral equation describing the above relationship. The integral equation using bivariate normal distribution function can be applicable to Fe requirements for certain populations excluding premenopausal women. For premenopausal women, the convolution integral between basal Fe loss and menstrual Fe loss was computed to obtain total Fe loss that was used to describe the distribution function of the requirement and the dietary intake. The Fe status data obtained from the literature were analyzed as an illustration. We conclude that mathematical approach can be useful to estimate mineral requirements and to elucidate dietary guidelines of minerals.

集団データに基づくミネラル必要量の推定

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Lecture 05 Contamination by lead and its human health effects in India, Vietnam and Cambodia

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[Introduction] Recently, contamination by environmental contaminants has become alarming in many Asian developing countries in which economic growth and population increase are marked. Lead is widely distributed in the environment and is toxic even at low exposure levels, especially to fetuses and children. However, information on contamination status by lead in Asian developing countries is still limited. Furthermore, despite the significant public health effects by exposure to lead, few investigations were conducted on lead contamination in Asian developing countries. In the present study, lead concentrations were determined in human blood and potential sources of lead such as air dust, gasoline, food and paint from India, Vietnam and Cambodia. To estimate lead exposure source(s) to human, the lead isotopic compositions were also measured.
[Materials and Methods] Human blood, air dust, gasoline, food and paint samples were collected from Chennai and its suburbs in south India, Kolkata and its suburbs in east India, Hanoi and its suburbs in Vietnam, and Phnom Penh and its suburbs in Cambodia. Collected samples were kept at -20 °C until chemical analysis. Lead concentrations and isotopic ratios were measured with an inductively coupled plasma-mass spectrometer (ICP-MS).
[Results and Discussion] Concentrations of lead ranged from 2.33 μg/dl to 27.4 μg/dl. Especially, concentrations in blood of residents from Perungudi (waste dumping site) and Palaverkadu (farming village) in India were significantly higher than those from other regions or those reported previously. Concentrations of Pb in blood of some residents exceeded the threshold levels for hypertension in adult and development of inhibition of intelligence in fetus. Furthermore, significant negative correlations between blood lead levels and δ-aminolevulinic acid dehydratase (ALAD) activities were observed in the residents from all the three countries, suggesting that heme biosynthesis is suppressed by Pb in those residents. Levels of lead in some air dust samples from Vietnam and India exceeded the guideline values of World Health Organization or Environmental Protection Agency in USA associated with health risk. Although low concentrations were observed in gasoline and cereals in India, paints had extremely high concentrations. The lead isotopic ratios of some paints are similar to those of human blood in India, indicating that the residents in India may be exposed to lead from paints. In contrast, the ratios in air dust samples were similar to that in blood of Vietnamese. This suggests that the residents in Vietnam are exposed to lead from the air dust.

インド、ベトナム、カンボジアの鉛汚染とヒトへの健康影響

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Lecture 06 Effect of vanadium-containing ground water from Mt. Fuji on human health: Mortarity data around Mt. Fuji

○Yoshiyuki SEKO , Kaori WATANABE , Tatsuya HASEGAWA

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Ground waters from Mt. Fuji contain relatively high concentration of trace element vanadium; the concentrations are about 50 μg/L, and those in other area are about 1 μg/L. The ground water is widely utilized for tap water around the mountain, and residents are drinking and using the water for cooking. Recent reports of clinical investigations suggest that the drinking the water have some health beneficial effects on human health. If the effects are strong enough to improve public health, health statistics around Mt. Fuji may be better than other area. In this preliminary investigation regional health statistics are analyzed in relation to vanadium concentration in ground water in Yamanashi prefecture. Vital statistics (1998-2002) and adult health check data (2003) of Yamanashi prefecture were available, and we used standardize mortality rate (SMR) and rate of persons with outlying health check data. There are eight secondary medical districts in Yamanashi prefecture, and one of them, Fuji-hokuroku (No.7), corresponds to high vanadium area, where vanadium concentrations in tap waters are relatively high. Therefore, we compared data of the area with other districts, where vanadium concentrations in tap water are low. SMRs from all heart diseases and from acute myocardial infarction of both male and female in No.7 district were highest among the eight secondary medical districts. Blood glucose is checked in an adult health check conducted by local authorities. Percentage of person whose blood glucose exceeded the normal range was highest in the district No.7. As the expected health benefit of the ground water is to alleviate symptoms of diabetes, our results are opposite of this expectation. This may suggest disbenefit of the ground water for human health, or the presence some local factor(s) that exceeds the possible benefit of the ground water. As we can not obtain firm conclusion from ecologic studies, further epidemiological studies are required.

バナジウムを含む富士山地下水の健康影響に関する研究：富士山周辺市町村の死亡統計

○瀬子 義幸、渡邊 かおり、長谷川 達也

山梨県環境科学研究所

Lecture 07 Effects of cadmium administration on reproductive performance in Japanese quail (Coturnix japonica)

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Abstracts: The effects of cadmium administration on body weight, egg production and development of the next generation were investigated using laying Japanese quail. Single intraperitoneal injection of 0.1, 0.3, 1.0, 3.0 and 10.0 mg $CdCl_2$ / kg body weight (BW) were administered to egg-laying female quail caged with male quail. Body weight and mortality of the birds, and egg production, egg weight, yolk weight, shell membrane weight, shell thickness were recorded daily up to 14 days after injection. Approximately half of eggs laid by each cadmium dose group were incubated at 39 °C and 65% humidity for examination of fertility, embryonic death, hatchability and hatch-out chick weight. The results showed that injection of 10.0 mg cadmium induced 41.7% mortality while all birds survived in other groups. Body weight was significantly decreased in a dose dependent manner after cadmium administration. Higher the doses of cadmium injection, greater the depression in body weight and longer the time to reach the level before injection. In addition, egg production was reduced significantly in more than 0.3 mg cadmium received birds compared to that of control and the 3.0 mg cadmium injection was most effective to lower the egg production. The rate of egg production declined on the next day of injection in all cadmium-injected groups following dose dependent relation and the birds those received less than 0.3 mg cadmium retained to previous rate very quickly. Whereas, the birds injected with more than 0.3 mg cadmium recovered the production very slowly and did not reached to the level before injection till the end of experiment. Egg weight and yolk weight were found to be reduced in all cadmium-injected groups up to 5 days after injection but there was no clear relation between dose of cadmium and loss of weight while 10.0 mg cadmium injection caused the more yolk weight loss. Cadmium injection did not affect shell membrane weight of the birds except those received 1.0 mg cadmium. Cadmium injection significantly lower the egg shell thickness when compared to that of control and the 3.0 mg cadmium injection was most effective to thinning egg shell. Either period after injection or doses did not affect the chick weight or fertility rate although the evidence seemed to be weaker due to getting very few eggs from birds above 0.3 mg cadmium injection. In the next experiment, mRNA expression of very-low-density-lipoprotein, vitellogenin, previtelline membrane protein and estrogen receptors α and β were measured in liver after cadmium injection. Total RNA were extracted and reverse-transcribed to perform PCR using sense and anti-sense primers designed for each gene. Single intraperitoneal injection of various doses of cadmium showed dose dependent suppression of vitellogenin, previtelline membrane protein, and estrogen receptors α and β mRNAs expression after 48 h of injection. The present study suggests that avian egg production ability might be remarkably threatened by cadmium exposures via major egg yolk proteins at transcription levels.

日本ウズラの生殖機能に及ぼすカドミウムの影響

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Lecture 08 Acclimatization to dissolved lead of carp (*Cyprinus carpio*)

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The fluctuation of symptoms of poisoning in carp (*Cyprinus carpio*) exposed to lead in water is discussed. In Exposure test-1, carp were exposed to 50,100,300,500,800,1000, and 2000 ppb for 4 weeks. The blood lead concentrations of the test fish increased with increasing water lead concentrations up to 800 ppb, but decreased in water lead concentrations of 1000 and 2000 ppb. Symptoms indicative of acute lead poisoning, such as hyperactivity, erratic swimming, muscular spasms, loss of equilibrium, wave-type muscular movements and lethargic sleeping were observed on the test fish exposed to lead concentrations greater than 800 ppb. Hyperactivity and erratic swimming were most frequently observed throughout the exposure period in fish exposed to this concentration. On the other hand, fish exposed to 1000 and 2000 ppb most frequently developed symptoms of acute lead poisoning within about 10 - 14 days after the beginning of the exposure tests, but later improved by the end of the experiment. This improvement could be due to secretion of coagulation film by the highly lead-contaminated fish. In Exposure test-2, carp exposed to 600 ppb lead for 114 days did not show acute lead poisoning such as swimming damage, etc., but had serious anemic responses. The blood lead concentrations of the fish increased with the longer exposure time. On the other hand, anemic responses were most serious after 18 days from the start of exposure, and then the symptoms gradually subsided. The anemic responses were characterized as being hypochromic and microcytic, and basophilic stippling of the erythrocytes was also observed. These results suggest that the anemic responses of carp might subside in spite of the continuous accumulation of lead in the blood, and the carp might be able to acclimatize to lead, at least temporarily, when the concentration is lower than the level that causes acute lead poisoning.

Sato,T.*et al.* Evaluation of lead toxity in carp *Cyprinus carpio* using its blood 5-aminolevulinic acid dehydratase and erythrocyte indices. *ITE Lett.Batt.New Tech.Med.* 2,840-845,2001.

Sato,T.*et al.* Acclimatization to dissovled lead in anemic responces of carp (*Cyprinus carpio*). *ITE Lett.Batt.New Tech.Med.* 4,829-835,2003.

コイの鉛への順応

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Lecture 09 Analysis of copper and zinc in the breast milk from mothers with Wilson's disease

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This is only a model abstract. Female patients with Wilson disease who are being treated with a chelating agent or zinc can become pregnant. Most of the mothers want to breastfeed their infants while continuing their treatment for Wilson disease. However, the copper concentration has not been investigated in the breast milk of mothers with Wilson disease. We report here copper and zinc concentrations in the breast milk of mothers with Wilson disease and the effect of the milk on their infants. Materials and Methods: Using atomic absorption spectrometry, the copper and zinc concentrations in the breast milk from three patients with Wilson disease were analyzed at several times. A patient was sequentially treated with zinc and trientine. In addition, the infant's serum levels of copper and zinc were analyzed. Results and Discussion: Although the serum copper levels of the patients were significant lower that that of control subjects, the copper and zinc concentrations in the breast milk from the patients while they were taking zinc or trientine were normal levels. The serum copper and zinc levels were also normal in the infants receiving the breast milk from the patient taking zinc and trientine. However, the copper and zinc concentrations in the breast milk from the patients taking penicillamin were lower than those of controls. These results indicate that mothers with Wilson disease receiving zinc or trientine may safely breastfeed their infants.

ウィルソン病の母からの母乳の中の銅と亜鉛の分析

○加賀 文彩[1]、志賀 勝秋[1]、藤澤 千恵[1]、児玉 浩子[1]、玉井 浩[2]、清水 教一[3]、小林 健司[4]、勝矢 陽子[4]、Abdulah Rizky[4]、小山 洋[4]

[1]帝京大学 医学部 小児科、[2]大阪大学 医学部 小児科、[3]東邦大学 医学部 小児科、[4]群馬大学 大学院医学系研究科 社会環境医療学講座

Lecture 10 Diagnosis of patients with Menkes disease, carriers and fetuses by gene analysis

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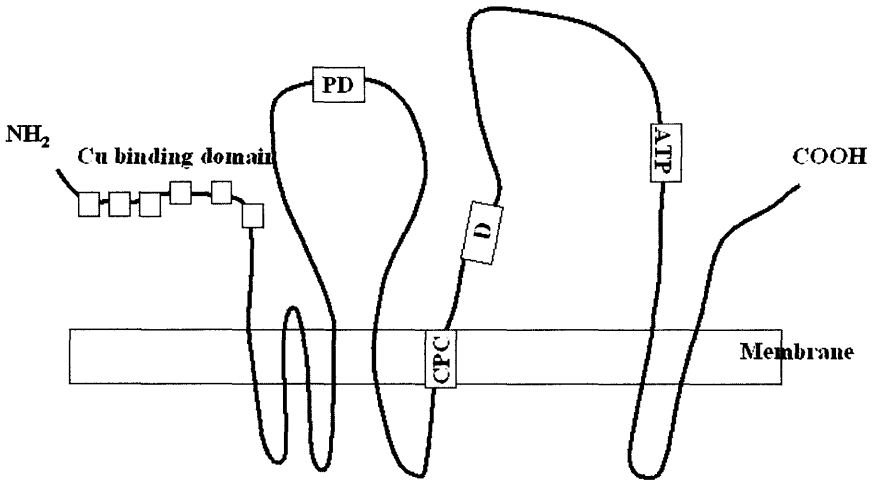
Menkes disease (MD) is an X-linked recessive disorder of copper metabolism characterized by progressive neurological degeneration and connective tissue abnormalities. The disease is caused by mutation in the *ATP7A* gene, which encodes a copper-transporting P-type ATPase. *ATP7A* is a large gene, and consists of 23exons spanning a genomic region of about 150 kbp. We examined mutations in the *ATP7A* gene in 34 unrelated Japanese patients with MD. Further, carrier detection was performed in 16 mothers of the patients, and prenatal or neonatal diagnosis was performed in 6 male siblings. Genomic DNA was prepared from peripheral blood lymphocytes, cultured fibroblast or amniocytes, and amplified by PCR. The direct sequencing of exons was performed with a 3700 DNA analyzer. Thirty-three different mutations were identified in the 34 patients; 8 nonsense mutations, 6 missense mutation, 8 splice-site mutations, and 12 insertion/deletion mutations. Most of *ATP7A* mutations in the patients were identified in important functional/structural domains of protein products such as copper biding domains, transmembrane domains, CPC motif, and ATP binding domains. Twelve of 16 mothers (75%) were carriers of Menkes disease. Four of 6 siblings suffered from Menkes disease. These results indicate that gene analysis is useful for the early diagnosis of MD and for quality of life of patients and families.



CPC: aCys-Pro-Cys motif, D: Phosphorylation domain, ATP: ATP binding domain

Schematic Structure of ATP7A

Menkes 病患者、保因者及び胎児の遺伝子解析を用いた診断

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帝京大学 医学部 小児科

Lecture 11 Wilson disease patients with atypical psychiatric symptoms

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Wilson disease is an autosomal recessive disorder of copper metabolism. Copper accumulates primarily in the liver and subsequently in the brain, cornea, kidney and other organs. The disease phenotype includes chronic liver disease (cirrhosis), neurological impairment (extra pyramidal signs), Kayser-Fleischer ring and others. Some patients show psychiatric symptoms. The first manifestations of Wilson disease are caused by hepatic disturbances in about 40% of patients (usually with onset in childhood and adolescence), another about 40% are neurological (usually in adolescence or early adulthood), and the remainder present with psychiatric, renal or another symptoms. In this paper, we report two cases with Wilson disease who presented with atypical psychiatric symptoms as first symptoms. Case-1: A 40-year-old female. When she was 26 years old, she presented with hallucination and delusion. She was diagnosed as schizophrenia and was treated. At 29 years old, tremor, dysarthria and gait disturbance were appeared. And Kayser-Fleischer ring was found by ophthalmologist, then she was diagnosed as Wilson disease. The treatment with copper chelating agent was started.Case-2: A 28-year-old female. When she was 17 years old, she had symptoms of osmophobia for her own odor. She was diagnosed as autosmia due to schizophrenia. Then she was treated with antipsychotic agents. At 24 years old, she presented gait disturbance, tremor and dysarthria. She was diagnosed as Wilson disease due to Kayser-Fleischer ring and hypoceruloplasminemia. She took copper chelating agents. Her psychiatric symptoms were decreased and almost disappeared by aggressive treatment for copper accumulation and by antipsychotic agents.Discussion: In these cases, initial symptoms were hallucination. Both of them were diagnosed as schizophrenia first. And a several years later, typical neurological symptoms of Wilson disease were appeared. Most common psychiatric symptoms are mental retardation and depression in Wilson disease. Hallucination is rare symptom. The clinical courses of these patients were very atypical as this disease. Thus, the diagnosis of Wilson disease was unsuspected until they developed obvious symptoms, and it took long time to reach final diagnosis. We should alertly suspect Wilson disease and screen it for the patients with psychiatric symptoms.

非定型的な精神症状を呈したウイルソン病症例に関する検討

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Lecture 12 The association between trace metals and the etiology of patients with hepatocellular carcinoma

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Aims: The aim of this study was to investigate the relationship between trace metals and the etiology of patients with hepatocellular carcinoma(HCC).

Methods: We measured the contents of copper, iron, and zinc in liver parenchyma in patients with HCC.Metals were quantified in thin-needle biopsy specimens using the particle-induced X-ray emission method(PIXE).

Results: Copper level in liver parenchyma was significantly higher in HCV-positive patients than in HBV-positive patients. Iron contents was significantly higher in patients with alcohol abuse than in HBV-positive patients.There were not significant difference in Zinc level among all groups.

Conclusions: In HCV-positive patients, hepatic copper level in liver parenchyma was higher than that of patients with other chronic liver desease. In patients with alcoholic liver injury, Iron level in liver parenchyma tended to be higer than that of patients with other etiology.

肝細胞癌症例における疫学別にみた微量金属

○桝谷 佳生、江原 正明、岡部 真一郎、福田 浩之、行澤 斉悟、吉川 正治

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Lecture 13 Analysis of cisplatin behavior in a non-small cell lung cancer(NSCLC) cell line

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[Introduction] Cisplatin(CDDP) is an effective anticancer agent that is widely used in the treatment of testicular, ovarian, bladder and small cell lung cancers. However the development of resistance to CDDP by tumor cells is a major obstacle to treatment. We established CDDP resistant cell lines and characterized them to elucidate the mechanism of the resistance. Decreased accumulation of CDDP was observed in CDDP resistance cell lines from NSCLC, and a good correlation was found between the amount of intracellular platinum and the sensitivity of lung cancer cell lines to CDDP. We also measured the uptake of platinum using ^{199}Pt tracer. The amount of platinum uptake shows a trend similar to that of CDDP uptake in CDDP resistance cell lines. In this study, to investigate the CDDP resistance mechanism, we measured the total platinum, CDDP and CDDP metabolites using ICP-MS and ^{199}Pt tracer.

[Materials and Methods] We used a human NSCLC cell line, PC-9, and its CDDP-resistant subline, PC-9/CP^r. ^{199}Pt tracer solution was obtained from the Au target irradiated in the RIKEN Ring Cyclotron. For the uptake experiments, the PC-9 and PC-9/CP^r were exposed to the ^{199}Pt tracer or several non-radioactive platinum compounds. After incubation, radioactivity was measured using a Ge detector, and total platinum was determined by ICP-MS and AAS. CDDP was measured by using LC-ICP-MS.

[Results] The uptake experiments of several platinum compounds were carried out. As shown in Fig. 1, the accumulations of platinum in the PC-9/CP^r were less than that of in PC-9 for the tested platinum compounds (Fig. 1). The reason is now investigating.

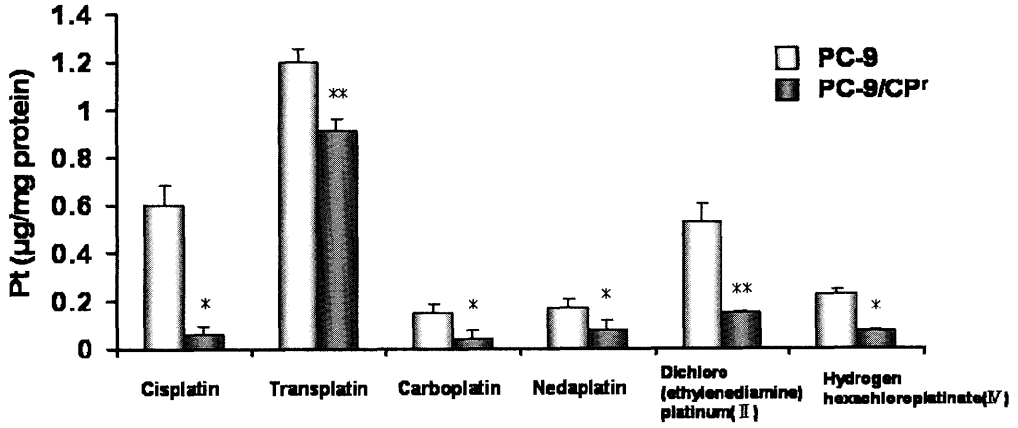

Fig.1 Accumulation of platinum in PC-9 cells and PC-9/CP^r cells after 4 h of exposure to 100 μM Pt compounds.
Each bar represents the mean of three independent experiments. *Vertical bars*, ±SD $^*P<0.01$, $^{**}P<0.05$

非小細胞肺がん細胞株中におけるシスプラチンの挙動解析

○高橋 雅樹[1]、鈴木 俊宏[1]、湯本 敦[1]、兎川 忠靖[1]、羽場 宏光[2]、榎本 秀一[2]、西尾 和人[3]、田邉 信三[1]
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Lecture 14 Anticancer activity of high selenium broccoli sprouts in the human esophageal cell lines

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Anticancer effect of high-selenium (Se) broccoli sprouts was investigated in the human esophageal cell lines. In this study, we compared the activity between high-Se broccoli sprouts and normal broccoli sprouts. The high-Se broccoli sprouts were germinated in the 10 ppm of sodium selenite solution. Speciation of selenium compounds in the high-Se broccoli sprouts was performed using HPLC-ICP-MS with size exclusion column and phosphate buffer (pH 6.8) as the mobile phase. The most abundant form of selenium in sprouts was Se-methylselenocysteine (Se-MSC). The other compounds were sodium selenite, sodium selenate, and selenocystine.High-Se broccoli sprouts extract was then applied on human esophageal cancer cell line, TE-13, and non-cancerous cell line, CHEK-1. Cell proliferation inhibition was observed using MTT assay. There was no differential sensitivity of both high-Se and normal broccoli sprouts in esophageal cells at higher concentration. On the other hand, at lower concentration, the effect of high-Se broccoli sprouts in cell proliferation inhibition was superior then normal broccoli sprouts. We speculate that the same effect given by both high-Se and normal broccoli sprouts was due to sulforaphane, as an active compound in the normal broccoli sprouts. The effect of Se-MSC in the high selenium broccoli sprouts was not optimal since the β-lyase enzyme, which is needed by many selenoamino acids in their anticancer activity, was poorly available in our *in vitro* experiment.

食道がん細胞を用いたセレン強化ブロッコリースプラウトによる抗がん作用の検討

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Lecture 15 Zinc action in hippocampal mossy fiber long-term potentiation

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Synaptic plasticity such as long-term potentiation (LTP) underlies learning and memory. The LTP in hippocampal mossy fiber synapses is independent of N-methyl-D-aspartate (NMDA) receptor activation. The LTP is expressed by presynaptic mechanisms leading to persistent enhancement of neurotransmitter release. The induction of mossy fiber LTP is critically dependent on the rise in presynaptic calcium induced by high-frequency (tetanic) stimulation. As a characteristic of mossy fibers, all giant boutons of mossy fibers contain zinc in the presynaptic vesicles and the zinc concentration is estimated to be approximately 300 μM. Zinc is released by tetanic stimulation (10-100 Hz), which induces the mossy fiber LTP. On the basis of the evidence that vesicular exocytosis during tetanic stimulation (10 Hz, 180 s) is negatively modulated by zinc, in the present study, zinc action in hippocampal mossy fiber LTP was examined using rat hippocampal slices.
Tetanic stimuli at 10 Hz for 180 s, which was used for the exocytosis experiment, were delivered to the dentate granule cells and the mossy fiber-CA3 pyramidal neuron responses were checked by the electrophysiological method. The mean amplitude of fEPSP was 146 ± 16% of the basal level. It was confirmed that tetanic stimuli at 10 Hz for 180 s induces the mossy fiber LTP. Thus, it is possible that zinc negatively modulates the mossy fiber LTP. The effect of CaEDTA, a membrane-impermeable zinc chelator, on the mossy fiber LTP induced with tetanic stimuli at 10 Hz for 90 s was examined to clarify the role of zinc released from mossy fibers in the mossy fiber LTP. Addition of 1 mM CaEDTA to hippocampal slices minimally enhanced the mossy fiber LTP induced with tetanic stimuli at 10 Hz for 90 s. In the case of the mossy fiber LTP induced with tetanic stimuli at 100 Hz for 1 s, on the other hand, the mean amplitude of fEPSP in the presence of 1 mM CaEDTA was approximately 108% of the control level (140 ± 5%). Furthermore, the increase in calcium orange signal in mossy fiber terminals during delivery of tetanic stimuli at 100 Hz for 1 s to dentate granule cells was significantly enhanced by addition of I mM CaEDTA, suggesting that zinc released from mossy fibers suppressively acts on vesicular exocytosis during tetanic stinulation (100 Hz, 1 s). The present study demonstrates that zinc released from mossy fibers may be a negative-feedback factor against the mossy fiber LTP.

海馬苔状線維－CA3 錐体細胞間での長期増強に対する亜鉛の作用

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Lecture 16 Zinc release in the lateral nucleus of the amygdala by stimulation of the entorhinal cortex

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Approximately 90% of total brain zinc is bound to zinc metalloproteins. The rest exists in the presynaptic vesicles and is reactive histochemically (as revealed by Timm's sulfide-silver staining method). The presence of zinc-containing glutamatergic neurons that sequester zinc in presynaptic vesicles and release it in a calcium- and impulse-dependent manner has been demonstrated in the brain, especially in the telencephalon. The amygdala is a subcortical component of the limbic system that is critical for emotional expression and possesses zinc-containing neuron terminals. The behavior for passive avoidance is impaired during amygdalar perfusion with CaEDTA, a membrane-impermeable zinc chelator, suggesting that amygdalar extracellular zinc is involved in the behavior for passive avoidance. In the present study, zinc release in the lateral nucleus of the amygdala was examined using brain slices, in which the entorhinal cortex was stimulated electrically. The lateral and basolateral nuclei in the amygdala and the lateral entorhinal cortex were evidently stained by Timm's sulfide-silver staining method. To observe endogenous zinc release in the amygdala, the amygdala was stimulated with 100 mM KCl by using in vivo microdialysis. Extracellular zinc concentration was significantly increased by the stimulation. Furthermore, to examine zinc release in the lateral nucleus of the amygdala, brain slices were prepared from rats and double-stained with zinc and calcium indicators. Extracellular zinc signal (ZnAF-2) in the lateral nucleus was increased with intracellular calcium signal (calcium orange) during delivery of electrical stimulation (100 Hz, 5 s) to the entorhinal cortex. Both the increases were completely inhibited by addition of 1 mM tetrodotoxin, a sodium channel blocker. The entorhinal cortex has glutamatergic projections to the lateral amygdala. The present study suggested that zinc is released in the lateral nucleus of the amygdala by depolarization of the entorhinal neurons. The lateral nucleus is a critical site for the acquisition and storage of fear-conditioning to emotionally arousing sensory stimuli. It is likely that zinc released in the lateral nucleus is involved in the behavior for passive avoidance.

嗅内野刺激に伴う扁桃体外側核からの亜鉛の放出

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Lecture 17 Does zinc deficiency cause repeated infections in elderly? : a pilot study

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Background and Aims: Zinc (Zn) deficiency cause a variety of disorders including dermatological, neurological and immunologic abbnormalities. We sometimes observe elderly patients with repeated infections and deficiency of trace elements. To investigate the relationship between serum level of Zn ion and suppression of protective immunity, we measured the levels of 10 elderly patients with repeated infections (group 1), and compared with those of elderly without infections.

Methods: 23 consecutive elderly patients who admitted to our hospital between June 2001 and December 2001 for rehabilitaion of post cerebral infarction or hemorrhage, who hospitalized and survived at least a half year. Eight of them were dieted normal foods. and remaining 15 patients were fed by gastric tube because of their poor status. After a half year observation, they weparated into two groups according to the criteria as followe; Group 1, 10 patients who suffered documented infections which were treated with intravenous transfution of antibiotics at leased twice during last half year, and Group 2, 10 patients who did not suffer severe infections during last half year. Three patients were excluded form the study because they suffered a infection once. After the half year observation, serum levels of Zn ion and other routine laboratory parameters were evaluated. Fisher's exact test was used for the comparisons of chracteristics between two groups. Mann-Whitney U-test was used for the comparisons of mean value of parameters between two groups. All values were represented as mean ±standard error (SEM).

Resuts: Significant difference was not seen between two groups about age, gender and feeding methods. However, serum levels of Zn in group 1 were significant low compared with group 2 (58.0 ± 5.2 μg/dl vs. 72.3 ±4.1 μg/dl, P=0.0375; n=10, 10, respectively). Our results suggested that Zn deficiency might be a risk factor of infections in elderly.

高齢者における亜鉛欠乏は反復性感染の原因となる？：パイロット試験

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Lecture 18 Effect of cobalt and vanadium on the liver glycogen content in the streptozotocin-induced diabetic rats

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- **Introducton** - The metabolism of glucose as a substrate for energy production is indispensable to cellular homeostasis. The concentration of blood glucose taken into cells is carefully maintained within a narrow range through various hormones which regulate the glucose transport, gluconeogenesis, glycogenesis and glycogenolysis. Recent studies have revealed that cobalt as well as vanadium activate hypoxia-inducible factor-1α (HIF-1α), and that it enhances the expression of several proteins which serve glucose metabolism such as glucose transporter isoforms. Thus, these trace elements most likely affect glucose metabolism through HIF-1α activation. In deed, cobalt decreased blood glucose in diabetic rats due to the suppression of gluconeogenesis. However, whether glycogen metabolism, as one of major factors by which blood glucose level was regulated, contributed to the glycemia-lowering effect was not clarified. In the present study, to determine the involvement of glycogen metabolism, glycogen contents in liver and muscle were measured when cobalt or vanadium was administrated to the streptozotocin-induced diabetic rats.

- **Experiment** - Diabetes was induced by the intraperitoneal administration of freshly prepared solution of streptozotocin (STZ) in 50 mM citrate buffer (pH 4.5) at 100 mg/kg of body weight. After 1 week of the STZ administration, a part of the normal rat group and a part of the diabetic group drank water containing 2 mM CoCl2 (Co) or 3.6 mM VOSO4 (V) for 2 weeks. After the treatment with trace elements, blood was sampled from the carotid artery of rats anesthetized with pentobarbital. In order to maximize physiologically insulin release and glycogen content, blood and tissue samples were prepared without any fasted period.

- **Results and Discussion** - After 2 weeks of both treatments, blood glucose in diabetic rats decreased significantly (Co: 340, V: 210 mg/dl), although it still remained higher than that in normal rats (66 mg/dl). These treatments did not modify blood glucose level in normal rats. We next examined the effect on plasma insulin level. These treatments changed the plasma insulin levels of neither control (2.3 ng/dl) nor diabetic rats (0.5 ng/dl). Liver glycogen contents were 1.5 mg/g tissue in the diabetic rats and 47 mg/g tissue in the normal rats, and the difference was significant. In the case of soleus muscle glycogen, normal and diabetic rats had 3.0 and 4.0 mg/g tissue, respectively, and there was no significant difference. The changes in glycogen content of liver and muscle due to STZ injection was consistent with the previous report. In both treatments, the liver glycogen remained unchanged in normal rats. Interestingly, in diabetic rats, the liver glycogen contents significantly increased (Co: 29, V: 67 mg/g tissue) whereas the muscle glycogen remained unchanged. The increase in liver glycogen of diabetic rats accompanied with lowering blood glucose, which may indicate that these trace elements increased the glucose intake of the liver from blood stream. However, as to the role of HIF-1α, a detailed analysis is required.

コバルトとバナジウムが糖尿病ラットの肝グリコーゲン含有量に及ぼす影響

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Lecture 19 Effect of LiCl evaluated by customized DNA microarray in *C. elegans*

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Lithium (Li) is widely distributed in nature with low concentration, and is contained in rock, sea water, food, such as marine and agricultural products. It is also clinically used as an effective drug in the treatment of manic-depressive illness (bipolar disorder), and is taken by many patients now. On the other hand, the therapeutic range of Li is narrow, and it is reported that toxic effect level of Li causes vomiting, unusual muscular action and pulse abnormalities, etc. Although it is well known that the main acting points of Li as drug for mental disease are synapses, other aspects such as interaction with other drugs, food composition and mechanism of side effects are unknown. The physiological functions of Li attract attention. In this study, we assessed the effects of lithium chloride (156-10000 μM) on *C. elegans* using bioassays targeting several endpoints and the gene expression patterns of metabolism related gene using DNA microarray. The wild-type *C. elegans* Bristol strain (N2), L1 larvae-stage was used as model animal, and cultured at 20°C. Calculation of lethality after 24 h exposure, length measurement and calculation of the rate of maturity after about 60 h exposure, and calculation of reproduction rate were performed, and LOEC was determined. Therefore the DNA microarray was set at 156 μM Li exposure, poly(A)$^+$RNA was extracted, and the labeling of the samples was done with Cy3 for the control sample and with Cy5 for sample treated with lithium chloride (156μM). The hybridization was done for 16 h at 42°C. No lethal effect was confirmed at this exposure concentration. LOEC of growth, maturity, and reproduction effects were determined as 625 μM Li, 156 μM Li, and 5000 μM Li. Moreover, the worm bag (egg-lying) fertilized ovum hatched in the body of nematode was seen after these doses of Li exposure. Analysis of the gene expression patterns showed that there were no gene inducible by Li exposure, but depression of CYPs, ABC transporters including mrp-1 and mrp-2, and lipid metabolism related gene was observed. These results suggested that metabolism may be depression by lithium chloride exposure.

線虫 *C. elegans* に及ぼす塩化リチウムの影響

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Lecture 20 Actions of lithium, magnesium and trace elements on neural stem cell differentiation

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Lithium has been known as a potent mood-stabilizing drug. Although it is widely used to treat manic depressive disorders, its target and actions are still unclear. We have investigated actions of lithium and magnesium on neural stem cells (NSC), defined as self-renewing and multipotent cells. Cells that possess the properties of NSC have been isolated and expanded in the presence of EGF and bFGF recombinant protein as neurospheres from C57Bl/6 mouse embryo. Using a combination of two neurotrophic factors, lithium chloride (LiCl) at 1 to 10 mM (within the therapeutic range) with or without magnesium chloride (MgCl2) at 1 to 10 mM was added to the culture supernatant. As a control, KCl, NaCl, CsCl, and RbCl at the same dose were also added to the culture supernatant. A dose responsive lithium effect for self-renewality of NSCs was observed from the growth rate of neurospheres. For these conditions, we have analyzed mineral concentrations of the culture medium including Mg, Ca, Fe, Cu, Zn, V, Ti, Sr, Rb, Mn, Co and Al. Among these elements, magnesium level was well correlated with lithium effect on self-renewality of NSCs. Further studies will be needed to know the possible involvement of lithium / magnesium interaction for the maintenance of NSCs

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リチウム、マグネシウムおよびその他の微量元素による神経幹細胞の自己複製能の制御

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Lecture 21 Changes in bone mineral density of zinc-deficient rats

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Objective: Zn deficiency causes a reduction in bone mineral contents. Recently, it has been shown that osteoclastogenesis is strictly regulated by receptor activator of nuclear factor kappaB ligand (RANKL) and osteoprotegerin (OPG) *in vitro*. We thus investigated the effect of Zn deficiency on RANKL and OPG expressions in rats.

Methods: Forty male SD rats (5 wks. old, 95-105 g b.w.) were divided into two groups on Zn-deficient (ZnD) or pair-fed standard (ZnS) diet by matching the individual body weight. All animals were maintained under the same conditions for four weeks. Samples of femur and blood were taken under anesthesia at 1, 2, 3 and 4 weeks following the dietary conditioning. Bone mineral density (BMD), activities of ALP and tartrate resistant acid phosphatase (TRAP) in bone, and expression levels of RANKL and OPG were measured with the colorimetric, DXA, and ELISA methods, respectively.

Results & Discussion: As shown in the table, serum Zn and Cu concentrations clearly indicated that the ZnD group of rats was in a Zn-deficient state. BMD was significantly decreased in the ZnD group, while there was no difference in femur weight between two groups. ALP activity in bone, an index of osteoblastogenesis, was significantly decreased in the ZnD group. TRAP activity, an index of osteoclastogenesis, was also decreased by Zn deficiency. Levels of bone OPG as an osteoclast inhibitory factor were slightly increased in the ZnD group, whereas RANKL levels did not change in both groups of rats. These results suggested that a reduction in osteoblast activity is involved in reduced BMD in Zn deficiency.

Effect of Zn deficiency on serum Zn, Cu, and ALP and TRAP in bone after 4 weeks of feeding (mean±SEM; *, p<0.01; **, p<0.05)

	Standard	Zn-deficient
Serum Zn (μg/dl)	104.2±3.84	35.6±1.12*
Serum Cu (μg/dl)	89.0±3.62	112.6±9.82**
Bone ALP activity (μmoles pNPP/min/mg protein)	0.56±0.014	0.33±0.030*
Bone TRAP activity (μmoles pNPP/min/mg protein)	0.017±0.0020	0.015±0.0011

亜鉛欠乏ラットでの骨密度の変化

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Lecture 22 Role of Zinc and Bone Metabolism: Zinc Enhances Synergistically Anabolic Effect of β-Cryptoxanthin on Bone Components in Rat Femoral Tissues

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Bone mass is decreased with aging. Osteoporosis is induced by decrease in bone mass. Pharmacological and nutritional factors may prevent bone loss with aging. Zinc has been shown to have stimulatory effects on osteoblastic bone formation and inhibitory effects on osteoclastic bone resorption, thereby increasing bone mass. Recently, carotenoid β-cryptoxanthin has been shown to stimulate bone formation and to inhibit bone resorption. Whether zinc can enhance the anabolic effect of β-cryptoxanthin on bone components has not yet been clarified. This study was undertaken to determine whether the combination of zinc and β-cryptoxanthin reveals an anabolic effect on bone components.

The effect of the combination of β-cryptoxanthin and zinc sulfate in rat femoral tissues in vitro was examined. Bone tissues were cultured for 48 h in a serum-free medium containing either vehicle, β-cryptoxanthin (10^{-9}-10^{-7} M) or zinc sulfate (10^{-6}-10^{-4} M). The presence of β-cryptoxanthin (10^{-9} M) or zinc (10^{-6} M) did not have a significant effect on calcium content in the femoral-diaphyseal (cortical bone) or -metaphyseal (trabecular bone) tissues. Culture with combination of β-cryptoxanthin (10^{-9} M) and zinc (10^{-6} M) caused a significant increase in calcium content in the diaphyseal and metaphyseal tisseus. Such an effect was not observed by the combination of β-cryptoxanthin plus genistein or menaquinone-7, or zinc plus genistein or menaquinone-7. The combination of β-cryptoxanthin (10^{-9} M) plus zinc (10^{-6} M) caused a remarkable increase in alkaline phosphatase activity and DNA content in the diaphyseal and metaphyseal tissues, while their application alone did not have an effect on the enzyme activity or DNA content in the femoral tissues. The effect of the combination of β-cryptoxanthin plus zinc in increasing bone components was prevented in the presence of inhibitor of protein synthesis, or inhibitor of transcriptional activity.

The effects of β-cryptoxanthin and zinc on bone components in the femoral tissues of rats in vivo were investigated. Rats were orally administered either vehicle, β-cryptoxanthin (5 or 10 μg/100 g body weight), zinc sulfate (0.1 or 0.5 mg Zn/100 g), or their combination once a day for 7 days. Calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal tissues was not significantly altered by the administration of β-cryptoxanthin (5 μg/100 g) or zinc (0.1 or 0.5 mg/100 g). Combined administration of β-cryptoxanthin (5 μg/100 g) and zinc (0.1 or 0.5 mg/100g) caused a synergistic increase in calcium content, alkaline phosphatase activity, and DNA content in the diaphyseal tissues. The effect of β-cryptoxanthin (5 or 10 μg/100 g) in increasing calcium and DNA contents was significantly enhanced by the combination with zinc (0.1 or 0.5 mg/100g) in the metaphyseal tissues. The metaphyseal alkaline phosphatase activity was markedly increased by the combination with β-cryptoxanthin (5 μg/100 g) and zinc (0.1 or 0.5 mg/100 g).

Thus we demonstrates that zinc enhances synergistically the anabolic effects of β-cryptoxanthin on bone components in vitro and in vivo.

骨代謝調節における亜鉛の役割－亜鉛はβクリプトキサンチンの骨同化効果を増強する

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Lecture 23 Acute renal injury and harmful systemic effects after intravenous administration of cadmium nitrate

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It has been generally reported that acute exposure to high dose of Cd compounds produced liver injury whereas chronic exposure to low dose of Cd compounds induced renal dysfunction. It was confirmed that $Cd(NO_3)_2 4H_2O(CN)$ was more toxic less soluble Cd compounds by LD in our previous study. Dose-response effect of hepatic damage after 300 minutes and kinetics of cadmium after i.v single dose were reported at the last presentation. However there were few reports about renal injury caused by CN in early stage of acute exposure. Therefore the acute harmful effects of CN on kidney were mainly investigated in this study. Rats were injected CN (2.7, 5.4 or 8.1(LD_{90}) mg/kg) or saline. Blood samples were obtained 300 minutes after infusion. Urine accumulated for 300 minutes after injection was obtained from an indwelling catheter. A remarkable decrease in urine volume in the 8.1 mg/kg group indicated acute renal failure. A decrease in creatinine clearance suggested acute glomerular dysfunction at doses greater than 5.4 mg/kg. Increases in β_2-microglobulin, N-acetyl-β-D-glucosaminidase creatinine ratio and urinary glucose in the 8.1 mg/kg group indicated proximal tubule injury. Secretion of K was also severely affected by proximal tubule injury and severe decreases in urine volume, and an increase in serum K was identified at doses greater than 5.4 mg/kg. It was suggested that severe kidney dysfunction was dramatically caused by acute exposure to high concentrations of CN that exceed the capacity for detoxification, aggravating harmful systemic effects. Especially severe hyperkalemia would be associated with cardiac-derived lethal toxicity of CN.

Urinary variables for 300 minutes after single intravenous injection of saline or Cd(NO3)2 •4H2O (2.7, 5.4 or 8.1 mg/kg).

	Saline	Dose (mg/kg)		
		2.7	5.4	8.1
Volume (ml)	4.0 ± 0.05	3.2 ± 0.04	3.0 ± 0.04	1.0 ± 0.01*
Ccr (ml/min)	1.3 ± 0.08	1.3 ± 0.03	0.6 ± 0.03*	0.2 ± 0.08*
β_2-MG (pg)	-	-	-	22.5 ± 3.5
NAG/Cr (10^2U/g)	0.3 ± 0.08	0.2 ± 0.05	0.3 ± 0.07	0.5 ± 0.15*
K (mEq)	0.8 ± 0.20	0.6 ± 0.19*	0.5 ± 0.04*	0.2 ± 0.04*

Mean ± SD n = 5; ANOVA; Fisher's protected LSD; *P < 0.05 vs. saline

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硝酸カドミウム静脈内投与後の急性腎障害および有害全身影響

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Lecture 24 Severe hepatic damage and renal dysfunction after intravenous administration of cadmium fluoride

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Cadmiun fluoride (CdF) is using as an industrial application for the insulator of super high speed telecommunication equipments because of its resistance against heat and water. However there were no reports about harmful effects by acute exposure of CdF. Therefore lethal toxicity and dose-response relationship of hepatic and renal damage were investigated in this study. Rats were injected CdF (1.33, 2.67 or 4.01(LD_{88}) mg/kg), saline or NaF. The influence of fluoride ion (F) was also investigated. NaF solution is theoretically corresponded to the F concentration of CdF 4.01 mg/kg. Blood samples were obtained from the right carotid artery 5 hours after infusion. Urine accumulated for 5 hours after injection was obtained from an indwelling catheter. AST, m-AST, ALT and BUN were increased and glucose was decreased dose-dependently in CdF groups. Cr was not significantly increased. Urine volume was dose-dependently decreased. The lethal toxicity of CdF was stronger than those of other Cd compounds such as cadmium nitrate (CdN) and cadmium chloride (CdL). It was confirmed that CdF caused severe hepatic injury because there were remarkable increases of hepatic enzymes and dose-dependent decrease of serum glucose. The degree of hepatic damage by CdF would be stronger than those caused by CdN or CdL. Exposure of CdF would also cause acute renal failure. There would be no significant harmful effects on liver by F.

Dose-response relationships of serum parameters 5 hours after single intravenous injections of saline, NaF or CdF_2.

Parameter	Saline	NaF (2.23mg/kg)	CdF_2 (mg/kg)		
			1.33	2.67	4.01
AST (IU/l)	142±29	153±65	291±79.54	760±244*	2017±888*
m-AST (IU/l)	13.3±3.0	12.9±8.7	25.2±9.08	76.9±26.6	722±372*
ALT (IU/l)	51±7.3	55±135	188±62.8	796±281*	1530±746*
LDH (IU/l)	1960±798	2119±857	1567±632	2594±219	6835±3324*
BUN (mg/dl)	15.9±6.6	17.0±2.5	17.2±2.6	21.7±1.6	26.8±6.2*
Cr (mg/dl)	0.49±0.04	0.53±0.18	0.42±0.04	0.42±0.04	0.55±0.09
Glu(mg/dl)	120.3±11.7	140.0±55.7	94.3±19.1	80.5±20.3*	51.6±23.1*

Mean ± SD; N=6; ANOVA; Fisher's protected LSD; *$p < 0.05$ vs. saline.

フッ化カドミウム静脈内投与後の重篤な肝障害および腎機能低下について

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Lecture 25 Acute renal dysfunction, metabolic acidosis and electrolyte abnormalities after intravenous injection of low concentrations of hydrofluoric acid in rats

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The acute toxicity of hydrofluoric acid (HFA) was investigated in a 24-hour lethal dose study of intravenous infusion in rats. Harmful systemic effects were also studied 1 hour after acute lethal exposure to HFA. Rats were injected with HFA, saline, sodium fluoride (NaF), or HCl solution. NaF and HCl solution concentrations corresponded to the F^- and H^+ concentrations of 9.6 mg/kg HFA. BUN and Cr were significantly increased in response to HFA concentrations greater than 3.2 mg/kg. Acute glomerular dysfunction also occurred at HFA concentrations greater than 3.2 mg/kg. HCO_3^- and base excess (BE) were significantly decreased in the 6.4 and 9.6 mg/kg groups. Ca^{2+} was significantly decreased, and K^+ was increased in the 9.6 mg/kg group. BUN was significantly increased in the NaF and HFA groups and was increased in the HFA group compared to that in the NaF group. Cr was significantly increased in the HFA group only. Ca^{2+} was significantly decreased in the NaF and HFA groups, and K^+ was significantly increased in the NaF and HFA groups. F^- exposure directly affected serum electrolytes. Metabolic acidosis and renal failure were more severe in response to HFA exposure than in response to NaF exposure because of more free F^- which has strong cytotoxicity, in the HFA group than in the NaF group. Lethal effects of HFA are promoted by exposure routes such as inhalation that cause rapid absorption into the body. Even low exposure to HFA can cause acute renal dysfunction, electrolyte abnormalities and metabolic acidosis. These complications result in a poor prognosis.

Serum variables 1 hour after single intravenous injection of saline, NaF, HCl, or HFA (1.6, 3.2, 6.4, or 9.6 mg/kg)

				HFA(mg/kg)			
	Saline	NaF	HCl	1.6	3.2	6.4	9.6
BUN(mg/dl)	11.8±1.9	18.2±2.5*	14.4±0.9	15.0±2.0	20.0±2.7*	20.8±3.7*	20.4±3.6*†
Cr (mg/dl)	0.4±0.0	0.5±0.0	0.4±0.0	0.5±0.0	0.6±0.0*	0.6±0.1*	0.6±0.2*
HCO_3^- (mmol/l)	24.5±1.9	21.3±1.8*	24.2±0.6	22.7±1.4	22.6±2.1	20.1±2.4*	16.3±3.1*†
BE (mmol/l)	-2.0±1.6	-4.5±0.7*	-2.6±0.7	-2.1±0.7	-3.8±1.9*	-4.9±1.4*	-7.6±2.0*†
Ca^{2+}(mEq/l)	2.6±0.1	1.7±0.4*	2.9±0.0	2.4±0.2	2.4±0.4	2.4±0.2	1.9±0.2*
K^+(mEq/l)	5.6±0.5	6.6±1.3*	5.9±0.3	5.5±0.3	5.6±0.4	6.3±0.3	6.7±0.2*

Mean ± SD n = 5; ANOVA; Fisher's protected LSD; *P < 0.05 vs. saline, †P < 0.05 vs. NaF

低濃度フッ化水素酸静注後の急性腎機能障害、代謝性アシドーシスおよび電解質異常

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Lecture 26 Inhibitory effects of dibutyltin (DBT) dichloride on cytokine productions in murine macrophage cell line J774.1

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Objectives: Dibutyltin (DBT) compounds are widely used as stabilizers and catalysts for synthetic polymers, and are also known as degraded compounds of tributyltin (TBT). The immune system is a target of DBT intoxication. The cytotoxic effects of DBT on thymic lymphocytes have previously been studied. We evaluated the effects of DBT dichloride in macrophages using murine macrophage cell line J774.1 in vitro.

Materials and Methods: Cells were cultured in RPMI 1640 containing antibiotics with 5% FBS in an incubator at 37 degree at 5% CO_2. Cells were aliquoted in 24-well culture plates at $1X10^6$ cells/well. DBT dichloride was dissolved in ethanol and diluted with culture medium. J774.1 cells were exposed to DBT dichloride at concentrations of 0, 0.5, 1.0, 1.5 and 2.0 μM in 24-well cell culture plates (n=6/group). Lipopolysaccharide was added after 18 hours. Cells were incubated for an additional 6 or 24 hours, then cell viability was determined using trypan blue exclusion. Total RNA was extracted from the cells after an additional 6-hour incubation. cDNA was synthesized from mRNA. Real-time PCR was used to analyze the mRNA expression for tumor necrosis factor α (TNFα), interleukin 1β (IL-1β) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in J774.1 cells. A calibrator normalized relative ratio for each cytokine to GAPDH was calculated as the index of mRNA expression. Supernatants of J774.1 cells were sampled after an additional 24-hour incubation. The concentrations of TNFα and IL-1β in the supernatant were determined by ELISA.

Results: Mean values of cell viabilities in the DBT-exposed groups were significantly lower than those of respective controls after both additional 6- and 24-hour incubations. Mean values of viabilities in the 2.0 μM group were marked low and significantly lower than those in other groups. After an additional 24-hour incubation, 1.0 and 1.5 μM group viabilities were significantly lower than that of 0.5 μM group. Mean relative mRNA expression of TNFα in the 0.5 μM group was significantly higher compared with other groups. There were no significant differences in relative IL-1&beta mRNA expression among groups. Mean concentrations of TNFα in the supernatant in the 1.0, 1.5 and 2.0 μM groups were marked low and significantly lower compared with those in the control or the 0.5 μM group. Mean concentrations of IL-1β in the supernatant in DBT exposed groups were also marked low and significantly lower than in those in the control.

Discussion: The level of DBT that caused macrophage cell death was similar to that of TBT in a previous study, as were the levels at which lymphocyte cell death was induced in previous studies. Concentrations of cytokines in the supernatant were marked low and not fully explained by the low cell viability of the DBT-exposed groups. Since the mRNA expressions of cytokines in higher-dose groups were similar to those in the control, translation from mRNA may be inhibited by DBT. At 0.5 μM, mRNA expression of TNFα increased, and the protein level of TNFα in the supernatant was similar to that in the control. Low-level exposure to DBT may increase mRNA expression and compensate effects of cell death.

二塩化ジブチルスズのマクロファージ系細胞のサイトカイン産生に対する抑制効果

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Lecture 27 Improvement in Trace Element Deficiency with Nutritional Supervision and Guidance to Take Powder Tea Leaves

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Our previous study revealed the deficiency of trace elements, Manganese, Magnesium, Iron, Calcium and dietary fiber, in the daily foods consumed by young women. We have been proposing to take powder tea leaves to meet the intake of trace elements in the Japanese standard of nutritional daily intake. It is also considered that the nutritional supervision is required to improve the Iron intake. Thus, this study is conducted to examine the effects of the supervision and of the powder tea leaves on the nutritional intake, especially Manganese, one of the trace elements, and Iron of the subjects. Taking of the powder tea leaves for improving the intake of trace elements was also examined on the effect on the Iron concentration in blood serum.

The subjects were fourteen healthy females whose age were 20.07±0.27. The subjects were divided two groups. One group ate only recommended foods without tea and the other group ate recommended food and powder tea leaves as tea. Duration of the experiment was six months. All the subjects were supervised to have better food intake. The latter group of the subjects was guided to take powder tea leaves two hours after each meal and totally 1.5g a day during 6 months of the experiment. Before and after the experiment, the subjects were required to describe their food intake of the recent three days in the questionnaire, and their blood serums were examined to check the iron levels by a commercial clinical test center. Nutritionnal levels and the balance were calculated from the description of food intake of each subjects for the recent three days by using a software, Excel Eiyo-u Kun ver. 4.0 (Kenpakusha, Tokyo). Paired t-test was carried out by Excel Toke-I 2004 for Windows (SSRI, Tokyo).

The effect of supervision was observed to improve the nutritional balance of their daily food, especially Iron, Calcium and dietary fiber. Eating of the powder tea leaves increased the Manganese and folic acid intake. The effect of powder lea leaves on the Iron concentration blood serum was examined because tannin is a famous inhibitor of iron absorption. The blood test showed that the serum iron concentration was decreased when the powder tea leaves are taken with meal but was not decreased by delaying to take the powder tea leaves after two hours of each meal. This means that the powder tea leaves did not affect the iron absorption after two hours of each meal.Nutritional supervision was effective for the subjects to get good balance of daily in take. Taking the powder tea leaves did improve the intake of trace elements, represented by Manganese. The powder tea leaves did not decrease the concentration of blood serum iron when subjects took it after two hours of each meal. In addition, it is known that taking the powder tea leaves is effective to obtain vitamin C, anti-stress agent and folic acid, relation to the nerve disorder. Better food habit is considered to help young women achieve high quality of life.

栄養指導による微量元素摂取の改善と粉末茶摂取に対する指導

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Lecture 28 Effect of Administration mulberry leaves powder on improvement of anemia and serum lipid levels in the iron deficient rats

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Effect of administration mulberry leaves powder and spinach powder on improvement of anemia and serum lipid levels in the iron deficient rats. Weaning male Wister rats were divided into five groups. Group 1 was fed the control diet containing iron 2.2 mg/100g diet for 8weeks. Group 2 was fed iron deficient diet for 8 weeks. Groups 3, 4 and 5 were fed iron deficient diet for 4 weeks and after that fed following experimental diets for 4 weeks. That is, Group 3 fed diet supplemented with iron sulfate, group 4 fed diet supplemented with mulberry leave powder, group 5 fed diet supplemented with spinach powder. Supplemented diets included iron 2.2mg/100g diet. RBC, Hemoglobin, Hematocrit and serum triglyceride value (the eighth weeks) were shown in Table 1. In the iron deficient group 2, Hemoglobin and Hematocrit levels were decreased, while the serum triglyceride was increased significantly. An effect for recovery of red count, hemoglobin value by the mulberry leaf dosage and the spinach dosage did not have a difference. The iron content of mulberry powder was more than spinach powder. Therefore, it is considered that mulberry powder have higher effect if the same amount of mulberry and spinach is ingested. Serum triglyceride concentration was decreased in groups 3, 4 and 5 groups, especially it was decreased in groups 4 and 5 groups. We propose that the mulberry leaves and spinach have the effect on improvement of serum lipid levels.

Table1. Concentrations of Blood component, serum triglyceride in the iron deficient and supplement rats (8weeks)

	RBC ($10^3/\mu l$)	Hemoglobin (g/dl)	Hematocrit (%)	triglyceride (mg/dl)
1. control	667.5±59.2	12.6±1.0	39.3±2.1	404.3±51.1
2. -Fe	220.3±19.3***	2.0±0.2***	8.4±0.9***	840.0±366.3
3. +Fe	733.0±46.4*	13.2±1.2	42.4±3.1*	319.3±55.1
4. mulberry	692.8±38.4	7.8±0.5***	26.7±1.9***	94.5±48.2**
5. spinach	668.3±23.5	7.5±0.1***	26.2±0.9***	116.0±49.5*

***P<0.001 **P<0.01 *P<0.05

鉄欠乏動物における貧血および血清脂質に及ぼす桑葉粉末の改善効果

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Lecture 29 Ingested trace elements and their blood concentrations in inpatients with enteral liquid food

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Objective : It was often investigated that serum copper (Cu) level tended to be low in inpatients with conventional Cu-poor enteral liquid foods. Severe hypocupremia was associated with leucopenia, neutropenia and anemia. These findings were improved by adding of Cu-rich foods. The author adopted L-8 liquid food involving Cu adequately to prevent hypocupremia. The comparison was carried out between intakes of trace elements from L-8 liquid food and recommended dietary allowances of trace elements in Dietary Reference Intakes for Japanese, 2005.

Methods : In this study 9 inpatients (2 men and 7 women) with dysphagia aged 74 ± 15 years (Mean ± SD), were given with L-8 liquid food (Asahi Kasei Pharma) through nasogastric or gastric tube on the bed until 24 months. They were suffered from cerebrovascular diseases and motor disability. Fasting blood sample was obtained early in the morning, every 6 months. As some trace elements serum iron (Fe), Cu, zinc (Zn), selenium (Se), molybdenum (Mo) and blood manganese (Mn) were measured by standard methods.

Results : Inpatients recruited in this study ingested 994 ± 197 kcal/day. Practically ingested trace elements through enteral liquid foods of L-8 were shown in the followings; Fe intake was 9.5 ± 1.5 mg/day at the baseline (before) and 8.5 ± 1.7 mg/day after L-8, Cu intake 0.15 ± 0.09 and 0.80 ± 0.16 mg/day respectively, Zn intake 1.6 ± 0.9 and 12 ± 2.4 mg/day, Mn intake not available and 8.8 ± 0.66 mg/day, Se intake 1.0 ± 2.8 and 40 ± 7.9 μg/day and Mo intake not available and 20 ± 0.4 μg/day. On the other hand the concentrations of trace elements changed from baseline (before) to 12 months and 24 months as the followings: serum Fe (μg/dl) changed from mean 59.7 to mean 56.8 and then mean 60.7 respectively, serum Cu (μg/dl) from 52.0 to 120.8 and then 107.0, serum Zn (μg/dl) 66.3 to 58.5 and then 59.7, Blood Mn (μg/dl) from 0.48 to 0.84 and then 0.77, serum Se (μg/dl) from 7.6 to 12.4 and then 13.1 and serum Mo (ng/ml) from 0.62 to 1.84 and then 1.58 respectively. Though serum Cu and Se were moderately low on an average before taking L-8 liquid food, L-8 administration resulted in the significant increases of both serum Cu and Se to normal ranges in one or two months and maintained their normal ranges throughout 24 months. However serum Fe and Zn did not show any significant changes during the observation periods, though Fe and Zn contents were adequate in L-8 liquid food as compared to the recommended dietary allowance (RDA) in Dietary Reference Intakes (DRIs) for Japanese, 2005. Blood Mn and serum Mo showed low in normal ranges on an average even before L-8, and further significantly increased within normal ranges.

Conclusion : In inpatients with enteral liquid foods L-8 administration through nasogastric or gastric tube resulted in significant increments of serum Cu, Se, Mn and Mo, while serum Fe and Zn did not show any significant changes by L-8 administration. Therefore, the contents of trace elements in L-8 liquid food tended to be adequate in the clinical investigation and from the comparison to the RDAs in DRIs for Japanese, 2005.

経腸栄養患者における微量元素摂取量と血中濃度の変化

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Lecture 30 Maternal zinc deficiency during lactation period modify pups' salt taste preference after weanling.

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It has been well known that maternal dietary NaCl intake influences weanling rats' salt preferences, and brief exposure to NaCl during early postnatal development enhances adult intake of sweet and salty compounds. However, few data have been published concerning about the maternal milk nutrients such as zinc level and later NaCl preference in weanling and adult grown rats. We have already shown that short term zinc deficiency clearly causes the increase of NaCl preference, so we demonstrated whether or not the maternal zinc deficiency during lactation period cause weanling and grown rats' NaCl preference using this zinc deficient system with SD/Slc rats. Zinc deficient (0.7 mg Zn/Kg), Low zinc (4.0 mg Zn/Kg), and Pair-fed control (33.7 mg Zn/Kg, and fed against Zinc deficient rats) diets were fed to the lactating mother during lactation period only (for 3 weeks after birth), and zinc sufficient diet was fed to the all group rats after weanling. Water and 0.15M NaCl solution, 2-bottle preference experiment showed that maternal zinc-deficient and low-zinc diets during lactation period caused the decreased NaCl preference in weanling and grown rats, even though after their recovery from zinc deficiency. After 3 weeks of feeding with zinc-sufficient diet, significantly increased epinephrine concentration in amygdala and decreased plasma oxytocin concentration and decreased chorda tympani nerve response to NaCl were observed in Zn-Def and Low-Zn groups. These results suggest that not only central nervous system but also peripheral mechanism of taste reception were strongly involved in the grown rats' taste preference by early zinc deficiency during lactation period.

授乳期間中の低亜鉛食給餌は仔ラットの離乳・成長後の食塩嗜好に影響を及ぼす

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Lecture 31 Fluoride levels in various tea-based beverages of Japan and in Chinese tea infusions

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Background and Objective: We have studied on the accelerated bone absorption in the middle-aged women in the grassland area of Inner Mongolia, China, where the drinking waters are high in fluoride concentration. And what's more, they are always drinking the brick tea infusions which are very high in fluoride concentration in that area. In Japan, various tea-based beverages are widely on the market in refresh drinking water, and it is easy to buy Chinese tea leaves by Internet. We are afraid of large intake of fluoride from tea-based beverages and Chinese tea infusions. Therefore, to estimate fluoride intake, we determined fluoride levels in tea-based beverages on the market and in infusions of Chinese tea leaves to buy by Internet.Materials and Methods: We collected 70 samples of tea-based beverages in pet bottle, can and paper pack in the different trade names or manufacturers on the market in Toyama Prefecture, Japan, and 24 samples of Chinese tea leaves to buy by Internet. The infusions of Chinese tea leaves were made using distilled water (70-100°C) by the methods of the attached documents to them. Fluoride levels in beverage samples and Chinese tea infusion samples were measured by the ion-selective electrode method using the total ionic strength adjustment buffer.Results and Discussion: In tea-based beverages, oolong tea (17 samples) is the highest average of 1.12 mg/L (the range, 0.76-1.62 mg/L), the next average is green tea (27 samples) of 0.96 mg/L (the range, 0.62-1.78 mg/L), and black tea (7 samples) is the average of 0.70 mg/L (the range, 0.44-0.91 mg/L). We could not find fluoride in beverages made of barley and buckwheat in stead of tea leaves. We could detect fluoride from blending tea beverages (6 samples) of which tea leaves were used in part (the range, 0.23-2.04 mg/L).In fusions of Chinese tea, (black) tea (3 samples) is the highest average of 1.19 mg/L (the range, 1.01-1.53 mg/L), the next average is oolong tea (6 samples) of 1.09 mg/L (the range, 0.79-1.58 mg/L), and green tea (4 samples) is the average of 0.81 mg/L (the range, 0.48-1.00 mg/L). Chinese black tea (8 samples), the same kind of brick tea, is the low average of 0.67 mg/L (the range, 0.39-1.47 mg/L).Although there were not so high fluoride levels of tea-based beverages and infusions as the infusions of brick tea (2.61-10.9 mg/L) of Inner Mongolia, China, 70% of tea-based beverage samples and 60% of Chinese tea infusion samples were over the limiting level of drinking water (0.8 mg/L). Conclusions: Fluoride intake from tea-based beverages and infusions of Chinese tea leaves is suggested to be large, and it is necessary to conduct more advanced research on fluoride hazards.

市販の各種お茶飲料および中国茶浸出液中のフッ素濃度について

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Lecture 32 Nutritional availability of selenium in selenium-enriched radish sprouts and selenium-enriched pumpkin

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Selenium (Se)-enriched radish sprouts cultivated with selenite solution and Se-enriched pumpkin grown on selenate-fertilized soil were obtained and their Se species were identified by high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICPMS). In addition, nutritional availabilities of Se in these Se-enriched plant foods were evaluated by an animal experiment.

Most of Se in the Se-enriched radish sprouts was extracted with 0.2 M HCl and identified as *Se*-methylselenocysteine. On the other hand, about 40% of Se in the Se-enriched pumpkin was extracted with 0.2 M HCl. Four Se compounds, selenate, *Se*-methylselenocysteine, selenomethionine and unknown Se compound, were detected in this 0.2 M HCl extract. When the pumpkin was digested with protease, Se extracted with 0.2 M HCl was increased to 90% of the total Se. Chemical species of the 0.2 M HCl-extractable Se increased was selenomethionine; about 45% of Se in the pumpkin was estimated to be protein-bound selenomethionine.

Male weanling ddY mice were fed *Torula* yeast-based Se-deficient diet for 3 weeks. After then, mice were divided into 7 groups and fed the basal diet or a diet supplemented with a 0.05 or 0.25 mg/kg of Se as either sodium selenite, Se-enriched radish sprouts or Se-enriched pumpkin for a further 1 week. After feeding for 4 weeks, glutathione peroxidase (GPx) activities and Se content in plasma and liver were measured and nutritional availabilities of Se in the Se-enriched plant foods were estimated by a slope ratio analysis (Yoshida et al., 1999). The GPx activities and Se contents in mice supplemented with Se-enriched sprouts or Se-enriches pumpkin were somewhat lower than those in mice supplemented with selenite. When liver GPx activity was used as an index, nutritional availabilities of Se in the Se-enriched sprouts and the Se-enriched pumpkin were estimated to be 44% and 42% to that in selenite, respectively. On the other hand, when liver Se accumulation was used for an index, the nutritional availabilities in the Se-enriched sprouts and pumpkin were estimated to be 65% and 86%, respectively.

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セレン強化スプラウトおよびセレン強化カボチャに含まれるセレンの栄養有効性

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Lecture 33 Intracellular changes of trace elements by fucoidan extracted from *OKINAWA MOZUKU*

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Aim: The fucoidans exhibit different biological properties such as anti- coagulant,anti-inflammatory, anti-viral and anti-tumor activities, although the precise mechanisms of fucoidan activities remained to be determined. This study undertook to elucidate physiological effect of fucoidan on intracellular changes of trace elements.
Methods:1.Metallothionein (MT) induction by fucoidan Fucoidan extracted from the brown seaweed (*OKINAWA MOZUKU*),82.9kD molecular weight, was used in this study.TRL1215 cells(normal rat liver cell line) were treated with 0.1 and 1.0 mg/ml of fucoidan under 5% CO_2 at 37 ℃. After 24 h culture,MT protein was stained immunohistochemically in fucoidan-treated cells and non-treated control.
2. Analysis of intracellular trace elements using in-air micro-PIXETRL1215 cells were cultured on Mylar film over night. 100μM of BrdU was pulsed,then 0 and 1.0mg/ml of fucoidan were treated for 9 h. The cells were criofixed and dried. Elemental distribution of the samples were analysed using in-air micro-PIXE at JAEA-Takasaki.
Results:1.MT was stained in the cytoplasm and nuclei of TRL1215 cells. There was no significant induction of MT by fucoidan.2.X-ray spectra taken from the fucoidan-treated cells and the control revealed P,S,Cl,K, Ca,Fe,Zn,Mg and Br yields. Mg and Zn concentrations were increased in the fucoidan-treated cells compared to those of the control. Br was markedly accumulated in fucoidan-treated cells. Mg and Zn maps showed a special correlation with Br map (Figure). Conclusion: Fucoidan is supposed to tarnslocate Mg and Zn into nuclei of hepatocytes.

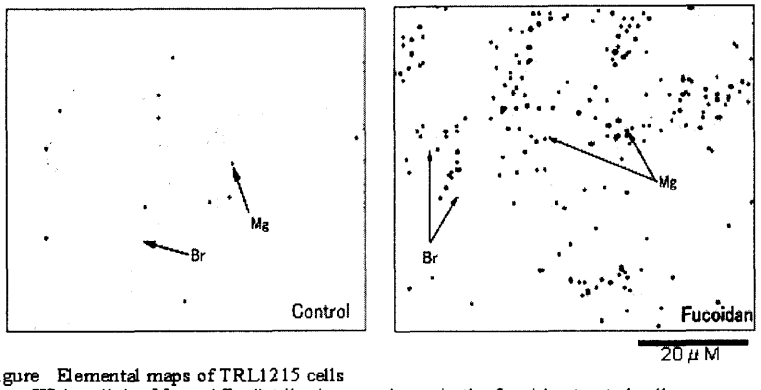

Figure Elemental maps of TRL1215 cells
Wide cellular Mg and Br distribution are shown in the fucoidan-treated cells.
Mg location sites are visible with special correlation followed by the accumulation of Br

フコイダン(オキナワモズク)による細胞内微量元素の変動

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Lecture 34 Effects of zinc deficiency on rat intestinal mucosa

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Objective: Zn-deficient rats exhibit parakeratic dermatitis. Parakeratosis also appears in the esophageal stratified squamous epithelium. Recently, it has been reported that intestinal ALP attenuates toxicity of lipopolysaccharide (LPS) by dephosphorylating monophosphoryl lipid-A moiety of LPS *in vitro* and *in vivo*. To investigate the effects of zinc deficiency on intestinal mucosa, we measured intestinal TNF-α expression as an inflammation-induced cytokine and nitro-tyrosine formation as an index of free radical generation in Zn-deficient rats.

Methods: Forty male SD rats weighing 95-105 g were used. The animals were divided into two groups on Zn-deficient (ZnD) or pair-fed standard (ZnS) diet by matching the individual body weight. All animals were maintained under the same conditions for four weeks. Intestinal mucosa and blood samples were obtained under anesthesia at 1, 2, 3 and 4 weeks following the dietary conditioning. ALP activity was determined by the colorimetric method. Expression of TNF-α and formation of nitro-tyrosine were detected by Western blot analyses.

Results & Discussion: As shown in the table, serum Zn and Cu concentrations clearly indicated that the ZnD group of rats was in a Zn-deficient state. ALP activities in serum and intestinal mucosa were significantly decreased in the ZnD group. Both TNF-α expression and nitro-tyrosine formation were substantially increased in the ZnD group. These results appear that intestinal mucosa under Zn deficiency is in a proinflammatory state by the augmented cytokine and free radical production and that TNF-α enhanced acts as a mediator of this feature.

Effect of Zn deficiency on serum Zn, Cu, serum ALP, and intestinal ALP after 4 weeks of feeding (mean ± SEM; *, p<0.01; **, p<0.05)

	Standard	Zn-deficient
Serum Zn (μg/dl)	104.2±3.84	35.6±1.12*
Serum Cu (μg/dl)	89.0±3.62	112.6±9.82**
Serum ALP activity (IU/l)	1165±36.4	342.8±52.9*
Intestinal ALP activity (μmoles PNPP/min/mg protein)	0.18±0.012	0.087±0.0055*

ラット腸管粘膜での亜鉛欠乏の影響

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Lecture 35 Effect of difference in zinc source and dietary zinc level on absorption and tissue concentration of zinc in rats

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Changes in the absorption rate and tissue distribution of zinc under different nutritional status of this element were examined. Five-week-old male Wistar rats were given a zinc-deficient basal diet (zinc content, 0.6 ppm) or the basal diet supplemented with graded levels (5.5 ppm, 10 ppm, 15 ppm, 20 ppm or 40 ppm) of zinc as zinc sulfate or oyster extract for 4 weeks. Feeding of zinc-deficient diet for 4 weeks induced poor growth and a significant decrease in zinc concentrations in many tissues. Rats fed a diet containing zinc at a level of 5.5 ppm showed lower growth and tissue zinc concentrations than those fed diet containing zinc at level of 10 ppm or more. In particular, the zinc concentration in tibia responded remarkably to the elevation of dietary zinc level. Apparent absorption of zinc was inversely correlated to the dietary zinc level; apparent absorption in rats fed diet containing 5.5 ppm zinc was about 90%, while that in rats fed diet containing 40 ppm zinc was about 30%. These results indicate that the minimum dietary requirement for zinc is 5.5 to 10 ppm in growing rats. In the zinc level of 15 ppm and 20 ppm, the absorption rate of oyster extract zinc was higher than zinc sulfate.Effect of changeover from a zinc-sufficient diet to a zinc-deficient diet on feed intake, body weights and tissue zinc contents was also examined. Five-week-old male Wistar rats were given zinc-sufficient diets containing 15 ppm of zinc as zinc sulfate or oyster extract for 21 days. Thereafter, rats in each group were divided into 2 groups: one group was given a zinc-deficient diet, while the other group was given a zinc-sufficient diet for a further 7 days. The changeover to a zinc-deficient diet decreased fecal zinc excretion remarkably and lowered feed intake and body weight within 3 to 4 days. Zinc concentrations in various tissues were also decreased by the changeover to a zinc deficient diet. The extent of the decrease varied with the type of tissue; zinc concentration in tibia was remarkably lowered. Differences in zinc source did not affect tissue zinc concentration. These findings indicate that the adverse effects of a zinc-deficient diet appeared within 3 to 4 days after starting the deficient diet even in zinc-sufficient rats because the amount of stored zinc is small.

ラットにおける亜鉛の吸収率と組織濃度に及ぼす飼料中亜鉛レベルの影響

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Lecture 36 Analysis of molecular mechanism of a copper intestinal absorption obstruction by zinc

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Zinc and copper are essential trace elements in humans and animals. It is known that zinc and copper compete in the case of intestinal absorption, but the precise mechanism is not clarified. Recently, membrane transport proteins (metal transporters), which were specific for zinc or copper, and existence of a copper chaperone have been clarified. But it is not clarified how these transporters, a metallochaperone and metal binding-proteins are concerned with a copper intestinal absorption obstruction by zinc. Therefore, in this study, we paid our attention to having a copper absorption obstruction by an excessive intake of zinc in an intestinal tract and tried it to clarify how these transporters, a metallochaperone and metal binding-proteins participated in absorption / excretion of zinc and a copper absorption obstruction to depend by zinc. Protein levels of zinc transporters, such as ZnT1 and Zip4, a copper transporter, Ctr1, and a copper chaperone for Cu/Zn-superoxide dismutase (SOD), and copper binding-proteins, Cu/Zn-SOD in the intestine and zinc and copper concentration in serum were analyzed in male Sprague-Dawley rats fed a standard diet containing 0.005% zinc or a high zinc diet containing 0.5% zinc for 8 weeks. Copper and zinc concentrations in serum were determined with an ICP-MS. Western blot analysis was carried out to evaluate protein levels of metal transporters, a metallochaperone and metal binding-proteins in intestine. Zinc concentration in serum was significantly higher in rats fed a high zinc diet relative to rats fed a standard diet. Inversely, copper concentration in serum was significantly reduced in rats fed a high zinc diet relative to rats fed a standard diet. A protein level of copper chaperone for Cu/Zn-SOD (CCS) in the intestine was significantly higher in rats fed a high Zn diet than in the control. However, a protein level of Cu/Zn-SOD in rats fed a high zinc diet was lower than in the control. Intestinal metallothionein was induced in rats fed a high Zn diet. There were no significant differences in protein level of intestinal Ctr1 between the two groups of rats. Our results indicated that copper absorption was obstructed by an excessive intake of zinc in an intestinal tract and that copper available for SOD via CCS diminished in the intestinal cells in rats fed a high zinc diet relative to rats fed a standard diet.

亜鉛による銅の腸管吸収阻害の分子機構の解析

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Lecture 37 CueO, cuprous oxidase concerned in the copper efflux system of E. coli

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E. coli, an enteric bacterium living in digestive tract of warm-blooded animals, has a two-component copper regulatory system, enabling it to survive high concentrations of copper generated proteolytic and acidic degradation of food in animal gut. One system activates expression of CopA and CueO by the MerR homologue, CueR, which is activated upon Cu(I) binding. CueO is a copper-regulated multicopper oxidase and functions as cuprous oxidase to convert Cu(I), transported from cytoplasm to periplasm by CopA, to less toxic Cu(II). Further, CueO is considered to oxidize numerous compounds including Fe(II), catechols and siderophores, although oxidase activities for them are considerably low. CueO is activated to holoenzyme by incorporating 4Cu ions into a type I Cu site, a type II Cu site and a pair of type III Cu cites of apoenzyme. Further, CueO contains a fifth Cu binding site comprised of 2Met2Asp residues for the binding of substrate. An α-helix (H5) covers this fifth Cu binding site so as to interfere the access of potential organic substrates to the fifth Cu binding site and to decrease polarity around the site, leading to increase in the specificity for Cu(I). Therefore, the considerably low acitivities for organic substrate is characteristic of CueO as a multicopper oxidase. In the present study, we performed the quantitative studies to reveal the substrate specificity of CueO as the sole cuprous oxidase in living systems.

The oxidase activity of CueO for Cu(I) was exceptionally high (187 unit/mg) compared to those for other substrates. However, the oxidase activities for those other than Cu(I) dramatically increased in the presence of 1mM $CuSO_4$, for example, the oxidase activities of ABTS was only 0.59 U/mg in the absence of Cu(II) but increased to 10.8 U/mg in the presence of Cu(II). This increase in activity was brought about by that a Cu(II) ion was bound to the substrate binding site so as to function as the mediator of electron transfer between type I Cu and substrate in the bulk or the weakly bound substrate on protein surface. Further, the ferroxidase activity of CueO also increased in the presence of 1 mM $CuSO_4$, indicating that the fifth Cu binding site of CueO functions not only as the binding site of Cu(I) as substrate but also as the binding site of Cu(II) as the mediator of electron transfer to effectively oxidize organic substances and Fe(II). In order to study these unique enzyme activities of CueO further in detail, we removed the α-helix covering the substrate binding site. Although the cuprous oxidase activity decreased to ca. 10% of that of the wild type CueO, the activities to oxidize organic substrates increased, and the substrate specificity of the mutant CueO became broad, indicating that the structural origin of CueO as cuprous oxidase is the presence of this shielding-helix. The structural similarity and difference of CueO as cuprous oxidase between ceruloplasmin and Fet3p as ferroxidase will be discussed.

大腸菌の銅排出系に関与するCu(I)酸化酵素CueO

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Lecture 38 Comparative study of behavior of bismuth and antimony administered intratracheally to rat

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Purpose: As bithmuth(Bi) and antimony(Sb) have similar properties to lead (Pb) physicochemically, they have been expected to be used as lead substitute in solder. But their toxicity by inhalation has not been clarified and more data are needed. In the present study, the behavior of Bi in rat administered once intratracheally with the particles was observed. Same experiment was also done with Sb. The distribution and retention of the particles in lung and time-dependent transfer to other organs or excretion were investigated, and the behavioral differences between Bi and Sb were discussed.

Experiment: Male SD rats were received single intratracheal administration of Bi particles (average diameter 1-2 micrometer) or Sb particles (average diameter 1-2 micrometer) suspended in 0.5% carboxymethylcellulose sodium solution. Doses of Bi were 0, 20, 100, or 500 mg/kg, and those of Sb were 0, 4, 20, or 100 mg/kg body weight. General state, body weights were checked for 2 weeks after administration. Five rats in each group were dissected at 2, 4, 8, and 15 days after administration. The concentrations of Bi or Sb in lung, liver, kidney, and spleen were determined by high-frequency plasma mass-spectrometry after microwave digestion. General hematology test of blood and general clinical test of serum were carried out.

Results and discussion: Bi concentrations in lung increased with dose and decreased with day after administration. Distribution amounts of Bi in lung showed individual differences, and corresponded to 30-60% of dose at 2nd day and 20-25% at 15th day. Bi concentrations in kidney, liver, and spleen also showed dose-dependency. In these 3 organs Bi concentrations were the highest in kidney and that at 15th day was higher than that in lung. Excretion in urine and feces also showed dose-dependent manner. First 2 to 4 days Bi was mainly excreted in feces and after that excretion amounts in urine increased suggesting that the excretion routes changed with days after administration. In the case of Sb, 85% rats of 100 mg/kg group died within 2 days after administration. The concentrations of Sb in lung were higher in 20 mg/kg group than that in 4 mg/kg group at 2 and 4 days after administration, however, the concentrations of these two groups were similar at 8th and 15th days. Sb concentrations in liver and kidney showed similar dose-dependency and time-dependency to those in lung. The concentration order of Sb in lung, liver, and kidney was same indicating that Sb in lung was rapidly transferred to other organs via blood flow. Excretion behavior of Sb in urine and feces are under investigation. Increase of relative lung weight was observed with both Bi and Sb administration. Pathological changes in lung were observed in 100 and 500 mg/kg groups of Bi and 4 and 20 mg/kg groups of Sb indicating much higher toxicity of Sb than Bi. These results suggested that Bi is suitable element for lead substitute in lead-free solder.

気管内投与したビスマスとアンチモンの体内挙動の比較

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Lecture 39 Changes of elements concentration and distribution in the liver and kidney of masked palm civet (Paguma larvata) infected with sarcoptic mange

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Introduction A sarcoptic mange is infectious dermatitis caused by mite parasitism in skin. Various infected animal species have been reported in Japan and other countries and its rampancy is concerned. A sharing living area with human and wild animals, a increase of contact opportunity with infected animals, and a suppression of immunity were considered as factors of its rampancy.

It is known that Zn play important role for immunity. Some elements such as Cu and Zn are involved in skin metabolisms. Therefore it may be suggested that element levels and/or distributions are changed in the infected animals.

And now various elements were emitted by human activity and then their concentrations were increased in urban area. Some elements such as Pb, As, Cd, Hg increase pathogen sensitivity. It is suspected that suppression of immunity in wild animals may be caused by environmental pollution. In this study, we attempt to reveal relationship among environmental pollution, changes of trace elements level/distribution and sarcoptic mange in masked palm civet.

Materials and methods 34 masked palm civet (*Paguma larvata*) were collected from Kamakura and Yokohama, Kanagawa. Their liver and kidney were used for chemical analysis. Sarcoptic manges were observed in 9 specimens in all. Severity of sarcoptic mange was divided into four groups by hair loss as follows; normal=no hair loss, light=partial hair loss, middle=half hair loss, heavy=generalized hair loss.

Liver and kidney samples were homogenized in 4 volumes of degassed 50mM Tris-HCl buffer solution containing 250mM glucose, pH 8.0 (25°C) under an atmosphere of nitrogen. Supernatant samples were prepared by ultracentrifuging the homogenates at 105,000×g for 60 min at 4°C. A 0.5 mL portion of the homogenate (whole tissue) and supernatant was wet-digested with 1.0mL HNO_3 by micro-wave oven. 19 elements concentrations were determined by the ICP-MS or CV-AAS. The distributions of Cu, Zn, Cd and Hg in the supernatants were determined on gel filtration HPLC column (Develosil 100Diol-5, Nomura Chemical) by eluted with 100mM ammonium acetate, pH 6.5 (25°C) at the flow rate of 1.0mL/min, with in-line detection with an ICP MS.

Result and discussion Compared concentrations date in normal animals with our past date of specimens collected from Tochigi Prefecture, significant higher concentration of hepatic V, Co, Ni, Zn, Se, As, Hg and renal V, Ni, Zn, As, Se, Hg in specimens from Kanagawa than Tochigi were obtained.

As severities of mange became serious, hepatic Cu, Zn concentrations were decreased. On the other hand, hepatic As and renal Ni, As, Se, Mo, Cd concentrations were increased. Moreover percentages of Cu and Zn bound to metallothionein (MT) in the hepatic and renal supernatants were decreased in affected animals. Cu and Zn are important elements for skin. It is known that MT play important role in storage, transfer and transport of Cu and Zn. It was suggested that stored Cu and Zn as MT binding form were used for skin protection and reproduction. Concentrations and distributions of Cu and Zn were disrupted in infected animals.

疥癬に感染したハクビシン(Paguma larvata)の肝臓と腎臓における元素濃度および分布の変化

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Lecture 40 Serum fluoride concentrations in healthy adult populations

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Purpose: To study serum fluoride concentrations of adult healthy persons by sex and ages.

Method: Adult participants were selected from two towns in Iwate prefecture using sex and age-specific stratified random sampling method. Participants of men and women were divided into three age groups (40's, 50's and 60's group), respectively. One hundred and sixty seven males and females were enrolled. Written informed consents were obtained from all subjects. Fasting blood samples were collected from all subjects. Serum samples were obtained by centrifuged and stored at -80 °C until measurements. Serum fluoride concentrations were determined by flow injection analysis with a fluoride ion-selective electrode. Tap waters prepared from 6 supplies were collected from two towns and fluoride concentrations were analyzed. Crude fluoride concentrations were compared between men and women or among age groups using Student-t test or ANOVA. Adjusted fluoride concentrations were also compared between the groups using ANCOVA.

Results: Fluoride concentrations of 6 tap waters ranged from 0.021 to 0.148 mg/L. The mean (standard deviation, SD) serum fluoride concentration of all subjects of men, 8.9 (1.4) μg/L, was significantly higher than that of women, 8.2 (1.4) μg/L. The mean serum fluoride concentration of each age group among men was 8.2 (1.4) μg/L in 40's, 9.3 (1.3) μg/L in 50's and 9.2 (1.4) μg/L in 60's, respectively. Concentrations were higher with an increment of age groups ($p<0.05$), but no differences were observed between age groups. Among women, serum fluoride concentrations were higher with an increment of age group (6.9 (1.4) μg/L in 40's, 8.5 (1.4) μg/L in 50's and 9.4 (1.3) μg/L in 60's; $p<0.001$). The concentrations of 40's were significantly lower than those of 50's and 60's among women ($p<0.05$). In a multiple regression analysis for the prediction of serum fluoride concentration, age and serum creatinine level were independently associated with serum fluoride concentrations among men. Age, creatinine level and fasting blood glucose level were independently associated with serum fluoride concentrations among women. Among women, serum fluoride concentrations in 40's group were significantly lower than other age groups after adjusting for creatinine level and fasting blood glucose level ($p<0.05$). The adjusted serum fluoride concentration (95% CI) of premenopausal women, 7.4 (6.4-8.3) μg/L, was significantly lower than that of postmenopausal women, 9.3 (8.7-9.8) μg/L ($p<0.01$).

Conclusion: Among women, the considerable difference in the serum fluoride concentrations between 40's group and other two groups or between premenopauseal women and postmenopausal women suggested that serum fluoride concentrations reflected bone metabolism in middle-aged women.

健常成人の血清中フッ素イオン濃度

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Lecture 41 The study for a effect of radiation-hormesis in Misasa spa

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Aim

It is described that having a spa every day is good for your health from effects of 3 viewpoint. The 3 viewpoints are effects of temperature, chemical reaction and low radiation. Then, we study the relation between cause and effect with by zinc and another many elements of hair in spa. Elemental concentration in hair was measured by nondestructive activation analysis.

Method

Hair was collected throughout the Misasa town office with answers to questionnaires to collected information on donors concerning occupation, conditions of residential areas, years of residence, eating habits, drug ingestion, a kind of used shampoo, date of the last permanent waving and hair coloring, history of dental treatment health condition, foods eating for 3 days and so on. Hair specimens which showed abnormal values, for example engineers using metals in occupation and people to be using shampoo to remove scurf, were excluded. Thus, 22 specimens which have a spa and take bath every day obtained from healthy 12 males and 10 women. The ages of them were from 29 years old to 82 years old. Hair was taken and washed by procedure recommended by IAEA. The hair specimen was irradiated in the pneumatic tube irradiation facility of the Kyoto University Research Reactor. Gamma-ray spectrometry of short-,middle- and long- lived nuclides was curried out with a 4096 channel pulse height analyzer equipped with pure Ge detector. Durations irradiation, cooling and measurement were combined appropriately. Concentrations were calculated in comparison with a value obtained from authentic samples. Then 23 elements in hair were quantitatively determined. Concentrations in taken minerals by health make program were calculated.
Radon concentrations in the each bath of 22 houses were measured by liquid scintillation counter.

Result and Discussion

Detected elements were Ag, Al, As, Au, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Na, S, Sb, Sc, Se, V and Zn. Detected minerals from nutrition in this region and national average were much the same. Elemental conc.in hair were treated in sexes and ages of 29~50 and 55~. Both man and female of high age group(55~) zinc conc.in hair were high value in comparison with normal value. It is suggested that have high immune activity.

三朝温泉のホルミシス効果に関する研究

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Lecture 42 Safety evaluation of inorganic antimicrobial agents (1)-Analysis of inorganic antimicrobial agents used in antimicrobial products-

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[Introduction]

With the recent increased interest in hygiene, antimicrobial products have been increasing. Antimicrobial agents used in antimicrobial products are classified into inorganic, organic, and natural organic compounds. Inorganic agents, such as Cu, Zn, and Ag compounds, are known to be relatively safe, and these agents are used in many kinds of products. Patients with metal allergy, and infants should avoid coming in contact with products in which metal compound agents are used at a high concentration, in particular regions treated with such agents at a high concentration. However, regions treated with metal compound agents and their concentration are not indicated in most commercially available products. In this study, to establish a simple method of measuring the concentration of metals in product regions, we evaluated screening methods using non-destructive X-ray fluorescence spectrometry (XFS). Qualitative analysis by XFS and quantitative analysis by inductively coupled plasma atomic emission spectroscopy (ICP-AES) were performed in the same regions, and the results were compared. Inorganic antimicrobial agents used in commercially available antimicrobial products were analyzed.

[Experimental]

(1) Samples: Forty antimicrobial products with which antimicrobial and deodorizing treatment and odor-neutralizing treatment were indicated were purchased, and 96 regions of the products were also analyzed. (2) XFS analysis: Cu, Zn, Ag, Ti, Fe, Cr, Ca, S, and Ni were examined. According to the CPS value, these metals were ranked qualitatively. (3) ICP-AES analysis: Samples were decomposed with acid by microwaves, and Cu, Zn, Ag, Al, Ni, Cr, Co, and Mg were analyzed.

[Results and discussion]

(1) XFS was useful for the screening of Cu and Zn. (2) XFS was not suited for the screening of Ag, but in the samples in which Ag was detected by ICP-AES analysis, Cu or Zn was detected at a higher concentration. Generally, Ag is not as a sole antimicrobial agent but together with Cu or Zn. Therefore, it is expected that Ag can be measured by quantitative ICP-AES analysis of regions in which Cu or Zn is detected. (3) Use of inorganic antimicrobial treatment was indicated on 11 products, but actually, 21 products were treated with inorganic antimicrobial agents. (4) Cu was detected in 6 products, and 5 products may have been treated with zeolite. (5) In 19 products, Zn was used for the antimicrobial treatment, and 18 products may have been treated with zeolite. (6) Ag was detected in 5 products, in which Al and Cu or Zn were contained at higher concentrations. The combination of Ag and Cu or Zn may enhance antimicrobial effects. (7) Inorganic antimicrobial agents were detected in 4 of the 13 products for infants. Although 2 products indicated the treatment with chitosan, Zn was detected. In these products, antimicrobial effects may have been obtained by the combination of chitosan and Zn or Zn alone. (8) Cr at a high concentration was detected in 3 products of socks.

無機系抗菌剤の安全性評価(1)－市販抗菌加工製品に使用されている無機系抗菌剤の分析－

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Lecture 43 Safety evaluation of inorganic antimicrobial agents (2)-Elution of metals with artificial sweat and saliva from antimicrobial agents and processed cloths-

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In Europe, the standard levels of heavy metals eluted with artificial sweat and saliva are specified in the self-imposed safety criteria (OEKOTEX Standard) for textile products. To establish standard values in Japan, we prepared metal zeolites (Ag, Cu, Zn, and Cr) and standard cloths processed with these zeolites and a silver antimicrobial agent, AG300. The agents, standard processed cloths, and regions of commercial textile products in which metals were detected at a high concentration were subjected to metal elution with artificial sweat(JIS L0848:2004), saliva(BS 6684 :1987), and ultrapure water according to the JIS shake-flask antimicrobial test method (JIS L1902-1900). The metal concentrations in the extracts were measured using an inductively coupled plasma-mass spectrometer (ICP-MS). A similar tendency was noted in elution from the agents, standard processed cloths, and commercial textiles. Small amounts of metals were eluted with water alone, while large amounts were eluted with artificial sweat and saliva. Large amounts of Cu and Zn were eluted, while the elution of Ag was low, and almost no Cr was eluted. The table shows the amounts of metals eluted from 1% processed cloths.
Furthermore, antimicrobial activity of the standard processed cloths was evaluated by the standard test (JIS1902) using *Staphylococcus aureus* and *Klebsiella pneumoniae*. Cu- and Ag (Ag zeolite and AG300)-processed cloths exhibited high antimicrobial activities against both bacteria. Zn-processed cloth also showed antimicrobial activity against *Staphylococcus aureus*.

Table 1 Elution of metals with artificial sweat and saliva from 1% processed cloths (μg)

No.	Type of processed cloth	Element	Acidic sweat	Alkaline sweat	Saliva	Water
1	Ag zeolite	Ag	2.12	8.95	6.21	1.26
		Cu	N.D.	N.D.	N.D.	N.D.
		Zn	N.D.	N.D.	N.D.	N.D.
		Cr	N.D.	N.D.	N.D.	N.D.
2	Cu zeolite	Ag	N.D.	N.D.	N.D.	N.D.
		Cu	514.95	353.09	530.70	N.D.
		Zn	N.D.	N.D.	N.D.	N.D.
		Cr	N.D.	N.D.	N.D.	N.D.
3	Zn zeolite	Ag	N.D.	N.D.	N.D.	N.D.
		Cu	N.D.	N.D.	N.D.	N.D.
		Zn	77.82	404.14	741.97	N.D.
		Cr	N.D.	N.D.	N.D.	N.D.
4	Cr zeolite	Ag	N.D.	N.D.	N.D.	N.D.
		Cu	N.D.	N.D.	N.D.	N.D.
		Zn	N.D.	N.D.	N.D.	N.D.
		Cr	N.D.	N.D.	3.37	N.D.
5	AG300	Ag	2.30	10.08	4.61	N.D.
		Cu	N.D.	N.D.	N.D.	N.D.
		Zn	N.D.	N.D.	N.D.	N.D.
		Cr	N.D.	N.D.	N.D.	N.D.

(N.D.: Less than 0.01 μg/g)

Eluant volume: 15 ml, sample amount: 0.75 g, elution time: 1 hour, temperature: room temperature
1% processed cloth: A standard processed cloth prepared by immersion of a cotton-added white cloth into a 100-times diluted aqueous solution of the original processing solution.
Original processing solution: Dispersion consisting of zeolite, binder, and water at a weight ratio of 2:8:90.
Zeolite: Crystalline mineral composed of aluminum, silicon, and sodium.
Antimicrobial zeolites: Zeolites added to antimicrobial metal ions, such as silver, copper, and zinc.

無機系抗菌剤の安全性評価(2)－人工汗・唾液による無機系抗菌剤及び加工布からの金属溶出－

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Lecture 44 Safety evaluation of inorganic antimicrobial agents (3)-Influences of inorganic antimicrobial agents on indigenous microorganisms on skin -

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The occurrence of mycosis due to an imbalance in indigenous microorganisms on the skin through the excessive use of antimicrobial processing agents is a matter of concern. We investigated the relationship between the amounts of metals eluted with artificial sweat and saliva from antimicrobial agents and processed cloths and their influences on indigenous microorganisms on skin. The relationships between 4 bacterial species (*Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis*, and *Propionibacterium acnes*) and 3 fungal species (*Candida albicans, Trichophyton mentagrophytes*, and *Aspergillus niger van Tieghem*), the metal concentrations in artificial sweat and saliva, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were investigated.

(1) The MIC values against (*Candida* and *Trichophyton* were similar to the bacteria, and were lower than that against *Aspergillus*.

(2) Table shows the MBC values against the bacteria and fungi exposed to metal ions in acidic and alkaline artificial sweat solutions and artificial saliva for 24 hours. Although survival rates varied among the microorganisms, fungi survived at a higher metal concentration. In the MBC measurement, microorganisms were exposed to metal ions for a specified time (2 or 24 hours), and then their survival was investigated by culture under conditions appropriate for each microorganism. Since the conditions were close to those of exposure of coexisting microorganisms on skin, these findings suggest that fungi survive at a metal concentration that kills bacteria, up setting the balance of indigenous microorganisms naturally present on skin, which may cause mycosis.

Table 1. Minimum bactericidal concentration (MBC) for 24-hour exposure in artificial saliva

Bacterium	Ag	Cu	Zn	Cr
Staphylococcus aureus	50mg/L	12.5mg/L	400mg/L	100mg/L
Escherichia coli	50mg/L	50mg/L	400mg/L	100mg/L
Staphylococcus epidermidis	12.5mg/L	50mg/L	400mg/L	400mg/L
Propionibacterium acnes	50mg/L	100mg/L	800mg/L	50mg/L
Fungus	Ag	Cu	Zn	Cr
Candida	800mg/L	200mg/L	Over 800mg/L	Over 800mg/L
Trichophyton mentagrophytes	200mg/L	Over 800mg/L	Over 800mg/L	Over 800mg/L
Aspergillus niger	Over 800mg/L	Over 800mg/L	Over 800mg/L	Over 800mg/L

Table 2. Minimum bactericidal concentration (MBC) for 24-hour exposure in artificial acidic sweat

Bacterium	Ag	Cu	Zn	Cr
Staphylococcus aureus	400mg/L	400mg/L	Over 800mg/L	800mg/L
Escherichia coli	200mg/L	400mg/L	800mg/L	800mg/L
Staphylococcus epidermidis	100mg/L	400mg/L	Over 800mg/L	800mg/L
Propionibacterium acnes	100mg/L	50mg/L	800mg/L	25mg/L
Fungus	Ag	Cu	Zn	Cr
Candida	Over 800mg/L	400mg/L	Over 800mg/L	Over 800mg/L
Trichophyton mentagrophytes	100mg/L	800mg/L	Over 800mg/L	Over 800mg/L
Aspergillus niger	Over 800mg/L	Over 800mg/L	Over 800mg/L	Over 800mg/L

Table 3. Minimum bactericidal concentration (MBC) for 24-hour exposure in artificial alkaline sweat

Bacterium	Ag	Cu	Zn	Cr
Staphylococcus aureus	50mg/L	800mg/L	Over 800mg/L	800mg/L
Escherichia coli	50mg/L	400mg/L	Over 800mg/L	Over 800mg/L
Staphylococcus epidermidis	50mg/L	400mg/L	Over 800mg/L	Over 800mg/L
Propionibacterium acnes	Under 6.25mg/L	800mg/L	800mg/L	400mg/L
Fungus	Ag	Cu	Zn	Cr
Candida	800mg/L	800mg/L	Over 800mg/L	Over 800mg/L
Trichophyton mentagrophytes	200mg/L	Over 800mg/L	Over 800mg/L	Over 800mg/L
Aspergillus niger	Over 800mg/L	Over 800mg/L	Over 800mg/L	Over 800mg/L

無機系抗菌剤の安全性評価(3)－無機系抗菌剤が皮膚常在菌に及ぼす影響－

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Lecture 45 Antioxidant activities of synthesized selenocompounds without selenol groups

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Introduction Antioxidant activity is an important function of selenium. Though selenol may be the most suitable chemical form for this activity, selenocompounds without selenol group may also show the activity. In this study, low-molecular-weight selenocompounds without selenol group, N-methyl, N-phenyl-(2-selenomethyl) benzoylamide, N-phenyl-(2-selenoallyl)benzoylamide, N-phenyl-(2-selenopropyl)benzoylamide, and bis[1-methyl-2-(N-phenylcarboxamido)ethyl] diselenide, dibenzyl diselenide, were synthesized and their antioxidant activities were evaluated.

Materials and Methods Reagents used for the synthesis of selenocompounds were reagent grade. Ebselen was a gift from Daiichi Seiyaku Co., Ltd.. 2-methyl-6-p-methoxyphenylethinyl-imidazopyrazinone (MPEC), a chemiluminescence probe, was purchased from ATTO Co.. In the assay of antioxidant activities of selenocompounds, they were dissolved in DMSO, and diluted with water or assay buffer to the concentrations studied. Glutathione peroxidase(GPx)-like activity was measured by a spectrophotometric method using hydrogen peroxide as a substrate. Superoxide anion scavenging activity of these compounds was measured by a chemiluminescent method. Superoxide anion was produced by hypoxanthine-xanthine oxidase system, and the chemiluminescence derived from superoxide anion was measured with MPEC and Type AB-2200 Luminescencer PSN(ATTO Co.). Superoxide anion scavenging activity was represented by EC50, the concentration reducing the chemiluminescence to 50% of the control.

Results and Discussion Synthesized selenocompounds showed low GPx-like activity though ebselen and diphenyl diselenide, which can be coverted to selenol compounds in the reaction mixture, had higher activity. The activity at 20μM of N-phenyl-(2-selenoallyl)benzoylamide, which was about 1/10 of ebselen, was the highest among the synthesized compounds, and the activity increased depending on the concentration. The activity of N-phenyl-(2-selenoallyl)benzoylamide was several times higher than that of selenomethionine. Methionine did not show the activity even at 160μM. These results suggest that selenocompounds which don't have selenol group can also behave as a GPx-like reductant and another reaction mechanism other than that for the compounds with selenol group is present. Spectrophotometric studies also supported this suggestion. It may be possible that selenium atom located between two carbon atoms in the molecule may be oxidized to selenoxide form and the selenoxide can be reduced to selenium by reduced form of glutathione, thus GPx-like reduction of hydrogen peroxide by non-selenol compounds occurs. N-phenyl-(2-selenoallyl)benzoylamide had comparatively low superoxide anion scavenging activity, about 1/6 activity of ascorbic acid although ebselen had 4 times higher.

These results indicated that selenocompounds without selenol group such as N-phenyl-(2-selenoallyl) benzoylamide can be reductants against peroxide or other oxidant species by forming selenoxide, though their antioxidant activity may not be high.

セレノール基をもたない合成セレン化合物の抗酸化活性

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Lecture 46 Normalizing effect of zinc(II) complex with 6-ethoxy picolinic acid on blood glucose levels in KK-A^y mice

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We examined normalizing effect for blood glucose levels of zinc(II) complex with 6-ethoxy picolinic acid ($Zn(6\text{-}EtOpa)_2$) on KK-A^y mice which used in this study as the model animals of type 2 diabetes, and compared with its ligand, 6-ethoxy picolinic acid (6-EtOpa).

These mice were divided into three groups after the onset of diabetes at 8 weeks of age, which were an untreated group, a 6-EtOpa treated group and a $Zn(6\text{-}EtOpa)_2$ treated group. They were given daily intraperitoneal (i.p.) injections of each material for a period of 2 weeks from 8th to 10th week of ages. The dose of $Zn(6\text{-}EtOpa)_2$ was 3 mg Zn/kg of body weight. Each animal was examined for its body weight, food intake, blood glucose levels, blood glucose tolerance, and HbA1c levels, hematological status, organ weight, zinc and copper concentrations in the organs. After 2 weeks of administration the blood glucose level of KK-A^y mice in $Zn(6\text{-}EtOpa)_2$ treated group showed a significant decrease compared to both the 6-EtOpa and control groups. The mice treated with $Zn(6\text{-}EtOpa)_2$ showed significant decrease in HbA1c levels and significantly lower level of blood glucose compared to the control group after 30 minutes of time in an oral glucose tolerance test. In the weight of the organs (g/100g body weight), the spleen resulted in significant increase in a $Zn(6\text{-}EtOpa)_2$ treated group compared with 6-EtOpa group, and the weight of the abdominal adipose tissue showed a low value significantly in a $Zn(6\text{-}EtOpa)_2$ treated group compared with 6-EtOpa and control groups. Zinc concentration in the femur was significantly risen in the $Zn(6\text{-}EtOpa)_2$ treated group compared to the both 6-EtOpa and control groups. Copper concentration was decreased significantly in both of femur and femoris muscle. These results indicate the possibility of normalizing effects for blood glucose disorder in diabetes mellitus by treating with $Zn(6\text{-}EtOpa)_2$.

2型糖尿病モデルマウスKK-A^{y}におけるエトキシピコリン酸亜鉛(II)錯体の血糖上昇抑制作用

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Lecture 47 A study on the mechanism relating to the inhibition of lipid peroxidation by copper histidine complex and Cu, Zn-superoxide dismutase activity in blood

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Background: Lipid peroxide is formed by lipid oxidation which occurs when reactive oxygen species (ROS) oxidize the lipid under copper catalysis. In the human body, the phenomena of lipid peroxide formation and suppression were simultaneously performed and observed. Role of Copper in catalytic oxidation and its contribution to the reduction of ROS by Cu, Zn-superoxide dismutase (Cu, Zn-SOD) is unclear.
Objectives: The purpose of this study was to identify a mechanism for the inhibition of lipid peroxide formation by copper amino acid complex, and for the reduction of ROS by Cu, Zn-SOD dismutase in blood.
Methods: The concentrations of copper, lipid peroxide, amino acids, and lipids in sera of twenty seven young women were determined. Different contributions for lipid peroxidation in amino acids of copper-amino acid complex were examined in vitro. Before and after vigorous exercise, the changes in the levels of copper concentration in serum and the erythrocytes of another seventeen young women were determined. The relationships among the change in levels, the Cu, Zn-SOD activity and the concentration of lipid peroxide were examined.
Results and Discussion: 1. In the case of high proportion of histidine concentration in serum, copper becomes a copper-histidine complex. On the other hand, if the proportion of histidine is low in serum, the copper becomes a complex with other amino acids.Data also suggests that an increase in copper-histidine complex inhibits the formation of peroxidized lipids in serum. 2. It was clarified that the copper concentration in serum and erythrocytes is higher after vigorous exercise than it is before. The copper and ROS in serum enter the erythrocytes and copper binding with SOD apoprotein increases, which reduces ROS activity. Therefore, this mechanism inhibits the formation of lipid peroxide in serum.
Conclusions: Copper and histidine in serum make a copper-histidine complex. This complex formation prevents the copper-catalyzed oxidation activity and inhibits the formation of lipid peroxide in serum. After vigorous exercise, the concentration of copper in serum increases. This copper enters the erythrocytes. The subsequent increase in copper binding with SOD apoprotein reduces ROS activity. This increase also helps to reduce the ROS generated during exercise. The result is suppressed lipid peroxide formation.

血液中銅・ヒスチジン錯体及び Cu,Zn-Superoxide Dismutase による過酸化脂質生成の抑制

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Lecture 48 Alleviation by long-chain fatty acids and their salts of inhibition by oxalates of a copper enzyme, tyrosinase

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It is known that in many lower animals, plants and microorganisms, tyrosinase is normally contained as its latent form, and activeted, in the presence of stress, by some agents including long-chain fatty acids, to produce melanin-type pigments in a defence process. It has also been revealed that oxalic acid and its salts (oxalates) non-competitively inhibit the tyrosinase activity due to formation of chelate linkage with functional copper in the enzyme active site (Hiraoka, A., BRTE, 16, 46, 2005). In this study, we investigated the effects of various saturated and unsaturated long-chain fatty acids with carbon numbers of 14-22 and their salts on the mushroom tyrosinase activity inhibited by oxalates. The tyrosinase inhibition by oxalates was significantly alleviated by addition to the reaction mixtures of the examined compounds. Their allviation effects were concentration-dependent within the ranges causing no turbidity in the reaction mixtures, and such reactivation effects by the more hydrophillic substances (i.e. polyunsaturated acids as the free fatty acids and triethanolamine salts as the salts) were the more remarkable due to the fact that they can be applied at the higher concentration. Similar results were also obtained employing EDTA-Na and citric acid instead oxalates as tyrosinase inhibitors. These results suggest that in many living organisms, long-chain fatty acids, especially polyunsaturated ones, released from the membranes as the stress response may participate the activation procedures of tyrosinase by attaching the enzyme molecules to remove endogenous inhibitor(s) which normally suppress(es) the enzyme activity by formation of chelate linkage with the functional copper.

シュウ酸による銅含有酵素チロシナーゼの阻害に及ぼす長鎖脂肪酸及びその塩類の緩和効果の検討

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Lecture 49 Effects of Hydroxy-monoazine- and -diazine-type Heterocycles on Removal of Depleted Uranium in Rats

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Uranium is a radionuclide, which causes severe renal dysfunction within a short time period due to chemical toxicity. It may also induces cancers such as leukemia and osteosarcoma in cases of long-term internal radiation exposure. The chelation therapy is an optimal method for accelerating the excretion of depleted uranium (DU) from the body. Although some experimental agents, such as EHBP, CBMIDA, and 3,4,3-LIHOPO, have efficacy to remove DU, they are still present in the experimental stage. Under these circumstances, the development of the chelating agent, that has an efficacy to remove DU as much and quickly as possible and with no toxic side effect after DU contamination and can use it for long term not to increase DU-induced risk, has been desired. We would like to describe here the synthesis of four heterocyclic compounds and effects of them on removal of DU in rats.

The following four chelating agents, AK-1, AK-2, AK-3 and AK-4, were synthesized. Male 8 week-old rats were pre-injected intramuscularly with 8 mg/kg of DU in aqueous nitric solution, and then divided into five groups. The rats of four groups were injected intraperitonealy with chelating agents, 50 times mol of DU, for three days. The following points became clear; 1) AK-4 showed significant DU excretion after one day of the injection as shown in Fig. 2 although total amount of DU excreted in urine and feces was almost same among chelating agents, 2) AK-4 showed the smallest weight loss among chelating agents during six days, and 3) judging from serum biological data such as creatinine and BUN, AK-4 showed the alleviation effect of renal dysfunction.

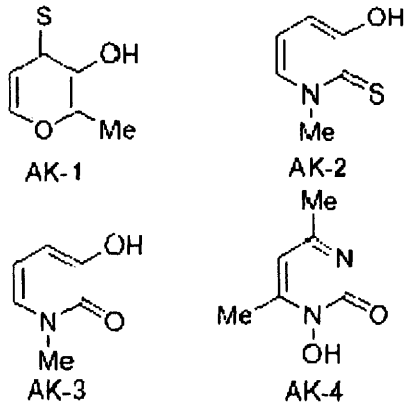

Fig.1 Hydroxy-monoazine- and -diazine-type heterocyclic compounds

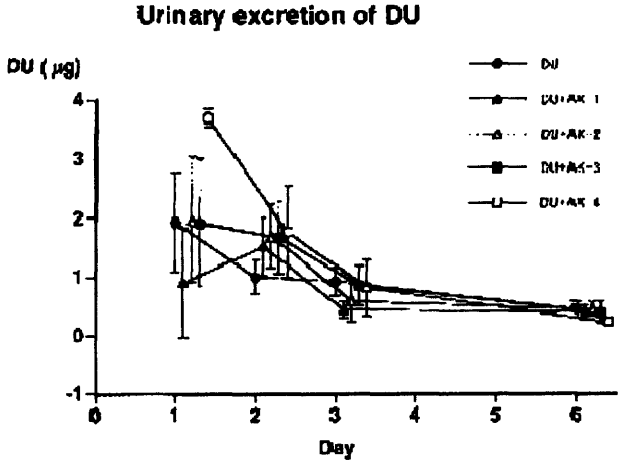

Fig. 2

ヒドロキシモノアジン及びジアジン型複素環化合物のラットにおける劣化ウラン除去効果

○加藤 明良[1]、池田 瑞代[2]、中村 麻利子[2]、福田 俊[2]

[1]成蹊大学 理工学部 物質生命理工学科、[2]放射線総合医学研究所緊急被ばく医療研究センター

Lecture 50 Aggregation of recombinant PrP provoked by coexistence of nicotinamide adenine dinucleotide coenzyme and metal

○ Noriyuki SHIRAISHI , Morimitsu NISHIKIMI

Department of Biochemistry, Wakayama Medical University, Wakayama, Japan

Prion protein (PrP) consists of an amino-terminal domain containing a series of octapeptide repeats with the consensus sequence PHGGGWGQ and a carboxyl-terminal domain composed of three alpha-helices and two short beta-strands. During the process of infection, its carboxyl-terminal region re-folds into protease-resistant, beta-sheet-enriched aggregates. The amino-terminal region of PrP is highly flexible, and its octapeptide repeats has recently been implicated in prion initiation. Here we found that PrP_{23-98}, an amino-terminal part of PrP, also aggregates in the presence of NADPH plus certain metals. Aggregation of PrP_{23-98} as assessed by turbidometry (550 nm) was provoked by incubation with Cu^{2+} plus by addition of NADPH, 3'-NADPH (analog of NADPH) or NADP (high concentration) but not NADH or NAD. Zn^{2+}, Cd^{2+}, and Ni^{2+} were effective besides Cu^{2+} whereas Al^{3+}, Ca^{2+}, Co^{2+}, Mn^{2+}, and Sn^{2+} were without effect. The reaction was dependent on the concentration of nicotinamide adenine dinucleotide coenzyme and retarded by metal chelators. In addition the aggregation was attenuated by treatment of PrP_{23-98} with diethylpyrocarbonate (modifier of histidyl residues). The aggregate formed seemed to be amyloid fibrils as judged by binding of thioflavin T as fluorescent probes for amyloid formation. Also the aggregates formed in the presence of Cu^{2+} acquired proteinase K resistance but those formed in the presence of Zn^{2+} did not.

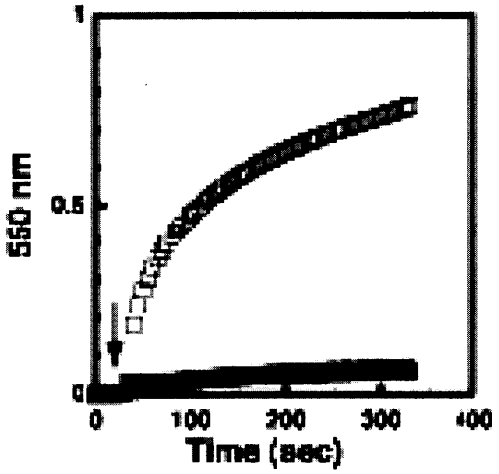

Fig. 1. Aggregation of PrP_{23-98} as assessed by turbidometry (550 nm). The arrow indicates the addition of NADPH.
○, Copper; ●, NADPH;
□, Copper + NADPH;
■, Copper + NADPH + EDTA

ニコチンアミド補酵素と金属の共存下で誘導されるプリオンタンパク質の凝集

○白石 則之、錦見 盛光
和歌山県立医科大学 生化学

Lecture 51 Copper binding of nuclear RNA- and DNA-binding proteins p54^{nrb}/NonO and PSF

○ Masaki WATANABE , Masakatsu TEZUKA

Department of Health Science, College of Pharmacy, Nihon University, Chiba, Japan

p54^{nrb}/NonO and PSF are nuclear RNA- and DNA-binding proteins that exhibit multiple nuclear functions such as splicing and transcriptional regulation. These proteins are known to exist as dimers in mammalian cells. Previously, using immobilized metal affinity chromatography with Cu(II) ion, we have shown that these proteins exhibit Cu binding properties and identified that the N-terminal histidine-rich region of p54^{nrb}/NonO is the copper binding site. In the first experiment of the present study, we investigated whether the p54^{nrb}/NonO and PSF proteins isolated from cultured cells bind to copper by using size exclusion and ion exchange chromatography with ICP-MS detection. Nuclear protein extracts in HEK293 cells were subjected to size exclusion fast protein liquid chromatography (FPLC) using a Superdex 200 column. Each fraction was separated by SDS-PAGE and p54^{nrb}/NonO, and PSF was detected by Western blotting. The amount of Cu in each fraction was determined by ICP-MS at m/z = 63 and 65. We observed that both p54^{nrb}/NonO and PSF eluted in high molecular weight fractions, which is consistent with the fact that these proteins form a large protein complex with other nuclear proteins. As expected, the majority of Cu was detected in the fractions containing p54^{nrb}/NonO and PSF. Subsequently, these fractions were combined and subjected to anion exchange chromatography (UNOsphere Q). The proteins were eluted separately using a stepwise gradient with acetate chloride. In the Western blot analysis, both p54^{nrb}/NonO and PSF were eluted in the flowthrough fraction. ICP-MS analysis then showed that Cu was detected only in the flowthrough fraction. Moreover, the fraction was separated by SDS-PAGE and stained with Coomassie brilliant blue, indicating that p54^{nrb}/NonO and PSF were well purified by the chromatographic method. These results suggest that Cu binds to endogenous p54^{nrb}/NonO and PSF in the nucleus of HEK293 cells. In the second experiment, to examine whether the copper binding affinity of p54^{nrb}/NonO could be determined by Scatchard plot analysis, we generated p54^{nrb}/NonO recombinant protein using the *E. coli* expression system. The recombinant protein (0.1 μM) was incubated with varying concentrations of $CuSO_4$ (2.5-40 μM) for 20 min at room temperature. The mixtures were placed in ultrafiltration devices and centrifuged at 10,000 × g for 40 min. Copper concentration in the filtered solutions was measured by ICP-MS. Scatchard plot analysis revealed that the dissociation constant was 0.13 μM and the Cu binding number was 2.5 atoms per molecule of the recombinant p54^{nrb}/NonO protein. Moreover, using the ultrafiltration technique, we confirmed that Zn did not bind specifically to the protein and did not competitively inhibit the binding of Cu to the protein. In conclusion, although the function and binding protein of intranuclear Cu in mammalian cells has not yet been clarified, our result strongly suggests that p54^{nrb}/NonO and PSF are nuclear Cu binding proteins.

核内 RNA/DNA 結合タンパク質 p54^{nrb}/NonO の銅結合性の解析

○渡辺 雅紀、手塚 雅勝
日本大学 薬学部 衛生化学研究室

Lecture 52 Detection of metallothionein isoforms in isoform-specific knock down cells by nano-speciation using 2D capillary HPLC-ICP-MS

○ Takamitsu MIYAYAMA , Yasumitsu OGURA , Kazuo SUZUKI

Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

[Introduction] Two major metallothionein (MT) isoforms, MT-I and MT-II, are ubiquitously expressed in all tissues and cell types. Because of their high homology, however, those two MT isoforms cannot be discriminated by analytical techniques that use an antibody. A gene knock-out mouse is one of the most powerful tools to elucidate protein function. However, the two isoforms in an MT knock-out mouse are defective simultaneously in the two currently available strains. In the present study, MT-I and MT-II genes were specifically knocked down with short interfering RNA (siRNA). Then, the effects of the siRNAs were evaluated by RT-PCR and two-dimensional (2D) capillary HPLC coupled with inductively coupled plasma mass spectrometry (ICP-MS). To enhance the sensitivity of ICP-MS to the metal bound to MT extracted from gene-modified cells, a low volume spray chamber with sheath flow was newly designed and an enriched stable isotope was used.
[Methods] Mouse hepatoma (Hepa 1-6) cells were transfected with or without siRNA targeting MT-I or MT-II, and exposed to ^{113}Cd-enriched $Cd(CH_3COO)_2$ for 1 h after the transfection. Then, the cells were harvested and subjected to RT-PCR and 2D capillary HPLC-ICP-MS. A 100 nL portion of the supernatant of cell lysate was applied to 2D (gel filtration plus anion exchange) capillary HPLC-ICP-MS.
[Results and Discussion] The fluorescence intensities of the RT-PCR products of MT-I and MT-II mRNAs were increased to 3.2- and 9.0-fold, respectively, by exposure to Cd. MT-I expression was decreased to 0.48-fold of the control without the Cd treatment by transfection with MT-I specific siRNA, suggesting that the siRNA interfered not only with the induction of MT-I but also with the basal expression of MT-I. In contrast, MT-II expression was marginally decreased by transfection with the MT-I specific siRNA. The transfection with MT-II specific siRNA gave similar results to that with MT-I siRNA, i.e., MT-II expression was decreased to 0.78-fold of the control. MT-I or MT-II protein expression, which was estimated by measuring ^{113}Cd intensity, was also decreased by the isoform-specific siRNAs, demonstrating the advantage of 2D capillary HPLC-ICP-MS over RT-PCR for the direct comparison of protein expression of the two isoforms.
[Conclusion] The isoform-specific knock down of MT was estimated by RT-PCR and 2D capillary HPLC-ICP-MS. Although the knock down of each isoform was not completely specific owing to the high homology of their sequences, 2D capillary HPLC-ICP-MS was shown to be effective and useful to separate MT isoforms prepared from cultured cells. In this system, the minimum requirement of cell number was calculated to be 2.0×10^3 cells. The combination of nano-speciation and RNAi technology may contribute to clarifying the physiological and/or biological roles of MT isoforms.

2D capillary HPLC-ICP-MSを用いたナノスペシエーションによるアイソフォーム特異的メタロチオネインノックダウン細胞中のメタロチオネインの検出

○宮山 貴光、小椋 康光、鈴木 和夫
千葉大学大学院 薬学研究院

Lecture 53 Direct determination of ceruloplasmin in human blood serum by HPLC-inductively coupled plasma-mass spectrometry for diagnose of Wilson disease

○ Kenji KOBAYASHI[1] , Yoko KATSUYA[1] , Rizky ABDULAH[1] , Chie FUJISAWA[2] , Takeaki NAGAMINE[3] , Akihiro MORIKAWA[4] , Masami MURAKAMI[5] , Hiroko KODAMA[2] , Hiroshi KOYAMA[1]

[1]Department of Public Health, Gunma University Graduate School of Medicine, Gunma, Japan , [2]Department of Pediatrics, Teikyo University School of Medicine, Tokyo, Japan , [3]School of health Science, Gunma University, Gunma, Japan , [4]Department of Pediatrics & Developmental Medicine, Gunma University Graduate School of Medicine, Gunma, Japan , [5]Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Gunma, Japan

[Introduction] Wilson disease (WD) is an autosomal recessive disorder of copper (Cu) metabolism which is caused by mutations in the *ATP7B* gene. WD is treatable, the delay of its diagnosis makes the treatment difficult. The establishment of early diagnosis is a very important for early treatment. 95% of plasma Cu is bound to ceruloplasmin (Cp) and excreted into the bloodstream in the form of hole-Cp in normal subjects. In the case of WD patients, it is excreted into the bloodstream in the form of apo-Cp. In the present study, we analyzed Cu distribution in the serum of WD patient by HPLC-inductively coupled plasma-mass spectrometry (ICP-MS). The goal of this works is routine diagnosis of WD in a clinical site. **[Methods]** Blood was collected with informed consent. After separation of blood cell, the serum was freeze-stored until analysis. Serum samples were filtered through a 0.45 μm membrane filter before analysis. The distribution of Cu in the serum were determined by gel filtration HPLC columns with 50mM Tris-HCl buffer (pH 7.4), with ICP-MS. A healthy human blood was used as a normal control. **[Results]** Figure shows the signal profile obtained from a HPLC-ICP-MS analysis in serum. Cp was detected as a Cu peak at a retention time of 12.2 min for the serum of control (upper). This results show that hole-Cp in the serum can detect using this method. In the case of two WD patients, no significant Cu peak was detected around a retention time of 12.2 min (middle and lower). This result indicated that the hole-Cp was not detected in the serum of WD patients. We reported here that the determination of Cp by HPLC-ICP-MS in human serum is a new tool for diagnosis of WD.

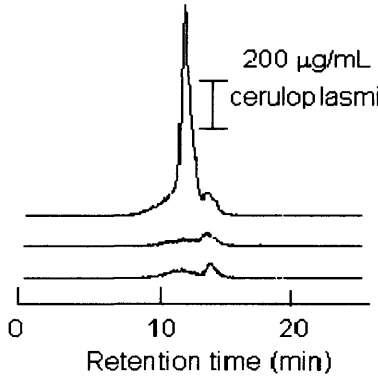

Figure HPLC-inductively coupled plasma-mass spectrometry signal profile of m/z 65 as copper in the serum of normal control (upper) and Wilson disease patients (middle and lower).

HPLC-誘導結合プラズマ質量分析による血清中セルロプラスミンの検出と Wilson 病診断への応用

○小林 健司[1]、勝矢 陽子[1]、アブドゥラ リズキー[1]、藤澤 千恵[2]、長嶺 竹明[3]、森川 昭廣[4]、村上 正巳[5]、児玉 浩子[2]、小山 洋[1]

[1]群馬大学 大学院 医学系研究科 生態情報学、[2]帝京大学 医学部 小児科、[3]群馬大学 医学部 保健学科、[4]群馬大学 大学院 医学系研究科 小児生体防御学、[5]群馬大学 大学院 医学系研究科 病態検査医学

Lecture 54 Determination of Fluoride Ion Concentrations by Flow Injection Method in Culture Medium Supplemented with NaF

○Tsutomu SATO[1] , Tomoko TANAKA[1] , Ken YAEGAKI[1] , Kazuyoshi ITAI[2]

[1]Dept. of Oral Health, School of Life Dentistry at Tokyo, Nippon Dental University, Tokyo, Japan , [2]Department of Hygine and Public Health, School of Medcine, Iwate Medical University, Morioka, Japan

Abstract: The biological effects of fluoride (F) have been studied using a cell culture system for past few decades, a physiological concentration of F ion in culture medium supplemented with NaF was not clearly determined. The purpose of this study was to measure F ion concentration in the culture medium by the flow injection method. F ion concentrations in both a purified water (resistance value>17Mohm·cm) and a culture medium were below the limit of sensitivity (0.3μg/L) of this method. F ion concentrations in five fetal calf sera were ranged from 6.23 -8.44μg/L. The F solutions of 5-500μg F/L adjusted with NaF were found to be 1-2% less than estimated concentrations. F ion concentration in the culture medium supplemented both with various concentrations of F including the physiological F concentration and with 10%(v/v) calf serum were between 75-78% of the final concentrations of F ion. The concentrations were not changed for 60 minutes after F addition. The F ion concentrations in the fetal calf serum were between 25-43% of the final concentration of F ion. The reduction rates of F ion concentration were increased with the concentration of the supplied F ion.

Conclusion: A physiological concentration of F ion was measured with the flow injection method, and it was found that F ion was readily bound to a certain component of serum.

フローインジェクション法によるフッ化ナトリウム添加培養液中のフッ素イオン濃度の検討

○佐藤 勉[1]、田中 とも子[1]、八重垣 健[1]、板井 一好[2]

[1]日本歯科大学 生命歯学部 衛生学講座、[2]岩手医科大学 医学部 衛生学公衆衛生学講座

Lecture 55 Metal contaminants in evacuated blood-collection tube used for trace metal assays

○Kenji KAWASAKI[1] , Mitsutoshi SUGANO[1] , Shozo NOMOTO[2]

[1]Department of Laboratory Medicine, Shinshu University Hospital, Nagano, Japan , [2]Department of Laboratory Medicine, Shinshu University School of Medicine, Nagano, Japan

Objective: To confirm their suitability for use in metal analysis, contaminants in 83 evacuated blood-collection tubes used widely for blood collection in laboratory medicine were quantitatively checked with a newly developed automated colorimetric method for zinc in serum and conventional automated methods for copper and iron in serum.

Method: The tubes were filled with serum, saline or 3 mmol/l hydrochloric acid solution to allow contact of the inner surface with the solution, and turned upside down 5 times during a 2-6 hour period. Then each contained solution was subjected to metal analysis.

Results: These are shown in Table 1.

Conclusion: It is essential to check for metal contaminants in tubes used for blood samples before undertaking any experiments in either fundamental or clinical biochemistry involving trace metals, especially zinc. The present survey indicated that Tube-4: Telmo VP-P052K, Tube-5: Telmo VP-AS073K, Tube-8: Nipro NP-SP0512, Tube-9: Nipro NP-PS0205, Tube-3: Becton D 369737(for trace metal), Tube-6: Sekisui SIM-N0705SQ, Tube-7: Sekisui SI-0707P are suitable for use in trace metal analysis.

Table-1 A magnitude of contaminants level expressed as an additive-concentration be expected

	Tube-1	Tube-2	Tube-3	Tube-4	Tube-5	Tube-6	Tube-7	Tube-8	Tube-9
Zinc									
Mean rise conc. (ug/L)	263	252	2	0.2	0.3	6	7	0.3	0.2
Standerd devia. (ug/L)	162	129	5	3	2	5	8	5	3
Copper									
Mean rise conc. (ug/L)	13	10	4	3	2	4	5	4	0
Standerd devia. (ug/L)	6	4	6	3	5	4	5	1	9
Iron									
Mean rise conc. (ug/L)	0.3	5	2	6	6	0	1	0.3	0.2
Standerd devia. (ug/L)	2	3	1	3	6	2	8	6	7

Tube-1 : Becton D, 367520, Tube-2 : Becton D, 368972, Tube-3 : Becton D, 369737(for trace metal)
Tube-4 : Telmo, VP-P052K, Tube- Tube-5 : Telmo, VP-AS073K, Tube-6 : Sekisui, SIM-N0705SQ
Tube-7 : Sekisui, SI-0707P, Tube-8 : Nipro, NP-SP0512 Tube-9 : Nipro, NP-PS0205

微量金属測定用真空採血管内の汚染金属

○川崎 健司[1]、菅野 光俊[1]、野本 昭三[2]
[1]信州大学病院 中央検査部、[2]信州大学 医学部 病態解析診断学講座

The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

第 17 回日本微量元素学会
プログラム・抄録集

会長　荒川　泰昭
（静岡県立大学教授）

会期: 2006 年 7 月 12 日（水）-14 日（金）

会場: グランシップ・静岡
〒422－8005 静岡市駿河区池田 79-4

主催: 日本微量元素学会

共催: 日本衛生学会、日本産業衛生学会、日本化学会、
日本分析化学会、日本薬学会、日本トキシコロジー学会、
日本免疫毒性学会、日本微量栄養素研究会、
文部科学省 21 世紀 COE プログラム推進拠点、
静岡総合研究機構

後援: （財）日本健康・栄養食品協会

連絡先: 第 17 回 日本微量元素学会 事務局
静岡市駿河区谷田 52-1（〒422-8526）
静岡県立大学・公衆衛生学・生体衛生学研究室内
担当: 栗山 孝雄
TEL&FAX: 054-264-5563
学術大会専用 URL: http://brte2006.umin.ne.jp
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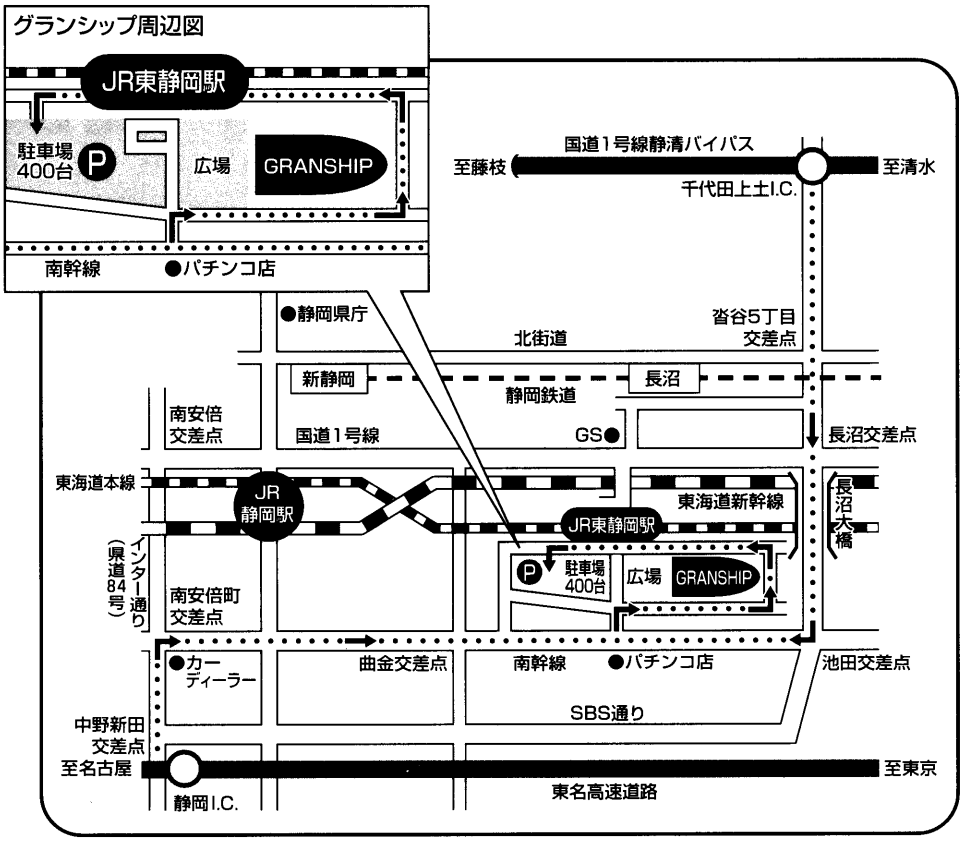
会場へのアクセス
Access to the Place of Meeting

◎**電車**‥‥新幹線（ひかり）で東京から1時間、大阪から2時間。静岡駅下車。
東海道本線（上り）へ乗換え、3分。東静岡駅下車。
東静岡駅南口、東側に隣接。

◎**車**‥‥‥東名高速静岡I.Cから20分。静清バイパス千代田上土I.Cから10分。



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グランシップ利用者＝1台につき1時間までごとに ……	100円
上記以外の方＝1台につき1時間までごとに ……	200円

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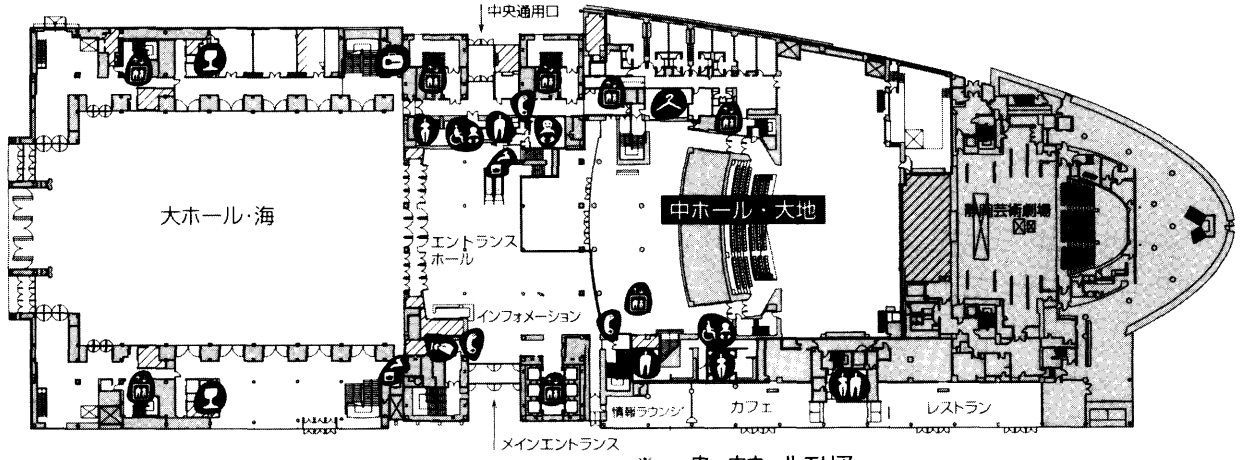
会場案内図
The Place of Meeting
—1—

■ 中ホール・大地（第1会場）
Medium Hall・Earth (Room 1)

1F全体配置図

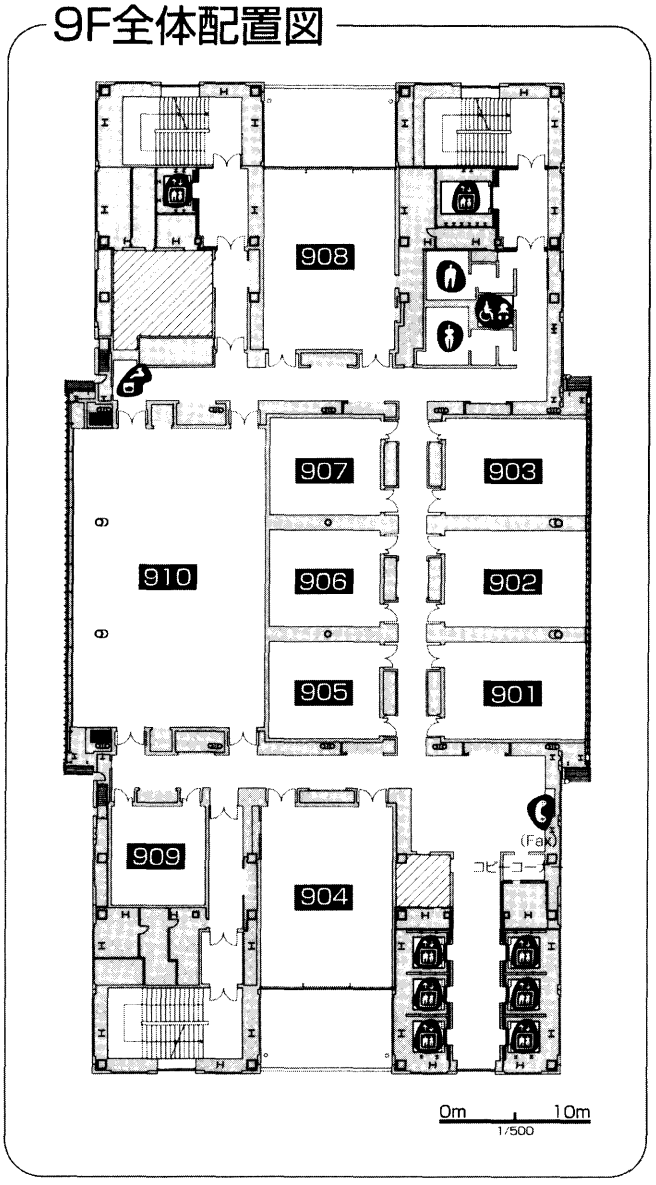


多目的トイレ　トイレ女　給湯室　クローク　自動販売機
トイレ　エレベータ　ドリンクコーナー　洗濯室　電話
トイレ男　託児室　ロッカー　水飲み場

会場案内図
The Place of Meeting
—2—

■ ９Ｆ会議室（Conference Room）

第４会場 910 (Room 4)
第９会場 901 (Room 9)
第１１会場 905 (Room 11)
第８会場 904 (Room 8)
第１０会場 902 (Room 10)
第１２会場 906 (Room 12)

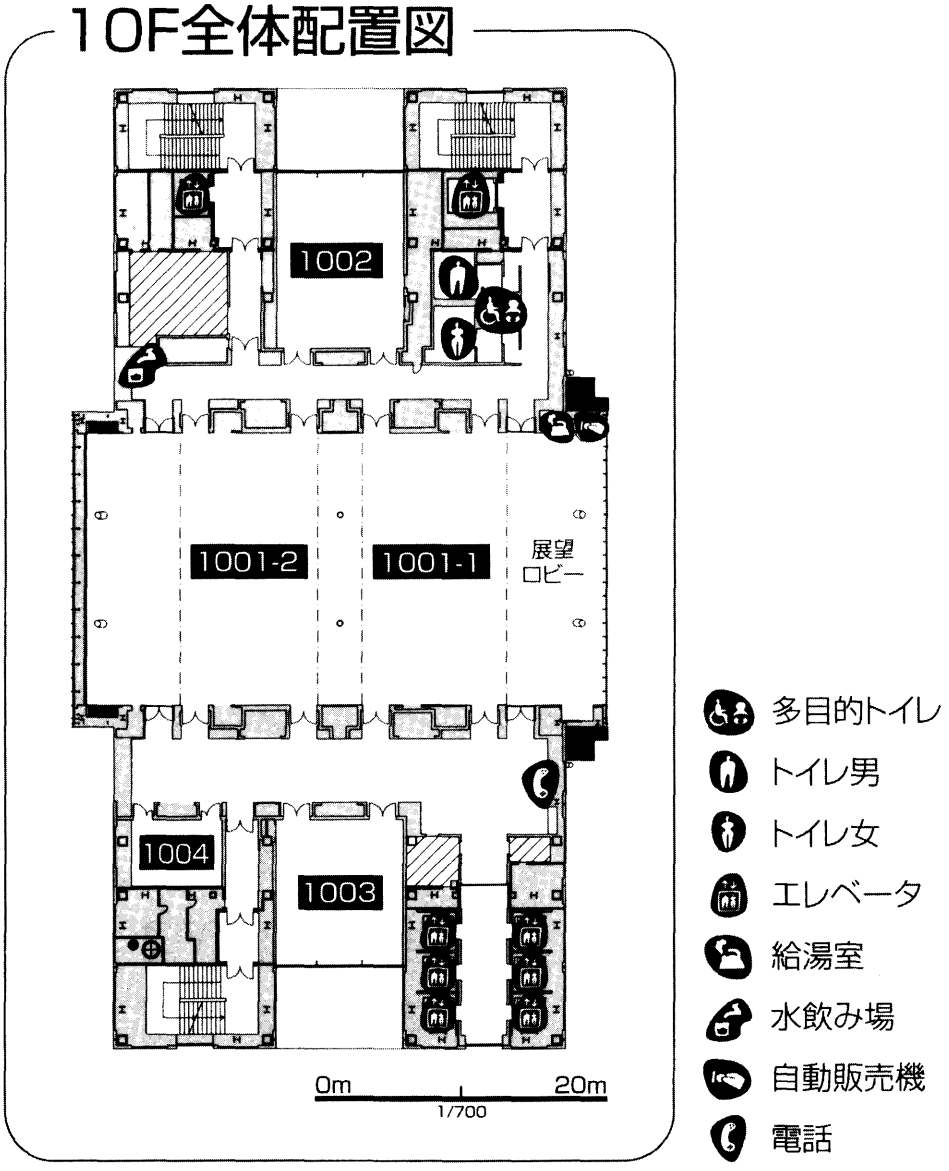


会場案内図
The Place of Meeting

—3—

■１０Ｆ会議室（Conference Room）

第２会場 1001-2 (Room 2)
第３会場 1001-1 (Room 3)
第５会場 1003 (Room 5)
第６会場 1002 (Room 6)
第７会場 1004 (Room 7)



参加者の皆様へ、ご案内とお願い

会期： 平成１８年７月１２日（水）１２：３０ ～ ７月１４日（金）１７：００

会場： グランシップ GranShip（ Shizuoka Convention & Art Center ）（JR 東静岡駅前）
中ホール・大地（１，２F）（第１会場）、会議室１００１－２（１０F）（第２会場）、
会議室１００１－１（１０F）（第３会場）、会議室９１０（９F）（第４会場）、
会議室１００３（第５会場）、会議室１００２（第６会場）、会議室１００４（第７会場）、
会議室９０４（９F）（第８会場）、会議室９０１（９F）（第９会場）、会議室９０２
（９F）（第１０会場）、会議室９０５（９F）（第１１会場）、会議室９０６（９F）
（第１２会場）
静岡市駿河区池田 79-4（〒422-8005） TEL：(054)203-5713 FAX：(054)203-6710

会場へのアクセス： 電車：新幹線（ひかり）で東京から１時間、大阪から２時間。静岡駅下車。
東海道本線（上り）へ乗換えて１駅目（３分）。東静岡駅下車。
車：東名高速静岡 I.C から２０分。静清バイパス千代田上土 I.C から１０分。

主催： 日本微量元素学会
共催： 日本衛生学会、日本産業衛生学会、日本免疫毒性学会、日本化学会、日本薬学会、
日本分析化学会、日本トキシコロジー学会、文部科学省 21 世紀 COE プログラム推進拠点、
日本微量栄養素研究会、静岡総合研究機構
後援： (財)日本健康・栄養食品協会

参加費： 事前登録がお済みの方は受付にてネームカードをお受け取り下さい。
当日登録の方は下記の参加費を納入し、ネームカードをお受け取り下さい。
会員：１０，０００円
学生：４，０００円
非会員：１２，０００円

※抄録集は会員には予め送付されますが、非会員の方は参加費に含まれておりませんので、別途にご購入願います。一部：１５００円です。

ネームカード： ネームカードには必要事項を記入し、会場内では必ずご着用下さい。

懇親会費： 事前登録がお済みの方は受付にてネームカードにシールの貼付を受けて下さい。
当日登録の方は下記の懇親会費を納入し、ネームカードにシールの貼付を受けて下さい。

会員・非会員：７，０００円
学生：４，０００円

理事会：日時：７月１２日（水）　１６：２０－１８：２０
会場：グランシップ１０階・会議室１００２（第６会場）

評議員会：日時：７月１３日（木）　１２：００－１３：００
会場：グランシップ９階・会議室９０４（第８会場）

総　　会：日時：７月１４日（金）　１３：００－１４：００（受賞講演３０分を含む）
会場：グランシップ１・２階　中ホール・大地（第１会場）

懇親会：日時：７月１３日（木）　１８：３０－２０：３０
会場：ホテルアソシア静岡ターミナル　３階　駿府の間（JR 静岡駅前）
TEL：054-254-4141（代）

各種委員会：日時：７月１２日（水）　１０：００－１２：００および１３：００－１５：００
会場：グランシップ９階　会議室９０１（第９会場）、会議室９０２（第１０会場）、
会議室９０５（第１１会場）、会議室９０６（第１２会場）

講演者および座長(司会者)の皆様へ

1. **講演時間と討論**
   1) 特別講演、教育講演では一講演あたり４０分（うち講師紹介３分）を予定しております。
   2) フォーカス講演、受賞講演では一講演あたり３０分（うち講師紹介３分）を予定しております。
   3) トピック講演は一講演あたり３０分（口演２５分、討論５分、計３０分）を予定しております。
   4) シンポジウム講演は一講演あたり２５分（口演２０分、討論５分、計２５分）を予定しております。
   5) 講演（口演発表）では、一演題あたり講演時間が１０分、討論時間が５分の計１５分を予定しております。

2. 特別講演、教育講演、フォーカス講演、受賞講演は、グランシップ１・２階　中ホール・大地（第１会場）で行います。

3. シンポジウム、トピック講演は、グランシップ１０階　会議室１００１－２（第２会場)、１００１－１(第３会場)で行います。

4. 講演の発表時間の計時に関しては、講演時間終了１分前および講演時間終了時にランプの点灯あるいはブザーでお知らせします。各自の責任で必ず発表時間を厳守して下さるようお願い致します。

5. 会場には**書画カメラ内蔵・液晶プロジェクター**（パソコン（Power Point）およびＯＨＣ使用可）を用意しております。
   1) OHC を使用される場合は、発表の**1時間前までに**会場受付で登録をして下さい。OHC は紙にプリントアウトしたものの方が映りが良いです。OHP フィルムは良くありません。
   液晶プロジェクターを使用される場合は、**調整の必要があるため、６月末までに Power Point ファイルを電子メール添付にて**事務局までご送付下さい。ご送付出来ない場合は当日、講演の少なくとも**2時間前までに**演題受付にご提出下さい。原則として当方が用意したパソコンを使用していただきますが、パソコン持ち込みをご希望の場合は必ず総合受付に立ち寄り、設定ミスのないように試写して下さい。(使用するコンピュータの OS は Windows-XP, Power Point 2003 が最も適している様です)。
   2) ご提出いただいたファイルは本会以外の目的には使用しません。コピーしたファイルは本会終了後に直ちに消去いたします。

6. 次演者および次座長（司会者）の方は予定時間の１５分前までに指定の席にお着き下さい。

第17回日本微量元素学会日程表

7月12日（水）；第1日目

会場番号	第1会場	第6会場	第9会場
会場	中ホール（1・2F）	1002（10F）	901（9F）
9:00－			
10:00－			各種委員会
12:00－	開場		
12:30－	開会の辞		
13:00－	市民公開講座 一部 冨田　寛 荒川　泰行 高木　洋治		各種委員会
13:40－			
14:20－			
15:00－	休憩		
15:10－	市民公開講座 二部 細谷　憲政		
15:50－	閉会の辞		
16:00－			
16:20－		理事会	
18:20－			

第17回日本微量元素学会日程表

7月12日（水）；第1日目

第10会場	第11会場	第12会場	会場番号
902（9F）	905（9F）	906（9F）	会場
			−9:00
各種委員会			−10:00
			−12:00
各種委員会			−13:00
			−15:00

第17回日本微量元素学会日程表

7月13日（木）；第2日目

会場番号	第1会場	第2会場	第3会場	第4会場
会場	中ホール（1・2F）	1001-2（10F）	1001-1（10F）	910（9F）
8:30－	開　場			
9:10－	開　会			
9:15－		トピック講演 （講演 I-V） 三原 久明 藤代　瞳 吉川　豊 小林 弥生 中西　剛 司会 櫻井 照明 長谷川 達也	セッションI （講演 1-4） 座長　西牟田 守	セッションIV （講演 12-14） 座長　江原 正明
10:00－				
10:15－			セッションII （講演 5-8） 座長　千葉 百子	セッションV （講演 15-17） 座長　川原 正博
10:45－				セッションVI （講演 18-20） 座長　山口 正義
11:15－			セッションIII （講演 9-11） 座長　遠藤 文夫	
11:30－				セッションVII （講演 21-22） 座長　池田　稔
11:45－				
12:00－	昼　食			
13:00－	フォーカス講演 大槻 剛巳、中野 孝司 司会　吉田 貴彦			
14:00－	教育講演 I 倉澤 隆平 司会　柳澤 裕之			
14:40－	教育講演 II 児玉 浩子 司会　鈴木 和夫			
15:20－	休　憩			
15:30－	特別講演 I 雨宮 次生 司会　石川　弘			
16:10－	特別講演 II 池澤 善郎 司会　大沢 基保			
16:50－	記念撮影			
17:10－	移　動			
18:30－	懇親会 （ホテルアソシア）			
20:30－				

第17回日本微量元素学会日程表

7月13日（木）；第2日目

第5会場	第6会場	第7会場	第8会場	会場番号
1003（10F）	1002（10F）	1004（10F）	904（9F）	会場
				−8:30
展示コーナー	呈茶コーナー	講師控室		−9:15
昼　　食			評議員会	−12:00 −13:00
展示コーナー	呈茶コーナー	講師控室		
				−16:50

第17回日本微量元素学会日程表

7月14日（金）；第3日目

会場番号	第1会場	第2会場	第3会場	第4会場
会場	中ホール（1・2F）	1001-2（10F）	1001-1（10F）	910（9F）
8:30－	開　　場			
9:00－		シンポジウムI （講演 1-5） 柳澤 裕之 池田　稔 大高 道郎 森山 光彦 山崎 雙次 司会　荒川 泰行 高木 洋治	シンポジウムII （講演 1-5） 加治 正行 奥野 智史 武田 厚司 堀口 敏宏 川原 正博 司会　斉藤　健 武田 厚司	
11:10－		移　動	移　動	
11:20－	特別講演III 佐治 英郎 司会　桜井　弘			
12:00－	昼　　食			
13:00－	総　会			
13:30－	受賞講演 櫻井 照明 司会　田村 遵一			
14:00－	移　動			
14:10－		セッションVIII （講演 23-26） 座長　圓藤 吟史	セッションXI （講演 34-37） 座長　森山 光彦	セッションXIV （講演 45-46） 座長　佐治 英郎
14:40－				セッションXV （講演 47-49） 座長　内海　昭
15:10－		セッションIX （講演 27-30） 座長　木村 美恵子	セッションXII （講演 38-41） 座長　姫野 誠一郎	
15:25－				セッションXVI （講演 50-51） 座長　千熊 正彦
15:55－				セッションXVII （講演 52-55） 座長　原口 紘忯
16:10－		セッションX （講演 31-33） 座長　角田 正史	セッションXIII （講演 42-44） 座長　米谷 民雄	
16:55－	閉　　会			
17:00－				

第17回日本微量元素学会日程表

7月14日（金）；第3日目

第5会場	第6会場	第7会場	会場番号
1003（10F）	1002（10F）	1004（10F）	会場
開　　場			−8:30 −9:00
展示コーナー	呈茶コーナー	講師控室	
昼　　食			−12:00 −13:00
展示コーナー	呈茶コーナー	講師控室	
			−16:00

第17回 日本微量元素学会
2006年7月12日(水)～14日(金)

市民公開講座

プログラム・アブストラクト

第17回日本微量元素学会 記念
市民公開講座

“サプリメントと健康”
—薬食同源と微量元素—

７月１２日（水）：第１日目
グランシップ・中ホール・大地（１，２F）（第１会場）

共催： 株式会社　ファンケル

第一部
「薬剤ならびにサプリメントによるミネラルの保健・予防・治療への処方」

特別講演Ⅰ　「いきいき生活と亜鉛　－その深～い関係－ 」　13:00－13:40
冨田　寛　日本大学医学部名誉教授

特別講演Ⅱ　「内科領域における処方の実際」　13:40－14:20
荒川　泰行　日本大学医学部消化器肝臓内科部門教授

特別講演Ⅲ　「外科領域における微量元素処方の実際—静脈栄養時において」　14:20－15:00
高木　洋治　甲子園大学栄養学部大学院栄養学研究科
大阪大学医学部名誉教授

第二部
特別講演Ⅳ　「サプリメントの展望　－有効性と安全性－」　15:10－15:50
細谷　憲政　厚生労働省
（財）日本健康･栄養食品協会理事長
東京大学医学部名誉教授

市民公開講座　特別講演　I

薬剤ならびにサプリメントによるミネラルの保健・予防・治療への処方
いきいき生活と亜鉛—その深〜い関係—

冨田　寛
冨田耳鼻咽喉科医院、微量栄養素研究所
（日本大学医学部名誉教授）

○味覚障害の患者は増えている

日本には味覚障害の患者はどれ位居るのだろうか？　日本口腔・咽頭科学会で、1990年アンケート方式で統計をとったところ、年間1千万人発生していることが分った。治療薬の治験を始めるにあたり、13年を経て2003年あらためて同じ方式で統計をとったところ、年間2千万人と1.8倍に増えていることが分った。

この10年高齢者の人口構成比が著増していることが、この増加の一因ではあるが、味覚のしくみから考えて「歳のせい」にすることは出来ないのである。

○味覚のしくみ

味覚は生きるための栄養確保と、死につながる毒の識別のために重要な感覚であり、甘味、旨味、酸味、苦味は生れたばかりの新生児でも分る。ネズミの味細胞は10日で、ヒトではおそらく4週間で新しくなると考えられ、新生交代の活発な細胞であり、視覚、聴覚のような老人性変化は起こり難い

この味再病の若返りに、食物から日々摂取する必須微量元素「亜鉛」が無くてはならないものなのである。

○味覚障害の主因は亜鉛の摂取不足と服用薬剤の副作用

味覚障害を起こす原因は15もあるが、必須微量元素「亜鉛」の欠乏が関わっているのが総症例の70%に及んでいる。

全体の30%強に当る食事性味覚障害は、亜鉛を含む食事の摂取不足が原因であり、孤食あるいは個食という食事スタイルが影響している。

壮年、老年の患者では、生活習慣病の治療薬の副作用による味覚障害も多い。

○老化予防と亜鉛

最近はアンチエージング（老化予防）の目的で、ビタミンA、C、E、アスタキサンチン、ポリフェノール、イチョウ葉、コエンザイムQ10など、活性酸素をおさえるサプリメントに人気が集っているが、強力な活性酸素除去酵素であるスーパーオキシドジスムターゼは、銅、マンガンとともに亜鉛が無いと効かない。老化防止にも亜鉛は重要な役目を担っているのである。

静岡名産の緑茶、うなぎ、みかんが、いきいき生活に果す役割についても触れてみたい。

【学位】　医学博士（日本大学）
【略歴】　日本大学医学部耳鼻咽喉科教授、日本大学付属板橋病院副院長、
日本微量元素学会初代理事長、第1回日本微量元素学会会長。

市民公開講座　特別講演　II

薬剤ならびにサプリメントによるミネラルの保健・予防・治療への処方
—内科領域における処方の実際—

荒川　泰行
日本大学医学部消化器肝臓内科部門　教授

わが国では、１９６０年～７０年の高度経済発展優先の裏で環境中に過剰に放出されたメチル水銀やカドミウムなどの重金属によってもたらされた健康障害（いわゆる公害病）、またミルクの製造過程で有害な砒素が混入して多くの乳児に重い後遺症を残した「森永砒素ミルク事件」など歴史的に不幸な出来事を経験しました。これらを契機にして、ヒトと金属の研究は、主として環境汚染や中毒学の分野において先行し、むしろ日陰の花でありました。しかし、近年生体内には多種類の金属が様々な濃度で存在して、生命機能の維持に重要な役割を担っていることが次々と明らかにされ、特に“微量元素（金属）の生化学的、および栄養学的な機能、欠乏症、過剰症”などに関する学際的な研究発展とともに微量元素の重要性が広く注目されるようになりました。まさに、微量元素に対する認識の負から正への転換が図られることになったと思います。

人体を構成する元素は、多量元素（酸素、炭素、水素、窒素、カルシウム、リン）、少量元素（硫黄、カリウム、ナトリウム、塩素、マグネシウム）、微量元素（鉄、フッ素、亜鉛、ルビジウム、ストロンチウム、臭素、鉛、銅）、及び超微量元素（アルミニウム、カドミウム、ホウ素、バリウム、錫、マンガン、ヨー素、ニッケル、モリブテン、クロム、コバルトなど）に大別されます。生体の構造と機能、潤滑なエネルギー代謝、細胞の増殖、自己複製等を正常に維持するために不可欠な元素を必須元素と呼ばれますが、ヒトでは現在１１種の常量元素（多量元素と少量元素）の他に、必須微量元素としては、鉄、亜鉛、マンガン、銅、セレン、ヨー素、クロム、コバルト、モリブテンの９種が広く知られています。この他にも数種類の元素の必須性が示唆されています。人体におけるこれらの微量元素の存在比は０．０２％にすぎませんが、大部分が酵素の活性中心ないし微量生体内活性物質として存在し、微量でも大きな働きをしています。“たかが微量元素、されど微量元素”の認識が大切であります。体内に含まれている量が少ないからといって重要度が低いということではありません。たとえ微量であっても、生体にとっては欠かせないものであり、どの微量元素もそれぞれ大切な役割を担っています。

マグネシウムは、環境中では炭酸塩、硫酸塩、塩化物として広く分布しいます。生体内では、カルシウム、ナトリウム、カリウムについで４番目に多い陽イオン金属で、細胞内ではカリウムについで２番目に多くあります。体重７０kgの成人でありますと、その体内には約２０～２８ｇのマグネシウムが存在しますが、そのうちの６０～６５％が骨中に、２７％が筋肉中に、６か～７％がその他の組織中に、そして残りの１％が細胞外液に存在します。したがって、臓器分布では、骨および筋肉の他に、代謝活性の高い脳・神経組織、および心筋、肝臓、消化管、腎臓、外分泌および内分泌腺、リンパ組織などに多く存在します。マグネシウムは、種々の代謝の基本的反応の必須イオンとして重要な役割を果たしておりますが、それは３００種類以上の酵素がその活性にマグネシウムを必要とする点にあります。特にリン酸伝達反応とＡＴＰ産生などエネルギー産生系の代謝に必須であります。最近、食生活の欧米化と飽食の時代を迎えて、マグネシウムについては十分には摂取できない食環境にあります。そして、この食事性マグネシウムの著しい接取減少（潜在性マグネシウム欠乏症）と、カルシウムとマグネシウムの摂取アンバランス（カルシウム／マグネシウム比の増大）が心臓病、糖尿病、骨粗鬆症など様々な生活習慣病に基盤であるメタボリックシンドロームの原因の一つになっていると考えられています。

また、亜鉛はヒトと動物に代表的な必須微量元素の一つであります。その生理作用として、①胎児の発育、②皮膚及びその付属器官の新陳代謝、③生殖機能，④骨格の発育、⑤味覚及び嗅覚の維持、⑥精神神経作用・行動への影響、⑦免疫機能維持、⑧その他が知られています。これまでに３００を超える亜鉛酵素、亜鉛要求酵素が単離されており、これらの酵素の活性中心となっていることが明らかにされていますが、さらに亜鉛は酵素活性とは関係しなくともたんぱくの高次構造を維持するための構造中心として、多くのたんぱくに含まれています。

１９６３年に、ヒトにおける亜鉛欠乏症の存在がPrasadらによってはじめて明らかにされ 、１９７３年には遺伝的な病気である腸性肢端皮膚炎やある種の脱毛が、翌年には味覚・臭覚障害がそれぞれ亜鉛欠乏によることが明らかになりました。また、１９７５年には高カロリー輸液で亜鉛欠乏症の起こることが発見されました。その後１９７０年代後半から１９８０年代前半にかけて、米国における人口の亜鉛状態調査、亜鉛欠乏症と免疫異常の報告、亜鉛欠乏とフリーラジカル・過酸化との関係報告、老人における低亜鉛状態の問題化、味覚低下に対する亜鉛補充療法、などの研究報告が相次ぎ、謎につつまれていた生体内亜鉛が生命の働きを支えるカギとして、亜鉛パワーの秘密の新たな知見や情報が大きくクローズアップされるようになりました。

亜鉛欠乏症の原因に関連して、今日飽食の時代、グルメの時代といわれる日常の食生活の中でインスタント食品や加工食品中心を中心とした偏食・奇食、あるいはダイエットなどによる欠乏症、薬剤や食品添加物のキレート作用による欠乏症である味覚異常、また高齢者が増加する中でがん、心臓病、糖尿病、動脈硬化症、高血圧症などの生活習慣病の病態の形成・進展と予防における必須微量元素欠乏状態の関係など、現代社会における新しい問題もでてきております。一方、亜鉛欠乏に対する補充療法についても、本来亜鉛をはじめとする生体に必要な微量元素は食事から摂るのが基本です。しかし、食品に含まれる亜鉛量そのものが少なくなっているという現状がありますので、現代の食生活でなかなか一日の必要量である１５ｍｇを摂取するのは難しい状況にあります。したがって、亜鉛を多く含む食品を意識的に多く摂取することが必要となりますが、亜鉛を大量に含んだ健康食品を慢性的な亜鉛不足を補う栄養補助食品として上手に使用することも可能であります。さらに、亜鉛とL-カルノシンから誕生したプロプレジンク製剤（商品名：プロマック顆粒、ゼリア新薬工業株式会社）が亜鉛含有胃潰瘍薬として汎用されていますが、組織修復促進作用や抗酸化作用を有します。したがって、今後亜鉛の幅広い生理作用のスペクトルムを考慮します時、保険適用外ながら味覚障害、C型慢性肝炎、肝硬変、肝不全、皮膚疾患、口腔・粘膜障害、褥瘡（じょくそう）、骨粗鬆症（こつそしょうしょう）、亜鉛欠乏による貧血、など様々な亜鉛欠乏状態を伴う病気・病態の治療への積極的な応用が期待されるところです。

私たちは、普段の食事（飲水）によって身体に必要なエネルギーや栄養素を獲得していますが、とかくすると三大栄養素（たんぱく、炭水化物、脂質）だけに関心が向かえがちですが、ミネラル、微量元素、ビタミンなどの微量栄養素の摂取にも関心を持つべきであります。したがって、さまざまな栄養素を満遍なく摂取するためには、それぞれの栄養素を細かく考えるというよりも、日常の不適切な生活習慣を改め、かつ普段から伝統的な和食を中心とした食生活に留意することが、結果として微量栄養素全般の摂取につながることを認識し、実践されることが大切であります。

【学位】 医学博士（日本大学）

【略歴】 最終学歴：日本大学大学院医学研究科内科学専攻卒業。日本大学医学部第三内科学講座助手、講師、助教授を経て現職。その間、日本大学医学部附属板橋病院長を３年間兼務（２００２年１１月～２００５年１０月）。

市民公開講座　特別講演　Ⅲ

薬剤ならびにサプリメントによるミネラルの保健・予防・治療への処方
—外科領域における微量元素処方の実際—静脈栄養時において

高木　洋治
甲子園大学栄養学部、大学院栄養学研究科　教授
（大阪大学医学部名誉教授）

外科においては術後長期間にわたって食事がとれないとか、小腸大量切除後の消化・吸収障害のため、通常の食事だけでは栄養維持ができないことがあり、このような場合に静脈から栄養素を投与する静脈栄養法(TPN)（高カロリー輸液法）が行われる。TPNにより従来不良であった予後が改善されるようになってきた。しかしTPNでは化学的に純粋な栄養素を強制的に投与するため、投与の仕方によっては欠乏症や過剰症を引き起こす可能性がある。経口摂取では通常起こらないとされる微量元素の異常の起こることが明らかとなり、微量元素の栄養学的重要性が認識されるようになった。TPN施行時に異常のみられる微量元素としては亜鉛、銅、マンガン、セレン、クロム、モリブデン等があり、亜鉛、銅の異常は比較的多くみられる。TPN時の亜鉛欠乏では顔面、会陰部より始まり漸次増悪する皮疹を主徴とし、口内炎・舌炎、爪変形、脱毛、下痢等の随伴症状を伴う。銅欠乏では貧血、白血球数、特に好中球数減少、骨髄白血球系障害、小児ではくる病様骨病変がみられている。マンガン欠乏の報告は少なく、小児の成長障害がマンガン投与で改善した１例あるのみで、むしろ過剰により、脳にマンガン蓄積がみられ、時にパーキンソン様症状をきたしたとの報告がある。セレンの欠乏により筋肉痛、心筋障害による突然死、不整脈等の起こることが報告されている。３価クロムの欠乏により耐糖能障害をきたし、クロム投与により改善がみられた報告がある。モリブデン欠乏で夜盲症、意識障害をきたし、血清メチオニン上昇、タウリン、システイン、尿酸低下、尿中硫酸塩減少、亜硫酸塩増加がみられ、モリブデン投与により改善がみられたとの報告がある。TPN時の微量元素投与量に関しては未だ明確な基準がなく報告者によりかなりのバラツキがある。わが国では高カロリー輸液用微量元素製剤としては１アンプル 2ml 中、鉄、亜鉛、銅、マンガン、ヨウ素を各 35,60,5,1,1μmol 含有したものと、これからマンガンを除いた２種が市販されている。セレン、クロム、モリブデンの製剤は未だ市販されていない。セレンにおいては血中セレン濃度低値、グルタチオンペルオキシダーゼ活性低値を示す患者が多く、突然死の可能性もあることから早急な製剤化が望まれる。クロムは必要量が少なく、輸液製剤中にも混入していることが多く、投与の必要性は意見の一致をみていない。モリブデンにおいても十分な検討がなされておらず今後の研究が待たれる。

【学位】　医学博士（大阪大学）
【略歴】　大阪大学講師（医学部小児外科）、助教授（同）、大阪大学教授（医学部保健学科）、
甲子園大学教授（栄養学部）

Civil Extension Special Lecture III

Supplementation of trace elements in surgical patients during total parenteral nutrition

○Yoji TAKAGI

College of Nutrition & Graduate School of Nutrition, Koshien University, Hyogo, Japan

Total parenteral nutrition (TPN) is useful for the surgical patients with difficulty of oral feeding for a long time postoperatively or with short bowel syndrome after massive resection of the intestine, and so on. However, TPN has some complications such as deficiency or toxicity in several nutrients, especially in trace elements such as zinc(Zn), copper(Cu), manganese(Mn), selenium(Se), chromium(Cr) and molybdenum(Mo), which makes us know the nutritional significance of them in a new fashion. Zn deficiency during TPN has symptoms such as dermatitis like acrodermatitis enteropathica, deformity of nail, alopecia and diarrhea. Anemia, leucopenia, neutropenia and bone changes like rachitis are seen in Cu deficiency during TPN. Mn deficiency occurs rarely, while its toxicity is sometimes seen during TPN. Excessive Mn is accumulated in basal ganglia, which is confirmed as the sign of high intensity by magnetic resonance imaging (MRI). Se deficiency during TPN shows the symptoms such as muscle pain, cardiomyopathy, color change in nail, and depigmentation of skin. The disturbance of glucose tolerance and negative nitrogen balance are seen as Cr deficiency during TPN. Although Mo deficiency is rare, there were reported such as scotoma, unconsciousness, and the increase of serum methionine levels, urinary sulfite excretion, and the decrease of serum uric acid, cystein and taurine levels, and urinary sulfate excretion as Mo deficient symptoms.

A proper balance of essential trace elements is important for the patients receiving TPN. However, their intravenously recommended daily dosages vary widely by various reports. In Japan, only two types of trace elements solution for TPN are commercially available; one contains 2mg of iron, 3.9mg of Zn, 0.3mg of Cu, 50μg of Mn and 120μg of iodine, and another does the same amounts except Mn which is excluded in each ampule. Se, Cr and Mo solutions are not yet commercially available. The production of Se solution on a commercial basis has to hurry up, because many patients receiving TPN show low plasma Se levels and decreased glutathione peroxidase activities and sudden death due to Se deficiency may occur. As Cr is considered to be contained to some extent in TPN solutions as contamination, the supplementation of Cr is still controversial. Mo administration should be more elucidated.

外科領域における微量元素処方の実際—静脈栄養時において

○高木 洋治

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市民公開講座　特別講演　Ⅳ

サプリメントの展望　―安全性と有効性―

細谷　憲政
（財）日本健康・栄養食品協会　理事長
（東京大学医学部名誉教授）

“特保マーク”や“JHFAマーク”の認知度が向上してくると、サプリメント等についての正しい理解が得られ、その妥当な活用に関心がもたれています。しかし、一方では、まがい物や好い加減のもの（薬事法などに触れるようなもの）も出回って、消費者を惑わしているとも言われています。それ故、適切な解かり易い消費者教育が必要とされています。

現在の日本においては、欧米先進国に見られるように、ヒト試験などを実施して安全性や有効性が検証され、また、それに伴って納得のできる消費者教育が実施されているとは思えない状況です。そこで、従来から存在している“保健大衆薬”と、現在、関心の持たれている特定保健用食品、健康補助成品（食品）、また、“機能性食品”、さらには“いわゆる健康食品”などについて、比較検証してみることにしました。

“保健大衆薬”は、ヒト試験（臨床介入試験）を実施して有効性を観察していないにも拘わらず、実験動物の生化学的知見などから、可能性を推察して、医薬品、医薬部外品扱いされて、各種各様の効能・効果が標榜できるとされています。しかし、特定保健用食品などは、その関与成分（いわゆるサプリメント）とそれを含有した製品について、ヒト試験を実施して、それぞれについての有効性を証明しているにも拘わらず、食品として取り扱われ、半健康状態を対象とするものとして、効能・効果は標榜できないとされています。食品類は、一般的に安全性は表示できたとしても、効能・効果は標榜できません。

そこで、ビタミン、ミネラル、アミノ酸などを主成分とする“保健大衆薬”（現在、先進国では日本だけに存在）の出現、並びに、その後の経過と、“機能性食品”の出現、並びに、特定保健用食品としての見直しの状況、さらにはその後の経過などについて、検証してみることにしました。

ところで、栄養素欠乏症が解消して、現在のように栄養素の過剰状態と欠乏状態の入り混じった“半健康状態”poor health statusが出現してくると、食品と医薬品の中間に位置するものが出現してきました。米国はこれを dietary supplements とし、欧州などは、これからハーブの一部を除外して food supplements としています。栄養の領域では、国際的には、経口栄養補助成品 oral nutritional supplements, ONSが保健・医療の領域で、幅広く活用されています。

現在の日本で最も必要とされていることは、食薬区分を、欧米先進国なみに、明確に区分して、裁量行政ではなく、ルール行政として確立することです。この問題は、薬害、医薬品公害に関係する医薬品—栄養素（成分）相互作用 drug-nutrient interaction の観点からも、国際的に注目されています。その上で、食品と医薬品との中間にあるとされる補助成品、成品 supplements を定義することが必要になります。また、保健領域の取り組み health care と医療領域の取り組み medical care を明確にし、これに対応する健康強調表示 health claim や医療強調表示 medical claim を適確に表示できるようにすることが必要です。

医薬品は、ある意味では、元々は毒物と見做されているものですから、副作用が問題になります。栄養素（成分）や食品は、栄養問題として取り組まれるので、過不足が問題になります。それ故、疾病の治療 cure や予防 prevention と生活習慣病のリスク回避、低減・除去 risk reduction とは、異なる取り組みとされています。現在、健康・栄養状態のリスク管理が叫ばれています。これに対しては、医薬品ではなく、サプリメントなどを活用して、対応することが、国際的には、常識になっています。

構造改革の一環として、保健・医療の改革の叫ばれている現在、私達の身体に、投与するもの、摂取するものについては、その原点に立ち戻り、国際的視点から検証して、国民、消費者に解かり易い、適切な取り組みのものとして、提示することが必要と思われます。

他国に例を見ない日本独自のあり方だけを続けていけば、いずれ日本は国際的に見向きもされなくなることは明らかです。その結果は、日本の健康食品業界は勿論のこと医薬品業界も、欧米はもとより、急速に追い上げてきているアジア各国から追い越されて、落ちこぼれていくことになります。

【学位】 医学博士（東京大学）

【略歴】 東京大学医学部教授、茨城県健康科学センター長、女子栄養大学大学院研究科委員長、
財団法人 日本健康・栄養食品協会理事長

第17回 日本微量元素学会
2006年7月12日(水)～14日(金)

プログラム

特別講演
教育講演
フォーカス講演
トピック講演
シンポジウム講演
受賞講演

特 別 講 演

７月１３日（木）：第２日目
グランシップ・中ホール・大地（１，２F）（第１会場）

特別講演Ⅰ　「微量元素と眼組織　—形態学的研究—」　15:30—16:10
雨宮　次生
長崎大学大学院　医歯薬学総合研究科　眼科・視覚科学分野

司会　石川　弘　（日本大・医）

特別講演Ⅱ　金属関連アレルギー反応の発症機序　16:10—16:50
池澤　善郎
横浜市立大学大学院　医学研究科　環境免疫病態皮膚科学

司会　大沢　基保　（帝京大・薬）

７月１４日（金）：第３日目
グランシップ・中ホール・大地（１，２F）（第１会場）

特別講演Ⅲ　金属薬剤の開発と医療への応用　11:20—12:00
佐治　英郎
京都大学大学院　薬学研究科　病態機能分析学分野

司会　桜井　弘　（京都薬大）

教 育 講 演

7月13日（木）：第2日目
グランシップ・中ホール・大地（1，2F）（第1会場）

教育講演Ⅰ　「住民調査に見る高齢者の亜鉛不足と亜鉛補充療法」　14:00－14:40

倉澤　隆平[1]，久堀　周治郎[1]
[1]東御市立みまき温泉診療所

司会　　柳澤　裕之　（埼玉医科大）

教育講演Ⅱ　「銅の体内動態と疾病」　14:40－15:20

児玉　浩子[1]，藤澤　千恵[1]，顧　艶紅[1]，志賀　勝秋[1]
[1]帝京大学　医学部　小児科

司会　　鈴木　和夫　（千葉大・薬）

フォーカス 講 演

7月13日（木）：第2日目
グランシップ・中ホール・大地（1，2F）（第1会場）

「アスベストと悪性中皮腫」
—発症の基礎と臨床の実際—　　13:00−14:00

講演 I

「アスベスト関連発癌と免疫影響」

大槻 剛巳[1]，三浦 由恵[1]，前田 恵[1]，林 宏明[1]，董 茂龍[1]，
西村 泰光[1]，富田 正文[2]，勝山 博信[3]
[1]川崎医科大学 衛生学 [2]川崎医科大学 医用中毒学
[3]川崎医科大学 公衆衛生学

講演 II

「悪性中皮腫：頻度と臨床的アプローチ」

中野 孝司[1]
[1]兵庫医科大学 呼吸器内科

司会　吉田 貴彦（旭川医大）

トピック講演

7月13日（木）：第2日目
グランシップ・会議室1001－2（10F）（第2会場）

トピックス「微量元素研究—若い力—」 9:15－11:45

講演Ⅰ

動物細胞におけるカドミウム輸送の分子メカニズム

○ 藤代 瞳[1]，奥垣 里美[1]，窪田 圭佑[1]，垣辺 真吾[1]，佐藤 雅彦[2]，姫野 誠一郎[1]
[1]徳島文理大学 薬学部 [2]愛知学院大学 薬学部

講演Ⅱ

セレンタンパク質の生合成とセレン特異的酵素

○ 三原 久明[1]，黒川 優[1]，近江 理恵[1]，栗原 達夫[1]，広津 建[2]，江崎 信芳[1]
[1]京都大学 化学研究所 [2]大阪市立大学大学院 理学研究科

講演Ⅲ

抗糖尿病作用を有する亜鉛およびバナジウム化合物によるα－グルコシダーゼの阻害と作用機構

○吉川 豊[1]，平田 良子[1]，桜井 弘[1]
[1]京都薬科大学 代謝分析学教室

講演Ⅳ

分析毒性学からみたヒ素の代謝機構

○小林 弥生[1]，早川 享[1]，崔 星[1]，平野 靖史郎[1]
[1]独立行政法人 国立環境研究所 環境健康研究領域

講演Ⅴ

核内受容体リガンドとしての有機スズ化合物とその毒性

○中西 剛[1]
[1]大阪大学 大学院薬学研究科 毒性学分野

司会 櫻井 照明 （徳島文理大・薬）
長谷川 達也 （山梨県環境科学研究所）

シンポジウム講演

7月14日（金）：第3日目
グランシップ・会議室1001－2（10F）（第2会場）

シンポジウムI「臨床における亜鉛の有効性の探索」 9:00－11:10

講演1

亜鉛の生理

○柳澤 裕之[1]
[1]埼玉医科大学 医学部 基礎医学部門衛生学

講演2

味覚障害と亜鉛

○池田 稔[1]，生井 明浩[1]
[1]日本大学 医学部 耳鼻咽喉科

講演3

消化管疾患と亜鉛 —亜鉛による大腸粘膜、胃粘膜におけるストレス蛋白質誘導作用と粘膜保護作用—

○大高 道郎[1]，渡辺 純夫[1]
[1]秋田大学 医学部 消化器内科

講演4

肝疾患と亜鉛

○森山 光彦[1]，荒川 泰行[1]
[1]日本大学 医学部 内科学講座消化器肝臓内科部門

講演5

皮膚疾患と亜鉛

○山崎 雙次[1]
[1]獨協医科大学 皮膚科学

司会 荒川 泰行（日本大・医）
高木 洋治（甲子園大院・栄養）

７月１４日（金）：第３日目
グランシップ・会議室１００１－１（１０F）（第３会場）

シンポジウムⅡ「微量元素の新規毒性の探索」 9:00－11:10

講演１

わが国の小児の血中鉛濃度 －受動喫煙の影響－

○加治 正行[1,2]，馬場 一徳[2]
[1]静岡市保健福祉局 保健衛生部 [2]静岡県立こども病院 内分泌代謝科

講演２

セレノメチオニンの解毒代謝ならびに生理学的利用

○ 奥野 智史[1]，長谷川 達也[2]，久保田 朝幸[1]，黒田 友則[1]，元林真二[1]，上野 仁[1]，中室 克彦[1]
[1]摂南大学 薬学部 [2]山梨県環境科学研究所

講演３

シナプス小胞亜鉛による海馬神経細胞死

○武田 厚司[1]
[1]静岡県立大学 薬学部 医薬生命化学教室

講演４

有機スズによる腹足類のインポセックス誘導機構の解析

○堀口 敏宏[1]，白石 寛明[1]，西川 智浩[1]，白石 不二雄[1]，西川 淳一[2]，太田 康彦[3]，森田 昌敏[1]
[1]国立環境研究所 環境リスク研究センター [2]大阪大学大学院薬学研究科
[3]鳥取大学農学部

講演５

古くて新しい金属、アルミニウム：その体内動態および細胞死メカニズムと疾患との関連

○川原 正博[1]
[1]九州保健福祉大学 薬学部 分析学講座

司会 斎藤 健（北大・環境医学）
武田 厚司（静岡県立大・薬）

受賞講演

7月14日（金）：第3日目
グランシップ・中ホール・大地（1，2F）（第1会場）

受賞講演　研究学術賞「浜理薬品賞」　13:30−14:00

「ヒ素の毒性および薬理作用の分子機構の解明・ヒ素のケミカルバイオロジー」

櫻井　照明
徳島文理大学　薬学部　衛生化学講座

司会　田村　遵一　（群馬大・医）

第17回 日本微量元素学会

2006年7月12日(水)～14日(金)

プログラム

講　　演

講　　演

7月13日（木）：第2日目
グランシップ・会議室1001－1（10F）（第3会場）

セッション　I　健康の維持・増進と微量元素　(9:15-10:15)

座長　　西牟田　守　（国立健康・栄養研究所）

講演1

ウクライナ国民の微量元素、Cu, Fe, Mn, Znの摂取量推定

○白石　久二雄[1], 幸　進[1], 新江　秀樹[1], 阿山　香子[1], ザモスチアン　パブロ[2], シガンコーフ　ニコライ[2], ロス　イワン[3], コルズン　ヴィタリー[3]

[1]放射線医学総合研究所　線量評価研究部　微量分析研究室 [2]ウクライナ放射線医科学研究センター [3]マルゼーフ衛生・生態医学研究所

講演2

アラル海沿岸地域調査における血中および尿中元素濃度と飲料水中元素濃度の関係

○千葉　百子[1], 篠原　厚子[2], 扇原　淳[3], 下田　妙子[4], 佐々木　敏[5], 平石　さゆり[2], 関根　美和[2], 稲葉　裕[2]

[1]国際医療福祉大学　薬学部 [2]順天堂大学 医学部 衛生学 [3]早稲田大学 人間科学部 [4]東京医療保健大学 [5]国立健康栄養研究所

講演3

長野県東御市住民の血清亜鉛濃度の実態～都市圏と農村圏での差異について～

○久堀　周治郎[1], 倉澤　隆平[1], 岡田　真平[2], 上岡　洋晴[3], 小切間　美保[4]

[1]東御市立みまき温泉診療所 [2]身体教育医学研究所 [3]東京農業大学 [4]同志社女子大学

講演4

集団データに基づくミネラル必要量の推定

○横井　克彦[1]

[1]聖徳大学大学院　人間栄養学研究科

セッション　II　生態系ならびに環境と微量元素　(10:15-11:15)

座長　　千葉　百子　（国際医療福祉大・薬）

講演５

インド、ベトナム、カンボジアの鉛汚染とヒトへの健康影響

○阿草　哲郎[1], 國頭　恭[2], RAMU Karri[1], CHARABORTY Paromita[3], CHAMNAN Chhoun[4], TRANG Pham Thi Kim[5], MINH Tu Binh[1], SUBRAMANIAN Annamalai[1], 岩田　久人[1], VIET Pham Hung[5], TANA Touch Seang[6], 田辺　信介[1]

[1]愛媛大学　沿岸環境科学研究センター [2]信州大学　理学部 [3]St. Thomas 高校 [4]カンボジア水産庁 [5]ハノイ国立大学 [6]カンボジア内閣官房

講演６

バナジウムを含む富士山地下水の健康影響に関する研究：富士山周辺市町村の死亡統計

○瀬子　義幸[1], 渡邊　かおり[1], 長谷川　達也[1]

[1]山梨県環境科学研究所

講演７

日本ウズラの生殖機能に及ぼすカドミウムの影響

○Rahman Shahidur[1,2], 笹浪　知宏[1], 森　誠[1], 望月　眞理子[3]

[1]静岡大学　農学部　応用生物化学科 [2]岐阜大学大学院　連合農学研究科 [3]日本獣医畜産大学

講演８

コイの鉛への順応

○佐藤　努[1], 中川　久機[2], 及川　信[2], 渡辺　美登里[3]

[1]静岡県立大学　食品栄養科学部　食品学科 [2]九州大学　大学院生物資源環境科学府 [3]九州大学　中央分析センター

セッション　Ⅲ　疾患と微量元素　(11:15-12:00)

座長　遠藤　文夫　（熊本大・医）

講演９

ウィルソン病の母からの母乳の中の銅と亜鉛の分析

○加賀　文彩[1], 志賀　勝秋[1], 藤澤　千恵[1], 児玉　浩子[1], 玉井　浩[2], 清水　教一[3], 小林　健司[4], 勝矢　陽子[4], Abdulah Rizky[4], 小山　洋[4]

[1]帝京大学　医学部　小児科 [2]大阪大学　医学部　小児科 [3]東邦大学　医学部　小児科 [4]群馬大学 大学院 医学系研究科 社会環境医療学講座

講演１０

Menkes 病患者、保因者及び胎児の遺伝子解析を用いた診断

○藤澤　千恵[1]，志賀　勝秋[1]，顧　艶紅[1]，加賀　文彩[1]，児玉　浩子[1]

[1]帝京大学　医学部　小児科

講演１１

非定型的な精神症状を呈したウイルソン病症例に関する検討

○清水　教一[1]，中村　道子[2]，青木　継稔[1]

[1]東邦大学　医学部　小児科学第二講座 [2]東邦大学　医学部　精神科学講座

７月１３日（木）：第２日目

グランシップ・会議室９１０（９F）（第４会場）

セッション　Ⅳ　癌と微量元素　(9:15-10:00)

座長　　江原　正明　（千葉大・医）

講演１２

肝細胞癌症例における疫学別にみた微量金属

○桝谷　佳生[1]，江原　正明[1]，岡部　真一郎[1]，福田　浩之[1]，行澤　斉悟[1]，吉川　正治[1]

[1]千葉大学　大学院　医学研究院　腫瘍内科学

講演１３

非小細胞肺がん細胞株中におけるシスプラチンの挙動解析

○高橋　雅樹[1]，鈴木　俊宏[1]，湯本　敦[1]，兎川　忠靖[1]，羽場　宏光[2]，榎本　秀一[2]，西尾　和人[3]，田邉　信三[1]

[1]明治薬科大学　分析化学教室 [2]理化学研究所 [3]国立がんセンター中央病院

講演１４

食道がん細胞を用いたセレン強化ブロッコリースプラウトによる抗がん作用の検討

○アブドゥラ　リズキー[1]，ファリエド　アハマド[2]，小林　健司[1]，勝矢　陽子[1]，桑野　博行[2]，小山　洋[1]

[1]群馬大学　大学院　医学系研究科　生態情報学 [2]群馬大学　大学院　医学系研究科　病態総合外科学

セッション V 生体機能と微量元素 (10:00-10:45)

座長 川原 正博 (九州保健福祉大・薬)

講演１５

海馬苔状線維－CA3 錐体細胞間での長期増強に対する亜鉛の作用

○櫻田 直美[1], 武田 厚司[1], 福家 さゆり[1], 南 彰[1], 奥 直人[1]

[1]静岡県立大学 薬学部 医薬生命化学教室

講演１６

嗅内野刺激に伴う扁桃体外側核からの亜鉛の放出

○今野 祐枝[1], 武田 厚司[1], 伊東 宏昌[1], 奥 直人[1]

[1]静岡県立大学 薬学部 薬学研究科

講演１７

高齢者における亜鉛欠乏は反復性感染の原因となる？：パイロット試験

○浮田 敏幸[1], 森田 豊穂[2], 田村 遵一[1]

[1]群馬大学 医学部附属病院 総合診療部 [2]二之沢病院

セッション VI 代謝・制御機能と微量元素－１ (10:45-11:30)

座長 山口 正義 (静岡県立大・生健)

講演１８

コバルトとバナジウムが糖尿病ラットの肝グリコーゲン含有量に及ぼす影響

○松本 智[1], 野村 保友[2], 馮 忠剛[1], 中村 孝夫[2]

[1]山形大学 工学部 応用生命システム工学科 [2]山形大学 大学院 医学系研究科 生命環境医科学専攻

講演１９

線虫 *C. elegans* に及ぼす塩化リチウムの影響

○井口 綾子[1], 守田 文代[1], 仁平 守俊[1], 平井 正巳[2], 有薗 幸司[1]

[1]熊本県立大学大学院 環境共生学研究科 [2]九州保健福祉大学 薬学部

講演２０

リチウム、マグネシウムおよびその他の微量元素による神経幹細胞の自己複製能の制御

○池川 雅哉[1], 本多 和人[2], 田代 啓[1], 木村 美恵子[3]

[1]京都府立医科大学 分子医科学部門 ゲノム医科学教室 [2]パーキン エルマー ジ

ャパン [3]タケダライフサイエンスリサーチセンター

セッション Ⅶ 代謝・制御機能と微量元素－２ (11:30-12:00)

座長 池田 稔 （日本大・医）

講演２１

亜鉛欠乏ラットでの骨密度の変化

○冨永 信子[1,3], 宮崎 孝[1], 荒木 隆一郎[1], 野寺 誠[1,2], 柳澤 裕之[1]

[1]埼玉医科大学 医学部 衛生学 [2]埼玉医科大学 保健医療学部 [3]埼玉医科大学 実験動物部門

講演２２

骨代謝調節における亜鉛の役割－亜鉛はβクリプトキサンチンの骨同化効果を増強する

○内山 聡志[1,2], 山口 正義[1]

[1]静岡県立大学大学院 生活健康科学研究科 代謝調節学 [2]日本学術振興会特別研究員 PD

７月１４日（金）：第３日目

グランシップ・会議室１００１－２（１０F）（第２会場）

セッション Ⅷ 毒性発現と微量元素 (14:10-15:10)

座長 園藤 吟史 （大阪市立大・医）

講演２３

硝酸カドミウム静脈内投与後の急性腎障害および有害全身影響

○土手 江美[1], 土手 友太郎[1], 三井 剛[1], 河野 公一[1]

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講演２４

フッ化カドミウム静脈内投与後の重篤な肝障害および腎機能低下について

○足立 和也[1], 土手 友太郎[1], 土手 江美[1], 三井 剛[1], 河野 公一[1]

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講演２５

低濃度フッ化水素酸静注後の急性腎機能障害、代謝性アシドーシスおよび電解質異常

○三井 剛[1], 土手 友太郎[1], 土手 江美[1], 足立 和也[1], 河野 公一[1]

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講演２６

二塩化ジブチルスズのマクロファージ系細胞のサイトカイン産生に対する抑制効果

○角田 正史[1], 山本 邦人[2], 伊藤 京子[1], 井上 葉子[1], 三木 猛生[1], 工藤 雄一朗[1], 佐藤 敏彦[1], 相澤 好治[1]

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セッション Ⅸ 栄養と微量元素 (15:10-16:10)

座長 木村 美恵子 （タケダライフサイエンスリサーチセンター）

講演２７

栄養指導による微量元素摂取の改善と粉末茶摂取に対する指導

住田 実穂[1], 森田 菜緒美[1], 松本 有加[1], 長井 明日美[1], 岩瀬 麻衣[1], 小島 沙耶香[1], 二宮 園子[1], 岩野 侑佳[1], 瀬古 友美[1], ○片山 直美[1,2]

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講演２８

鉄欠乏動物における貧血および血清脂質に及ぼす桑葉粉末の改善効果

○赤澤 典子[1], 鈴木 幸一[2], 世良 耕一郎[3]

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講演２９

経腸栄養患者における微量元素摂取量と血中濃度の変化

○齊藤 昇[1], 田畑 直人[2], 安藤 義久[2], 齊藤 三郎[1], 内野 勝子[3], 鵜島 雅子[1], 戸田 理一郎[1], 山口 泉[4]

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講演３０

授乳期間中の低亜鉛食給餌は仔ラットの離乳・成長後の食塩嗜好に影響を及ぼす

○駒井　三千夫[1]，田中　保憲[1]，白川　仁[1]
[1]東北大学　大学院農学研究科　栄養学分野

セッション　X　食品と微量元素　(16:10-16:55)

座長　　角田　正史　（北里大・医）

講演３１

市販の各種お茶飲料および中国茶浸出液中のフッ素濃度について

○新村　哲夫[1]，長瀬　博文[1]，荒谷　哲雄[1]，鏡森　定信[2]
[1]富山県衛生研究所　環境保健部　[2]富山大学　医学部　保健医学

講演３２

セレン強化スプラウトおよびセレン強化カボチャに含まれるセレンの栄養有効性

○吉田　宗弘[1]，佐野　菊江[1]，石幸　恵理[1]，福永　健治[1]，西山　利正[2]
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講演３３

フコイダン（オキナワモズク）による細胞内微量元素の変動

○高田　久嗣[1]，長嶺　竹明[1]，中里　亨美[2]，草壁　孝彦[1]，中島　克行[2]，鈴木　慶二[2]，酒井　卓郎[3]，及川　将一[3]，荒川　和夫[3]，伊波　匡彦[4]
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７月１４日（金）：第３日目

グランシップ・会議室１００１－１（１０F）（第３会場）

セッション　XI　微量元素の吸収・排泄機構　(14:10-15:10)

座長　　森山　光彦　（日本大・医）

講演３４

ラット腸管粘膜での亜鉛欠乏の影響

○野寺　誠[1,2]，宮崎　孝[1]，荒木　隆一郎[1]，冨永　信子[1,3]，柳澤　裕之[1]
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講演３５

ラットにおける亜鉛の吸収率と組織濃度に及ぼす飼料中亜鉛レベルの影響

○松田 芳和[1], 安部 麻美子[1], 小邨 奈未[1], 川島 朋絵[1], 福永 健治[2], 荒川 泰昭[3], 吉田 宗弘[2]

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講演３６

亜鉛による銅の腸管吸収阻害の分子機構の解析

○齋藤 健[1], 三浦 真理[2], 蔵崎 正明[3], 細川 敏幸[4], 柳沢 裕之[5]

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講演３７

大腸菌の銅排出系に関与するCu(I)酸化酵素CueO

○植木 優作[1], 今野 佑介[1], 片岡 邦重[1], 櫻井 武[1]

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セッション XII 微量元素の体内動態 (15:10-16:10)

座長 姫野 誠一郎 (徳島文理大・薬)

講演３８

気管内投与したビスマスとアンチモンの体内挙動の比較

○篠原 厚子[1], 千葉 百子[1,2], 佐藤 洋[3], 大前 和幸[4], 岡本 正英[5], 芹沢 弘二[5], 稲葉 裕[1]

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講演３９

疥癬に感染したハクビシン(Paguma larvata)の肝臓と腎臓における元素濃度および分布の変化

○鈴木 美成[1], 稲岡 恵[1], 鹿島 勇治[2], 斉藤 一三[3], 渡邊 泉[1], 久野 勝治[1]

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講演４０

健常成人の血清中フッ素イオン濃度

○板井　一好[1]，小野田　敏行[1]，野原　勝[1]，岡山　明[2]，大澤　正樹[1]，丹野　高三[1]，坂田　清美[1]

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講演４１

三朝温泉のホルミシス効果に関する研究

○大森　佐與子[1]，藤井　美代子[1]，堀内　公子[1]，御船　政明[2]

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セッション　ＸⅢ　微量元素の安全性評価　(16:10-16:55)

座長　　米谷　民雄　（国立医薬品食品衛生研究所）

講演４２

無機系抗菌剤の安全性評価（1）－市販抗菌加工製品に使用されている無機系抗菌剤の分析－

○中島　晴信[1]，大嶋　智子[2]，栗山　孝雄[3]，荒川　泰昭[3]

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講演４３

無機系抗菌剤の安全性評価（2）－人工汗・唾液による無機系抗菌剤及び加工布からの金属溶出－

○中島　晴信[1]，宮野　直子[1]，高塚　正[2]，栗山　孝雄[3]，荒川　泰昭[3]

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講演４４

無機系抗菌剤の安全性評価（3）－無機系抗菌剤が皮膚常在菌に及ぼす影響－

中島　晴信[1]，○高塚　正[2]，栗山　孝雄[3]，荒川　泰昭[3]

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7月14日（金）：第3日目
グランシップ・会議室910（9F）（第4会場）

セッション XIV 微量元素の新規機能性の開発 (14:10-14:40)
座長 佐治 英郎 （京都大・薬）

講演45

セレノール基をもたない合成セレン化合物の抗酸化活性

○齊藤 睦弘 [1], 梅本 大輔 [1], 松永 藍 [1], 佐藤 卓史 [1], 千熊 正彦 [1]
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講演46

2型糖尿病モデルマウスKK-A^{y}におけるエトキシピコリン酸亜鉛(II)錯体の血糖上昇抑制作用

○森下 美香 [1], 西出 緑 [1], 松本 衣代 [1], 安達 祐介 [2], 吉川 豊 [2], 桜井 弘 [2], 梶原 苗美 [1]
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セッション XV 微量元素関連の生体化学反応－1 (14:40-15:25)
座長 内海 昭 （フォルマン科学技術研究所）

講演47

血液中銅・ヒスチジン錯体及びCu,Zn-Superoxide Dismutaseによる過酸化脂質生成の抑制

井上 節子 [1], 中島 滋 [1], ○信太 直己 [2], 雨宮 有子 [2], 山倉 文幸 [3], 池田 啓一 [3], 岩井 秀明 [2]
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講演48

シュウ酸による銅含有酵素チロシナーゼの阻害に及ぼす長鎖脂肪酸及びその塩類の緩和効果の検討

○平岡 厚 [1], 白尾 美佳 [2], 笠井 博子 [3], 津吹 政可 [3]
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講演49

ヒドロキシモノアジン及びジアジン型複素環化合物のラットにおける劣化ウラン除去効果

○加藤　明良[1]，池田　瑞代[2]，中村　麻利子[2]，福田　　俊[2]
[1]成蹊大学　理工学部　物質生命理工学科 [2]放射線総合医学研究所緊急被ばく医療研究センター

セッション　XVI　微量元素関連の生体化学反応－２　(15:25-15:55)
座長　　千熊　正彦　（大阪薬大）

講演５０
ニコチンアミド補酵素と金属の共存下で誘導されるプリオンタンパク質の凝集
○白石　則之[1]，錦見　盛光[1]
[1]和歌山県立医科大学　生化学

講演５１
核内 RNA/DNA 結合タンパク質 $p54^{nrb}$/NonO の銅結合性の解析
○渡辺　雅紀[1]，手塚　雅勝[1]
[1]日本大学　薬学部　衛生化学研究室

セッション　XVII　微量元素の分析技術開発　(15:55-16:55)
座長　　原口　紘炁　（名古屋大・工）

講演５２
2D capillary HPLC-ICP-MS を用いたナノスペシエーションによるアイソフォーム特異的メタロチオネインノックダウン細胞中のメタロチオネインの検出
○宮山　貴光[1]，小椋　康光[1]，鈴木　和夫[1]
[1]千葉大学大学院　薬学研究院

講演５３
HPLC-誘導結合プラズマ質量分析による血清中セルロプラスミンの検出と Wilson 病診断への応用
○小林　健司[1]，勝矢　陽子[1]，アブドゥラ　リズキー[1]，藤澤　千恵[2]，長嶺　竹明[3]，森川　昭廣[4]，村上　正巳[5]，児玉　浩子[2]，小山　洋[1]
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講演５４

フローインジェクション法によるフッ化ナトリウム添加培養液中のフッ素イオン濃度の検討

○佐藤　勉[1]，田中　とも子[1]，八重垣　健[1]，板井　一好[2]

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講演５５

微量金属測定用真空採血管内の汚染金属

○川崎　健司[1]，菅野　光俊[1]，野本　昭三[2]

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The 17th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE2006) in Shizuoka, Wednesday, July 12 – Friday, July 14, 2006

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Abbreviation　略語

CESL : Civil Extension Special Lecture　市民公開講座
SL : Special Lecture　特別講演
EL : Educational Lecture　教育講演
FL : Focus Lecture　フォーカス講演
TL : Topic Lecture　トピック講演
S : Symposium　シンポジウム
AL : Award Lecture　受賞講演
SS : Session　セッション
Chair : Chairman/Chairperson　司会者／座長

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日本微量元素学会

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第17回日本微量元素学会発表内容の
学会誌「Biomedical Research on Trace Elements」への掲載について

日本微量元素学会 講演者各位

「特別講演」、「教育講演」、「フォーカス講演」、「トピック講演」、「シンポジウム講演」は特集号17巻3号以降に特集総説として順次掲載されます。

一般講演の内容は査読の上、17巻4号(平成19年1月1日発刊予定)以降に短報として順次掲載されます。発表時の討論を踏まえて原稿を作成し、平成18年7月31日までに、日本微量元素学会誌(BRTE)編集部に e-mail で御投稿ください。投稿形式は、学会発表研究(短報)の投稿規定 (BRTE 誌16巻3号の学会情報 app.22、および日本微量元素学会ホームページ: http://brte.umin.ne.jp/journal/contribution_society/) をご参照ください。

なお、一般講演の内容は例年全ての講演者に御投稿頂いておりますので、何卒宜しくお願いいたします。

記

学会発表研究の投稿締切日: 平成18年7月31日

投稿先: 日本微量元素学会誌(BRTE)編集部

e-mail: brte@saitama-med.ac.jp

Phone: 049-276-1168

Fax: 049-294-6907

以上

日本微量元素学会誌
Biomedical Research on Trace Elements
編集委員長 柳澤裕之

日本微量元素学会編集部
〒350-0495 埼玉県入間郡毛呂山町毛呂本郷38
埼玉医科大学・医学部・基礎医学部門衛生学内
Tel: 049-276-1168 Fax: 049-294-6907
e-mail: brte@saitama-med.ac.jp

第１８回日本微量元素学会のお知らせ（第１報）

The 18th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements

（BRTE 2007）

ホームページ：http://www.health-info.jp/brte/top.htm

会期：2007 年（平成 19 年）7 月 5 日（木）－ 7 月 7 日（土）

会長：糸川　嘉則（仁愛女子短期大学）

〒910-0124　福井市天池町４３－１－１

Tel：0776-56-1133　Fax：0776-56-2922

会場：福井県三国観光ホテル

〒913-0048　福井県坂井郡三国町緑ヶ丘４丁目４－８

TEL：0776-81-3111　　FAX：0776-81-3235

URL:：http://www.mikuni-hotel.com/index.htm

第 18 回日本微量元素学会事務局：
タケダライフサイエンスリサーチセンター
〒600－8813　京都市下京区中堂寺南町 134　京都リサーチパーク
Tel：075-315-7275　　Fax：075-315-7107
e-mail：tls@takedahp.or.jp
ＵＲＬ：http://www.health-info.jp/brte/top/htm
連絡係：山下智栄

プログラムその他の詳細は、順次、決定次第ホームページに掲載させていただきます。

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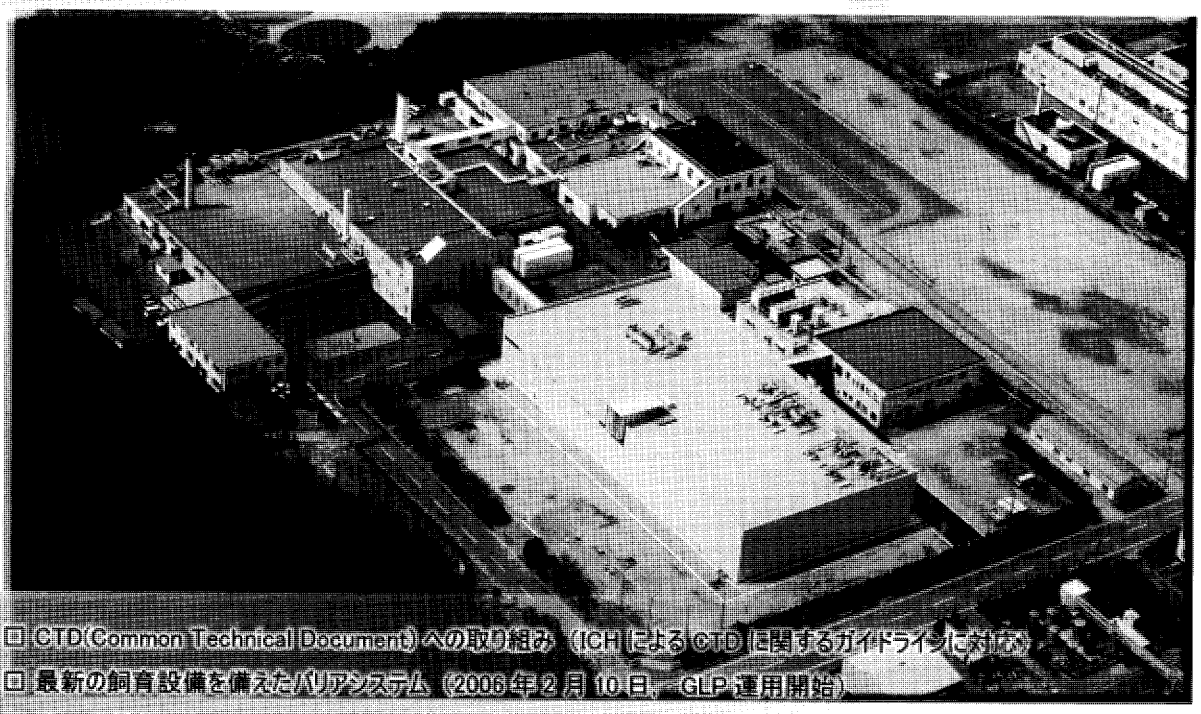
豊富な毒性病理学的知識に裏打ちされたメカニズム解析試験の実施、
確かな技術と信頼をベースにした皆様のパートナー



□ CTD(Common Technical Document)への取り組み（ICHによるCTDに関するガイドラインに対応）
□ 最新の飼育設備を備えたバリアシステム（2006年2月10日、GLP運用開始）
□ 第三者機関による Peer Review EPLとの業務提携
□ SAS transport file の提供

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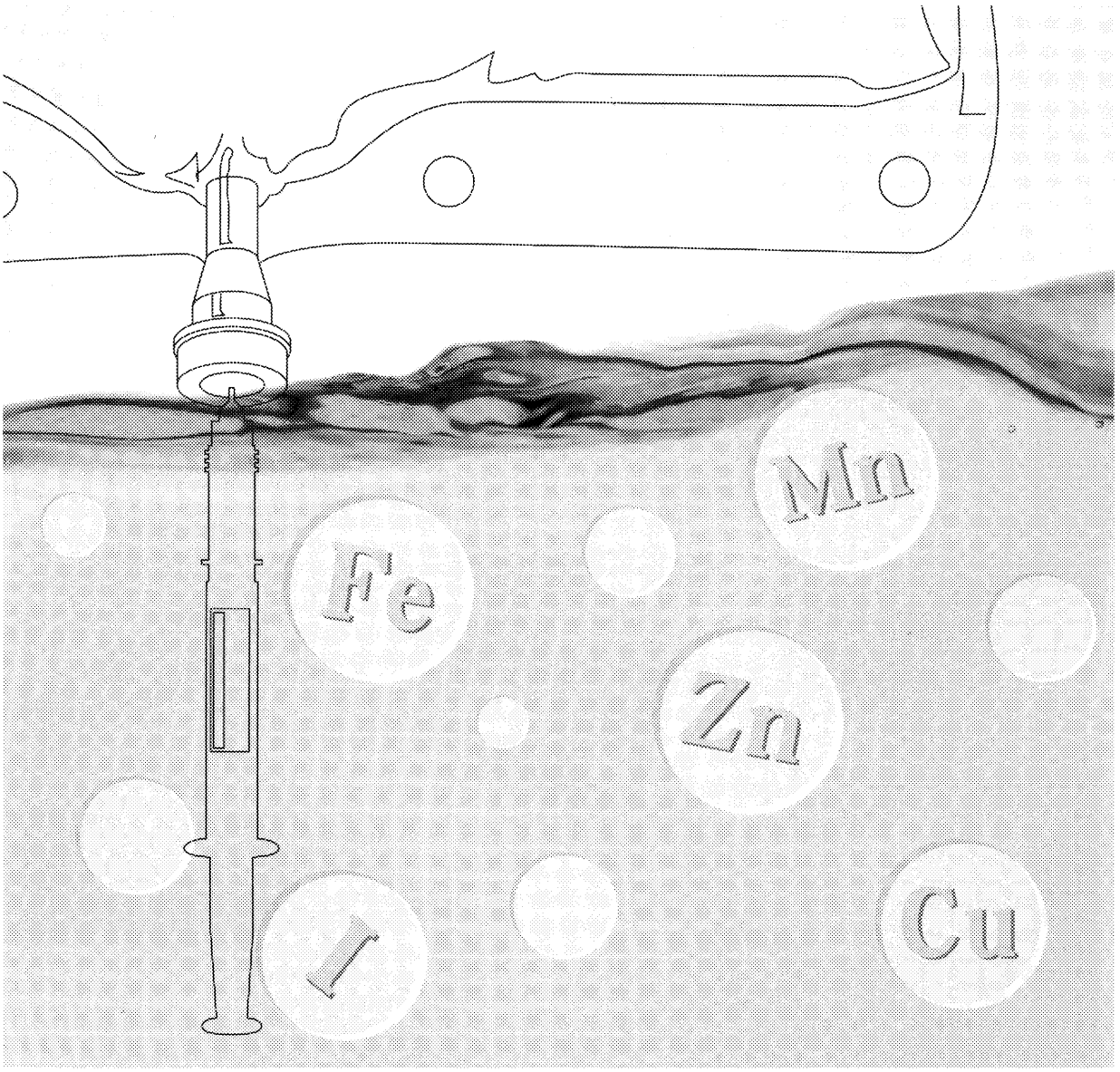

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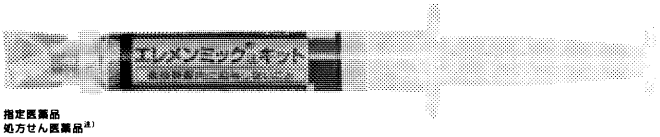
注）注意-医師等の処方せんにより使用すること



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★「効能・効果」、「用法・用量」、「禁忌を含む使用上の注意」等詳細は現品添付文書をご参照ください。

発売（資料請求先）
味の素ファルマ株式会社 学術研修部
〒104-8315 東京都中央区京橋一丁目15番1号

製造販売
味の素株式会社
〒104-8315 東京都中央区京橋一丁目15番1号

2005年6月作成
EMI・JB5・0605・DNP

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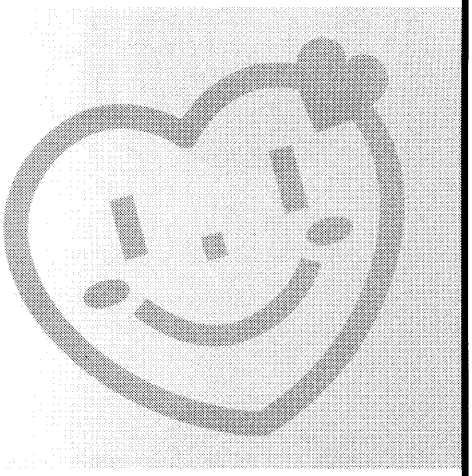

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お問い合わせ先
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TEL 054(664)0802　FAX 054(664)0803

富士山の Vanadis（バナディス） 水を超えた水

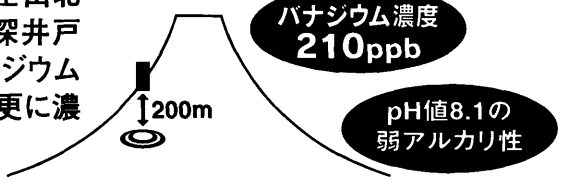
天然バナジウムウォーター

1000mℓあたりバナジウム210μg

カラダ舞い、健康が求める霊峰の水。

バナジウムは人間の健康を保つために欠かせない必須微量元素の可能性が提唱され、健康を気づかう現代に様々な研究が進められております。富士山麓の限られた地域では、雪どけ水や雨が長い年月をかけゆっくりと地層に浸透し地中深くに蓄えられ清澄な伏流水となります。その伏流水には貴重なバナジウムが豊富に含まれております。

「富士山のVanadis」は富士山北麓の標高1000mで200m深井戸より汲み上げた高濃度バナジウム水を、現地急速氷結方式で更に濃度を高めたものです。



栄養成分（1000mℓ当り）

エネルギー	0kcal
たんぱく質,脂質,炭水化物	0g
ナトリウム	11mg
カルシウム	15mg
マグネシウム	4.7mg
カリウム	1.0mg
バナジウム	0.21mg (210μg)
亜鉛	0.02mg (20μg)

硬度	56mg/ℓ
pH値	8.1

「富士山のVanadis」は製法特許申請中です。

株式会社 グリーンステージ
本　社／〒103-0014 東京都中央区日本橋蛎殻町二丁目6-6-204　TEL0120-640-611（フリーダイヤル）
<ホームページ>http://www.greenst.co.jp

田丸屋の わさび漬

愛され続けて１３０年、今も昔も変わらぬ味。

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株式会社 田丸屋本店
〒420－0852
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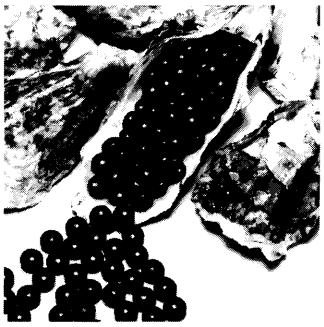
http://www.tamaruya.co.jp/

牡蠣

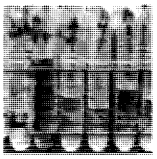
「栄養補助食品」であるということ。

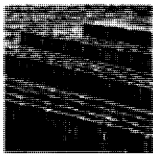
「健康食品」という言葉が日常にあふれて、既に久しくなります。また、新たに関連した様々な表現、表示、形容も生まれてきています。しかし規則正しい日常における、栄養豊かな食生活そのものが本来の「健康食品」であるという考えに基づき、日本クリニックのかき肉エキスは一九七四年に創業以来、今日まで一貫して、「栄養補助食品」と積極自主表示を展開して参りました。栄養とは何か、それを補助するとは如何なることかの素朴な観点に立って、遙かな時代から誰でも知っていた「牡蠣」、同時に誰もが知り得なかった「牡蠣」の素晴らしさを今後も真摯に見つめて参ります。



日本クリニックかき肉エキスの特許取得

1. かき肉エキス製法方法	第1770901号
2. 血小板凝集抑制作用物質（Z物質）	第1813693号
3. リチウム濃度増強剤	第1906437号
4. 血小板凝集抑制剤	第2007451号 第2007452号
5. 精神分裂病治療用組成物	第1932241号
6. かき肉エキスの低分子画分	第2120831号
7. 抗酸化組成物	第5925382号
8. 亜鉛等ミネラルW抽出型かき肉エキス製造方法	第3429726号
9. ミネラル吸収促進物質（FC物質）	第3267962号
● 牡蠣由来「純正タウリン」生成抽出方法	（出願済）
● かき肉エキス低吸湿パウダー	（出願済）
● 牡蠣の銅成分強化抽出方法	（出願済）
● 牡蠣成分による抗腫瘍剤	（出願済）





牡蠣 その主要栄養素

〈ミネラル〉
亜鉛・ナトリウム
カルシウム・鉄・カリウム
リン・マグネシウム・銅・マンガン
ヨード・セレン・総クロム
リチウム・コバルト

〈ビタミン〉
ビタミンB_1
ビタミンB_2・ビタミンB_6
ビタミンB_{12}・葉酸・ビオチン
イノシトール
コリン

〈炭水化物〉
グリコーゲン

〈アミノ酸〉
タウリン・アルギニン・リジン
ヒスチジン・フェニルアラニン
チロシン・ロイシン・イソロイシン
メチオニン・バリン・アラニン・グリシン
プロリン・グルタミン酸・セリン
スレオニン・アスパラギン酸
トリプトファン・シスチン

牡蠣の神秘を科学で探究する　かき肉エキスのパイオニア
日本クリニック株式会社
本社／〒616-8555 京都市右京区太秦開日町10番地の1
http://www.japanclinic.co.jp

ほら、未来が見えてきた。

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点滅するいま、より軽く、より強
く、よりリサイクルしやすい素材
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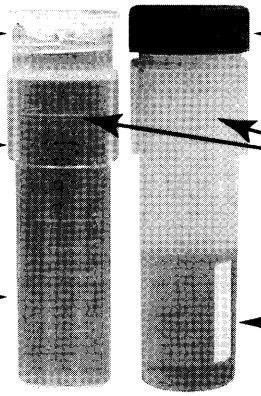
トータルコストを削減し、器具洗浄の手間からあなたを開放します

●専用時計皿(オプション)
還流分解に対応

●ラックロック機能
専用ラックで回転防止
片手でフタが開閉可能

●試料希釈機能
5mL間隔のメスシリンダー



●試料保管機能
専用スクリューキャップ

●試料計量機能50mLラインは、クラスAの精度保証計量証明書が添付します
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計量証明証添付 50mLクラスA 最大容量65mL	メスシリンダー機能 5mLメス入り	酸分解可能 専用ブロック DigiPREP対応	中期保管可能 スクリューキャップ 時計皿対応	オートサンプラーラック そのままセット

※詳しい資料をご希望の方は下記問い合わせ先まで請求してください。資料請求No. IO0005

GL Sciences ジーエルサイエンス株式会社

本社 営業企画課
〒163-1130 東京都新宿区西新宿6丁目22番1号 新宿スクエアタワー30F
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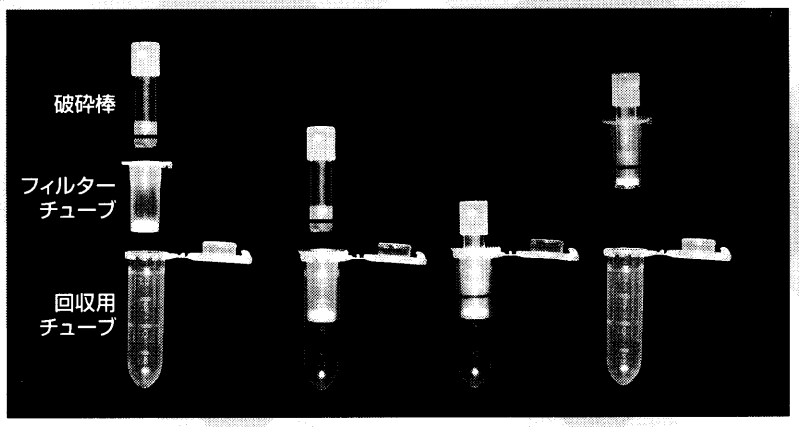
nippi 遠心分離機を用いて固形試料を簡単に破砕できる
ディスポーザブル・フィルターチューブ

研究用

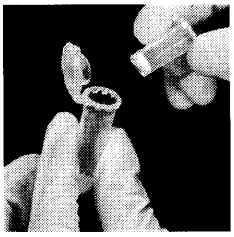
バイオマッシャー

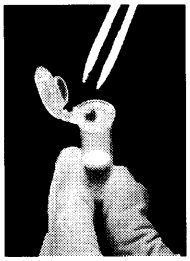


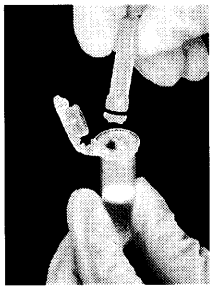
特長

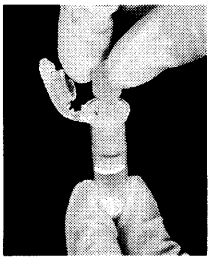
- ■短時間
- ■簡単操作
- ■遠心分離機を使用
- ■固形試料を簡単破砕
- ■固い試料のすり潰し O.K.

わずか数分間で固形試料をホモジナイズ可能

1

フィルターチューブの爪を回収用チューブのヒンジ部に合わせるようにセットします。

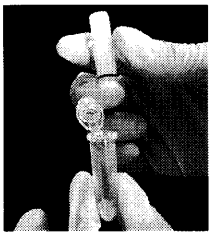
2

試料をフィルターチューブの中に入れます。

3

破砕棒を上から挿入して押し込みます。

4

固い試料の場合は，破砕棒を手で押し込みながら回転させて試料を破砕します。

5

遠心分離器にセットし，12,000 ～ 15,000 × g で 10 ～ 30 秒間の遠心分離を行います。

6

回収用チューブに破砕された試料が集められます。使用済みのバイオマッシャーは廃棄して下さい。

※詳細は，当社テクニカルサポート（下記参照）までお問い合わせ下さい。

発売元
フナコシ株式会社
〒113-0033 東京都文京区本郷2丁目9番7号
http://www.funakoshi.co.jp/ e-mail：info@funakoshi.co.jp

機器
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e-mail：sales@funakoshi.co.jp

YASUI KIKAI

実績
- ■厚生労働省・国立感染症研究所・Cancer Reserch UK・国立医薬品食品衛生研究所・農林水産省植物防疫所・動物衛生研究所・水産総合研究センター
- ■BSE検査：全国の食肉衛生検査所・家畜保健衛生所等
- ■DNA鑑定：科学警察研究所・全国の警察本部・科学捜査研究所
- ■その他：全国の大学・国立研究機関など多数
- ■マルチビーズショッカーの使用例文献：Nature.、PNUS.等多数

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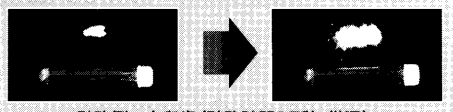
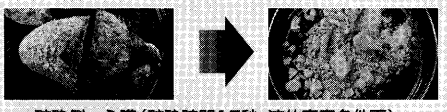
※別紙仕様書をご参照下さい

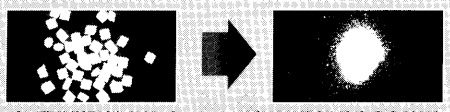
バイオハザード対策
サンプルホルダー
取り外し可能・
オートクレーブ
滅菌可能

- ●多試料を完全密閉容器内で同一条件で精密に粉砕！
- ●研究者に安全、確実に試料の粉砕が行えます！
- ●凍結、冷却、常温など破砕温度条件が選択可能。
- ●サンプル間のクロスコンタミなくほぼ完全回収可能！
- ●科学警察研究所はじめ全国の科学捜査研究所で実績！
- ●多試料のグリーン調達用前処理に最適！


破砕例　小臼歯（破砕時間15秒・常温）


破砕例　心臓（破砕時間15秒・液体窒素条件下）

メノウ（モース硬度：6.5-7）：10秒（常温）


ポリ乳酸樹脂（生分解プラスチック）：10秒（液体窒素条件下）

特徴
- ■硬い組織（臓器・歯・骨・珊瑚・昆虫・木材チップ・穀物・種子・イネ生葉・クロレラ等）から微生物まで市販のディスポーサブルチューブ内（密閉状態）で破砕可能！
- ■冷却破砕用サンプルホルダー（0～5℃で破砕可）を各種用意！
- ■DNA・RNA・酵素・蛋白質・脂質・糖質の抽出に最適！
- ■一度に多検体の破砕が可能。（2㎖：24～56本、12㎖：3～8本、50㎖：3～4本、100㎖：3本、96ディープウェル：2枚〈1.2㎖：192本〉等）
- ■サンプル量は2㎖のチューブの他、0.5㎖～100㎖等までの市販のチューブ・96ウェルがホルダーに取付可能（オプション）。特殊チューブも制作可能。（100㎖は微生物用）

用途
- ■生物体からの微量元素（重金属）の分析
- ■ChIP-chip法に用いる蛋白質の抽出・可溶化
- ■皮膚組織などから微生物のDNA抽出・臓器などからウイルスのRNA抽出
- ■イネ生葉・米粒・麦芽からDNA・RNA抽出（液体窒素による凍結破砕も可）
- ■ヒトがん組織・骨・歯からのDNA・RNA蛋白抽出（薬理試験・科学捜査研究等）
- ■大腸菌からの組み換え蛋白及び活性のあるS30画分の抽出等（低温破砕ホルダー使用による）
- ■酵母からの活性タンパク質抽出（低温破砕ホルダー使用による）
- ■各種動物組織からのプリオンタンパク質抽出（BSE検査キットに対応）
- ■酵母からの活性タンパク質抽出（4.0℃破砕）

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〒108-0014　東京都港区芝5-9-12　金沢ビル1階　TEL.03-5443-8489（代表）　FAX.03-5443-6430　http://www.yasuikikai.co.jp

∞∞∞ 食品化学新聞社　定期媒体のご案内 ∞∞∞

食品の機能と健康を考える科学情報誌

FOOD STYLE 21

フードスタイル21

毎月１日発行、Ａ4判、100ページ前後
年間購読 24,000円（税、送料込み）
１冊2,500円

「FOOD　Style　21」は、食品成分(素材)の生理機能や生活習慣病予防に関する最新の研究を分かりやすく解説するとともに、その応用技術、開発商品を積極的に紹介しています。
また東洋医学や伝統的な民間療法についても現代科学の眼で解明していき、より健全で快適な食生活の実現を志向する食品産業の新分野を拓く科学情報誌です。
1997年６月の創刊以来、健康食品および一般食品メーカーの研究者をはじめ、大学、研究機関などの多くの方にご購読いただいております。

健康食品新時代をリードする

健康食品新聞 HJ Health Food Journal

HJ 健康食品新聞

毎週水曜日発行、輪転オフセット大判
平均４～６ページ建て
年間購読 21,000円（税、送料込み）

健康食品市場をトータルに捉えた総合情報紙として、「健康食品新聞」は2003年4月から週刊化しました(2002年５月創刊)。正確な情報伝達、的確な製品選択が可能な信頼あるメディアとして、食品成分の生理活性機能、素材・商品開発、技術、行政動向、流通・市場動向、海外動向マーケティング、学術資料など最新情報を分かりやすく読みやすく解説した紙面作りをしています。

定期購読のお申込み ・ バックナンバーご購入
その他お問い合わせ

㈱食品化学新聞社　健康食品事業部

〒101-0051 東京都千代田区神田神保町3-2-8　昭文館ビル
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本当に患者さんのために役立つ情報誌を読んでいますか？

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2006 Vol.88 No.7

7月号 特集テーマ

日常臨床で知っておきたい 微量元素の知識

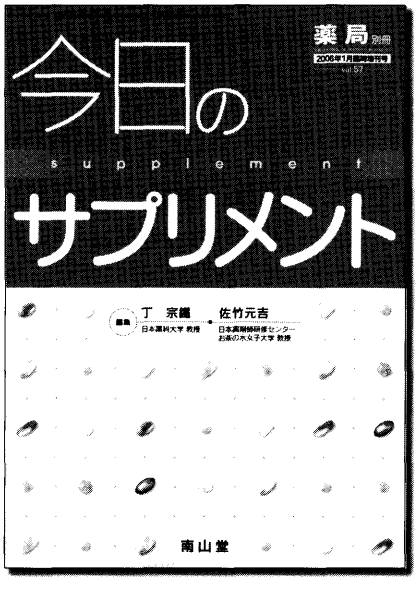
最近の荒れた食生活・ダイエットによる微量元素の欠乏．また，健康食品等による過剰摂取など問題となっており，疾患との関わりも示されています．本特集では，微量元素の知識と臨床への応用について取り上げます．

薬局 2006年1月別冊（臨時増刊号）

今日のサプリメント

編集　丁　宗鐵　日本薬科大学 教授

佐竹元吉　日本薬剤師研修センター／お茶の水女子大学 教授



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「サプリメント」解説書の決定版！！

本書の3つのポイント

① 本分野で活躍する第一線の医師・薬剤師等による執筆！
② 医薬品との相互作用など薬剤業務で活用できる情報満載！
③ 消費者・患者からのサプリメントの相談に役立つ付録が充実！

医療スタッフとしてサプリメントに関する的確なアドバイスや情報提供にお役立ていただける一冊です！！

南山堂　〒113-0034 東京都文京区湯島4-1-11　TEL 03-5689-7855　FAX 03-5689-7857（営業）
URL http://www.nanzando.com　E-mail eigyo_bu@nanzando.com　振替 00110-5-6338

本日の、"日本微量元素学会"でお使い頂いております、日本アビオニクス製のプロジェクタです。

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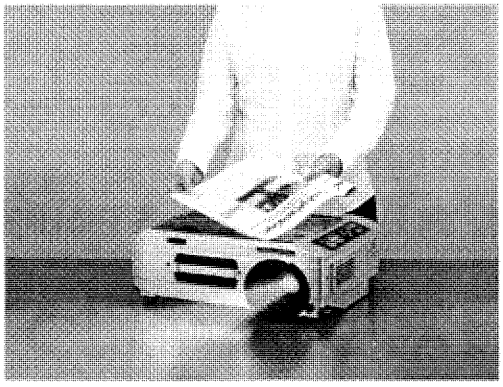

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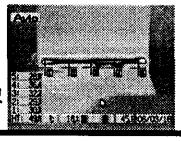
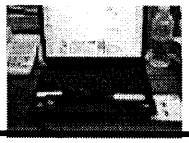
- 使いやすさを追求した斬新なエルゴノミックデザイン
- 研究開発や非破壊検査など多様なニーズにお応えします。

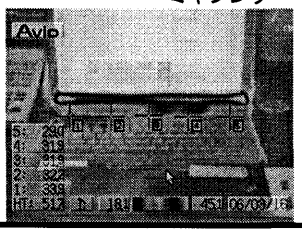


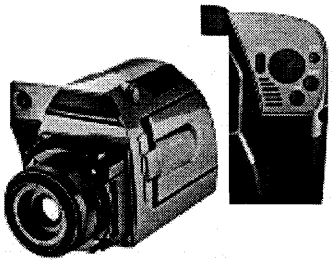
熱画像



可視画像

ミキシング





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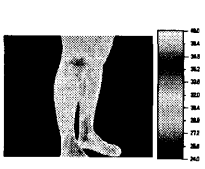
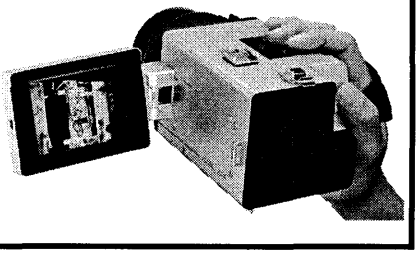
小型・軽量・簡単操作で優れたコストパフォーマンス！

測温現場での温度分布測定を強力にアシスト

- ☆ 超軽量1.6kgを実現
- ☆ 屋外でもはっきり見える半透過型カラー液晶モニタ
- ☆ 堅牢なアルミボディによる防塵防沫構造（IP54）
- ☆ 国産センサ採用による優れたメンテナンス性
- ☆ ロープライス

※非冷却型サーモグラフィの同等クラス品において





■ お問い合わせ先

日本アビオニクス販売株式会社　営業本部第一営業部　岡本

TEL (045) 304-8239/ FAX (045) 304-8248

弊社ホームページにて製品情報を提供しています。　http://www.aviohanbai.co.jp/

サプリメントの基本は、「安心・安全」だと、ファンケルは考えます。

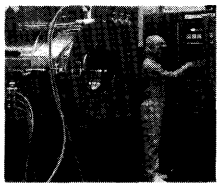
POINT 1 品質・管理

ファンケルのサプリメントは、合成着色料・香料・保存料すべて無添加。天然素材100%。
「植物由来カプセル」を採用。

BSEを避ける カプセル使用。

さらなる安全性の確保のために、牛などの動物由来のハードカプセルを全て植物由来へ変更。化学合成を行わない、天然素材100%を原料とするカプセルを、サプリメントに世界で初めて採用しました。

VEGETABLE CAPSULE 植物由来カプセル使用



◀最新鋭の工場で品質管理。

POINT 2 学術情報

サプリメントと薬の
「相互作用検索システム」
を開発しました。

安心してお飲みいただくために。

医師、薬剤師、管理栄養士、臨床検査技師などから構成される学術本部を組織し、サプリメントと医薬品の飲み合わせに関する情報を瞬時に検索できるデータベースを構築しました。

科学的根拠に基づいた膨大な情報量。

SDIシステムでは、国内外1400を超える学術論文を調査。全ての医薬品とファンケルサプリメントの相互作用情報が収められています。

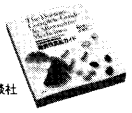
医療用医薬品 約19,000種類 ＋ 一般用医薬品 約13,000種類 × FANCL健康食品 約100種類

オペレーティングシステム〈SDI窓口を開設〉
※SDIシステムを活用した電話相談サービスを開始しました。

※SDI=Supplement&Drug Interaction

「医療従事者のための機能性食品ガイド」が誕生。

医師や薬剤師、栄養士の方にサプリメントの機能や科学的根拠を理解していただくための実用書です。 発行:講談社



ファンケル「安心・安全」へのこだわり

POINT 3 研究・開発

厳しい基準に添った
ハイレベルな研究・開発に取り組む
「健康食品研究所」。

最先端科学と研究者の情熱が結集。

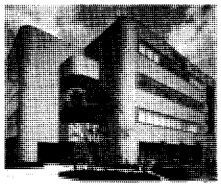
ファンケル独自の厳しい基準をクリアする独自の理論や処方の開発に取り組み、350件の特許を出願。研究機関との共同開発も積極的に行い、その成果を学会で広く発表しています。

妥協を排除し原料を厳選。

安全性情報の確認や刺激性試験の実施など、厳しい安全基準に合格した原料だけを抽出。目的にあった優れた機能を持つ成分だけを厳選配合しています。

安全基準確立のための寄附講座「生体安全医学講座」を開設。

最先端の遺伝子解析システムを用い、サプリメントによる生体へ及ぼす影響など、吉川敏一教授(京都府立医科大学内科学教室 生体機能制御学講座)を中心に構成されています。



◀ファンケル健康食品研究所

POINT 4 生産・製造管理

国際標準規格(ISO)認証、
厳しい管理基準の
「生産工場」で
一貫した管理体制。

自社工場だから安心。

最新鋭の設備、機械を導入した自社工場で、高い規格に基づき、一貫管理体制で生産。機械と人の目による厳正な品質管理など、あらゆるプロセスに妥協はありません。

信頼の国際規格を認証取得。

ファンケルでは本社、研究所、工場、お客様対応を含めグループ全体で品質マネージメントシステムを保証するISO9001、環境マネージメントシステムを保証するISO14001を取得しています。

2000年11月 ISO9001認証取得　2002年11月 ISO14001認証取得

あなたを想うサイエンス。 FANCL

株式会社ファンケル
横浜市中区山下町89-1
〒231-8528

ファンケルのミネラルサプリメント

イライラや骨密度が気になり始めたら

カルシウムマグ
ツイントース配合

牛乳や魚などが摂れないときに。ツイントース配合で吸収力アップ。
保健機能食品(栄養機能食品)

商品番号 5058　472円
180粒(1粒の重量335mg)/約30日分

商品番号 5058-03　1,260円
徳用タイプ540粒(1粒の重量335mg)/約90日分

顔色が悪い、朝起きられない方に

鉄
ツイントース配合

女性の2人に1人が鉄不足。サプリメントでしっかり補給しましょう。
保健機能食品(栄養機能食品)

商品番号 5059　945円
120粒(1粒の重量240mg)/約30日分

商品番号 5059-03　2,520円
徳用タイプ360粒(1粒の重量240mg)/約90日分

お肌も体も疲れてる?と感じる時に

亜鉛
ツイントース配合

新陳代謝やコラーゲン生成に必須の亜鉛。疲れてるかな?と思ったら。
保健機能食品(栄養機能食品)

商品番号 5066　840円
120粒(1粒の重量252mg)/約30日分

商品番号 5066-03　2,250円
徳用タイプ360粒(1粒の重量252mg)/約90日分

10種類のミネラルをバランスよく配合

マルチミネラル
ツイントース配合

必要なミネラルをマルチに配合。ツイントース配合で吸収力アップ。
保健機能食品(栄養機能食品)

商品番号 5061　892円
180粒(1粒の重量272mg)/約30日分

商品番号 5061-03　2,310円
徳用タイプ540粒(1粒の重量272mg)/約90日分

カルシウムを手軽においしく

「かんで食べるサプリメント」
どこでもカルシウム
ツイントース配合

カルシウムは骨形成に必須。手軽にしっかり補給!ヨーグルト風味
保健機能食品(栄養機能食品)

商品番号 5210　1,470円
7日分(42粒)X4袋(1粒の重量750mg)/約4週間分

商品番号 5210-03　3,885円
徳用タイプ1箱(4袋)X3(1粒の重量750mg)/約12週間分

鉄分を手軽においしく

「かんで食べるサプリメント」
どこでも鉄分
ツイントース配合

鉄の吸収を助けるツイントース、にビタミンB_{12}・葉酸を配合。プルーン風味
保健機能食品(栄養機能食品)

商品番号 5212　1,470円
7日分(21粒)X4袋(1粒の重量800mg)/約4週間分

商品番号 5212-03　3,885円
徳用タイプ1箱(4袋)X3(1粒の重量800mg)/約12週間分

8種の栄養素が5粒で摂れる

グッドチョイス
ベーシック

ビタミン、カロテン、カルシウム&マグネシウム、亜鉛、コエンザイムQ10など、8つの栄養素を1パックにしました。

商品番号 5186　1,365円
30袋(1袋中5粒)/10~30日分

商品番号 5186-03　3,570円
徳用タイプ90袋(1袋中5粒)/30~90日分

8種の基本栄養素とHTCコラーゲン・鉄を1パックに

グッドチョイス
W

基本的な8つのビタミン・ミネラルに、女性に不足しがちな鉄分と美容に役立つHTCコラーゲンをプラス。

商品番号 5188　2,205円
30袋(1袋中7粒)/10~30日分

商品番号 5188-03　5,880円
徳用タイプ90袋(1袋中7粒)/30~90日分

8種の基本栄養素とウコン・DHAを1パックに

グッドチョイス
M

基本的な8つのビタミン・ミネラルに、ウコンとDHAをプラスしました。飲み過ぎ、偏食が気になる男性に。

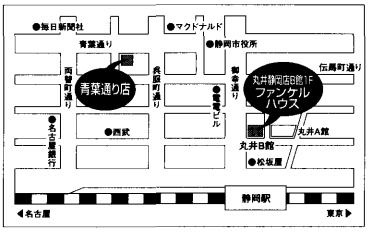
商品番号 5187　2,205円
30袋(1袋中7粒)/10~30日分

商品番号 5187-03　5,880円
徳用タイプ90袋(1袋中7粒)/30~90日分

FANCL HOUSE

ファンケルハウス 静岡青葉通り店
静岡市呉服町
営業時間 10:30~20:00
TEL.054-251-5789(直通)

ファンケルハウス 丸井静岡店
丸井静岡店B館1F
営業時間 10:30~20:00
TEL.054-255-3837(直通)



左記2店舗にて「公開講座に参加した。」とお申し出いただいた方に

「どこでもカルシウム」サンプル(6粒入り)差し上げます。

(お一人様1個1回限り)

期間:7月31日(月)まで。*無くなり次第終了。

商品のご注文 フリーダイヤル 0120-30-2222
[朝9時~夜9時/年中無休] *携帯電話・PHSからもご利用いただけます。

インターネット http://www.fancl.co.jp/
[24時間受付/年中無休] *携帯電話からもご利用いただけます。

サプリメントに関するご相談は[サプリメント相談室] フリーダイヤル 0120-750-210
[平日/朝9時~夜9時、日・祝日/朝9時~夕方5時] *携帯電話・PHSからもご利用いただけます。

あなたを想うサイエンス。
FANCL
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横浜市中区山下町89-1
〒231-8528

※「ツイントース」は(株)ファンケルの登録商標です。