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Abstracts of The 35th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE-2024)

JSBRTE
Japan Society for
Biomedical Research on Trace Elements

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Japan Society for Biomedical Research on Trace Elements

Abstracts of
The 35th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements
(BRTE-2024)

"Study on trace elements in society"

Tokorozawa Civic Cultural Centre, Saitama, Japan

20-21 September 2024

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Co-sponsored by

- The committee for Allergy and Immunotoxicology (AIT), Japan Society for Occupational Health (JSOH)
- Allergy & Immunotoxicology, International Commission on Occupational Health (ICOH)

Organizing committee

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Abstracts of the 35th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements (BRTE-2024)

"Study on trace elements in society"

Preface

It is my great pleasure to publish here the Abstracts of the 35th annual meeting of the Japan Society for Biomedical Research on Trace Elements (JSBRTE) as a supplement issue of Metallomics Research.

JSBRTE was established at 1990 to activate a broad range of research fields about metal/metalloids and to provide a platform of researchers. JSBRTE has organized annual meetings in every year from 1990. At 2024, the 35th annual meeting of JSBRTE (BRTE-2024) was held at MUSE, Tokorozawa civic cultural center, Tokorozawa, Saitama, Japan from September 20-21, 2024. In the annual meeting, approximately 100 researchers, doctors and students who were interested in metals/metalloids research gathers and discussed. In annual meetings, the book for Japanese abstracts has been published as an issue of Biomedical Research on Trace Elements (BRTE), the official journal of JSBRTE. In this meeting, we decided to publish the book for English abstracts as a supplement issue of Metallomics Research. Metallomics Research is an official journal of JSBRTE, and established at 2021. Metallomics Research is an international, peer-reviewed, open access journal publishing significant and novel contributions on the roles of metals, metalloids, and other trace elements in biological function. The journal welcomes investigations from a broad range of research fields including analytical chemistry, biochemistry, toxicology, epidemiology, nutrition, pharmacology, medical sciences, environmental sciences, health sciences, agriculture sciences, and plant biology. We believe that this abstract book will be effective for the international presentation for our research.

Masashi Tsunoda, M.D., Ph.D.
Congress Chair of BRTE-2024
Professor, Department of Preventive Medicine and Public Health
National Defense Medical College, Japan

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[1]The Jikei University School of Medicine, [2]Department of Public Health and Environmental Medicine, Faculty of Medicine, The Jikei University School of Medicine

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[1]Department of Epidemiology and Environmental Health Juntendo University Faculty of Medicine, [2]Juntendo University Faculty of Pharmacy Laboratory of Molecular Biology, [3]Department of Public Health Graduate School of Medicine Juntendo University

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Department of Preventive Medicine and Public Health, National Defense Medical College

S-08: The beneficial effect of zinc supplementation therapy on immune function decline caused by zinc deficiency

Takamasa KIDO[1], Machi SUKA[1], Hiroyuki YANAGISAWA[2]
[1]Department of Public Health and Environmental Medicine, The Jikei University School of Medicine, [2]The Jikei University School of Medicine

Special Lecture

I-01: Effects of immune challenges and the role of sex in the neurotoxicity of manganese

Nikolay M. FILIPOV[1], Helaina D. LUDWIG[1], Ryan S. MOTE[1], Jessica M. CARPENTER[1], Tuhina GUPTA[2], Kaori SAKAMOTO[3]

Departments of [1]Physiology and Pharmacology, [2]Infectious Diseases, and [3]Pathology, University of Georgia, United States

Manganese (Mn), while an essential trace metal and vital for key biological processes, is neurotoxic when overexposure takes place in both occupational and non-occupational settings. Mn overexposure leads to brain accumulation and basal ganglia dysfunction; (neuro)inflammation is viewed to be a major contributor for this dysfunction. In certain occupations, such as mining, Mn overexposure coincides with increased bacterial burden with the highly persistent pathogen *Mycobacterium tuberculosis* (Mtb). Because Mn accumulates in the basal ganglia, we conducted a study where male C57BL/6 mice were administered Mn into the globus pallidus (GP) and then inoculated with Mtb intravenously. One, 2, and 5 weeks after Mn/Mtb treatment, brains were dissected and processed for neurochemical and immunohistochemcal analyses of, respectively, monoamines, and microglia/astrocyte activation. There was a dose- and time-dependent (maximal at 2 weeks) decrease of striatal dopamine and its metabolites on the injected site of the brain and concomitant, predominantly unilateral, glial activation (increase of the microglia and astrocyte markers IBA-1 and GFAP) throughout the study. Thus, Mtb infection in the presence of elevated basal ganglia Mn leads to increased dopaminergic toxicity and accompanying neuroinflammation that is long lasting. Next, as non-occupational Mn overexposure occurs primarily through consumption of Mn-contaminated drinking water (DW) irrespective of gender/sex, we conducted a study where the focus was on sex differences in terms of nervous and immune systems responsiveness to excessive Mn in the DW and response modulation by the inflammagen lipopolysaccharide (LPS). Adult C57BL/6 mice with GFP-tagged monocytes/microglia were subjected to Mn DW treatment for up to 8 weeks. Behaviorally, after 6 weeks, Mn exposure resulted in decreased activity, gait deficits, and decreased fear/anxiety-like behavior. Two weeks after behavioral assessment, when mice were challenged with LPS, circulating inflammatory cytokines and acute phase proteins increased in both sexes post LPS administration. Mn levels were increased in the brain and liver, but Mn alone did not affect circulating cytokines in either sex. Notably, Mn-exposed/LPS-challenged males had potentiated plasma cytokine output, whereas the reverse was seen in females. Males, but not females, continued to exhibit decreased fear (increased risky behavior), even when challenged with LPS. Overall, our DW Mn study results suggest that while some behavioral alterations are sex-independent, males appear to be more susceptible to the effect of

Mn on mood even in the presence of an inflammagen challenge. Moreover, male-biased augmentation of post-LPS cytokine production by Mn, further points to the need to consider sex in the context of Mn neurotoxicity.

Partial Support:

R21ES026383 (NIH) and endowment funds from the Lalita and Raghubir Sharma Distinguished Professorship.

Educational lecture

I-02: The latest legal information necessary for occupational health physicians' activities

Kazuhiko UCHIDA
Uchida occupational health consultant office

The Ministry of Health, Labor and Welfare has formulated the 14th Industrial Accident Prevention Plan, which has a five-year planning period from April 2023 to March 2028. This prevention plan covers eight key measures, many of which are relevant to occupational health physicians when they work in companies. In this lecture, I will focus on measures against long working hours and chemical substances, which are particularly related to the work of occupational physicians, as well as efforts to support for balancing treatment and work.

1. Measures to prevent health problems caused by long working hours

In the workplace, the work style reform has regulated the upper limit of overtime hours, and various efforts have been made, such as promoting the introduction of an interval system between shifts and making it mandatory to take five days of paid leave per year. In addition, the certification standards for death from overwork have been revised based on medical evidence, and the questionnaires when interviewing people working long hours are also being revised.

2. Chemical substance measures

Since most of the causes of workplace accidents due to chemical substances were not subject to special regulations on chemical substances, new chemical substance management efforts targeting harmful chemical substances that are not subject to regulations have begun.

Occupational health physicians are expected to be involved in health examinations of risk assessment subjects after fully understanding risk assessments and measures based on the results, the usefulness of protective equipment and its use, etc.

3. Support for balancing treatment and work

As the workforce ages, the number of workers who work while undergoing treatment for illness is increasing. In order to balance treatment and work, it is necessary to utilize flexible working and vacation systems. Occupational health physicians are experts who play a part in supporting balance and are required to provide appropriate advice and opinions to both employers and employees after understanding the framework.

Award Lecture

SBRTE Young Investigator Award

A-01: Understanding the contribution of zinc transporters in maintaining mental homeostasis

Yukina NISHITO

Department of Analytical and Bioinorganic Chemistry, Division of Analytical and Physical Sciences, Kyoto Pharmaceutical University

Essential trace elements play important roles in various physiological functions. Their deficiency induces clinical disorders. Thus, maintaining metal homeostasis is important. I have been studying about how metal homeostasis are maintained by focusing on the function of zinc transports.

To maintain zinc homeostasis, two zinc transporter family proteins, Zrt-/Irt-like protein (ZIP) and Zn transporter (ZNT) play important roles. We recently reported that intracellular zinc homeostasis is strictly controlled by the coordinate expression of ZIPs and ZNTs [1, 2]. Moreover, we also showed coordinated expression of ZIP4 and ZNT1 play critical roles in zinc absorption processes [2]. These findings provide novel molecular insights into how zinc homeostasis are maintained by the zinc transporters.

Recent studied showed that several zinc transporters are involved in manganese homeostasis. For example, mutations of *ZIP14/SLC39A14* and *ZNT10/SLC30A10* cause Parkinsonism and dystonia with hypermanganesemia. Moreover, *ZIP8/SLC39A8* mutations result in type II congenital disorders of glycosylation (CDG) due to systemic manganese deficiency. Focusing on these facts, we examined the how these zinc transporters recognize these metals and how metal homeostasis is interacted through these transporters [3, 4]. Our results would facilitate the understanding of regulation systems of maintain metal homeostasis.

In this lecture, I would like to talk about the current research in our present studies.

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[1] Nishito Y, Kambe T. Zinc transporter 1 (ZNT1) expression on the cell surface is elaborately controlled by cellular zinc levels. *J Biol Chem*. 2019; 294(43):15686-15697.

[2] Nagamatsu S, Nishito Y, Yuasa H, et al. Sophisticated expression responses of ZNT1 and MT in response to changes in the expression of ZIPs. *Sci Rep*. 2022; 12(1): 7334.

[3] Nishito Y, Tsuji N, Fujishiro H, et al. Direct Comparison of Manganese Detoxification/Efflux Proteins and Molecular Characterization of ZnT10 Protein as a Manganese Transporter. *J Biol Chem*. 2016; 291(28): 14773-14787.

[4] Nishito Y, Kamimura Y, Nagamatsu S, Yamamoto N, Yasui H, Kambe T. Zinc and manganese homeostasis closely interact in mammalian cells. *FASEB J*. 2024; 38(7): e23605.

JSBRTE Award

A-02: Exploration of bio-trace elements based on speciation

Yasumitsu OGRA
Graduate School of Pharmaceutical Sciences, Chiba University

Bio-trace elements are actually present in trace amounts, thus determination of them was a research aim in biomedical research in trace elements. The quantitative analysis of what bio-trace elements are present in tissues and in what concentrations was the first question to be answered, then a qualitative analysis of what chemical forms they are present in has been also needed. Speciation has been developed and used for such quantitative and qualitative analysis in this research field. Chemical speciation consists of two techniques for separation by chemical species and specific detection of elements. In our research group, HPLC has been used as a separation technique, and initially atomic absorption spectroscopy has been used as a detection technique, and now inductively coupled plasma mass spectrometry (ICP-MS) is used. Although ICP-MS is highly sensitive, it has the disadvantage that it is an element-specific detection method, which does not provide molecular information of target biomolecules. To compensate for this disadvantage, electrospray ionization mass spectrometry has been also used. Using these analytical techniques, I would like to review our research we have conducted so far on the elucidation of the biological functions of trace elements.

A-03: Comprehensive study on the diverse physiological functions of zinc

Taiho KAMBE
Graduate School of Biostudies, Kyoto University

Living organisms obtain essential metals, such as iron, zinc, copper, and manganese, from food. Although these biometals are only present in trace amounts in living organisms, they play crucial roles in life processes, such as energy conversion and signal transduction. Consequently, the dynamics of absorption, excretion, membrane transport, sensing, and utilization of these biometals in the body are tightly regulated, and any disruption can lead to diseases. Given that up to 30% of the proteins in living organisms bind metals (from plants and animals to microorganisms and archaea), an intriguing question arises: how have living organisms evolved protein functions that utilize metals? My research focuses on molecules to address this fundamental question of life phenomena and applies the findings to real life. I have been researching zinc transporters since their early discovery by cloning the zinc

transporter gene before the human genome was fully decoded to conduct functional analyses. Specifically, this involved: i) elucidating the mechanism of zinc absorption in the gastrointestinal tract and developing ways to improve zinc nutrition; ii) investigating the cause of transient neonatal zinc deficiency (TNZD); iii) exploring the molecular mechanisms of secretory and organelle-localized zinc enzyme activation; and iv) clarifying the molecular mechanisms underlying various zinc deficiency disorders.

Recently, I expanded my research to include other essential trace metals such as copper, manganese, and iron. I aim to continue our research to elucidate the biological functions of these essential trace elements.

Joint Symposium
Immunotoxicity and neurotoxicity of trace elements related to workplaces

The committee for Allergy and Immunotoxicology (AIT), Japan Society for Occupational Health (JSOH) and Allergy & Immunotoxicology, International Commission on Occupational Health (ICOH)

Hiroo WADA[1,2], Masashi TSUNODA[2,3]

[1]Department of Public Health, Juntendo University Graduate School of Medicine, [2]The Committee for Allergy and Immunotoxicology, Japan Society for Occupational Health, [3]Department of Preventive Medicine and Public Health, National Defense Medical College

The Study Group on Allergy and Immunotoxicity is one of the research groups of the Japanese Society for Occupational Health, and originated in the Study Group on Occupational Allergy, which was established in 1976. The Study Group on Occupational Allergy was active in addressing the issues of allergy in occupational setting, however the name was changed to the Study Group on Allergy and Immunotoxicity in 1997, because the scientific goal of the study group should not only in the field of allergy but also in those of immunology and toxicology. Since then, the Study Group is actively in research and practices of the fields. Currently, our official activity is to organize symposia and/or meeting to discuss various topics of the field of allergy and immunotoxicology, including the basic science, clinical medicine and occupational perspectives during the Japanese Society for Occupational Health, which was published as a review paper in the SANGYO EISEIGAKU ZASSHI, an official journal of Japanese Society for Occupational Health, to make our activities in the field of allergy and immunotoxicology broadly known. Furthermore, the Study Group has been also supporting global activity of the scientific committee of Allergy & Immunotoxicology, the International Commission on Occupational Health (ICOH) by joint symposia and researches.

The topic of allergy and immunotoxicology, which is associated with environmental exposure, is one of the central issues of occupational health, although those researchers who are interested in 'occupational health' becomes smaller in number. On the 35th Annual Meeting of the Japanese Society for Trace Element Research, therefore, this symposium is jointly organized by the scientific committee, Allergy & Immunotoxicology of ICOH, and the Study Group of JSOH, to emphasize the importance of the trace element research in the field of allergy and immunotoxicology, as well as in occupational health, and to stimulate their research.

S-01: Risk of zinc deficiency in daily life and work –Relationship to lifestyle-related diseases–

Hiroyuki YANAGISAWA[1], Takamasa KIDO[2], Machi SUKA[2]

[1]The Jikei University School of Medicine, [2]Department of Public Health and Environmental Medicine, Faculty of Medicine, The Jikei University School of Medicine

Zinc is an essential trace element for humans. Recently, the relationship between lifestyle-related diseases and zinc has been the focus of much attention. It is known that lifestyle-related diseases include hypertension, diabetes mellitus, and chronic kidney disease, which are also recognized in zinc deficiency. In addition, the same pathological conditions are observed in zinc deficiency, such as increased free radicals, impaired immune function, abnormal lipid metabolism, and impaired glucose tolerance, which are pathological backgrounds of lifestyle-related diseases. In recent years, the following factors have become an issue as the causes of zinc deficiency: (1) cooking with heat, (2) nutritional imbalance, (3) food additives that are taken without knowing (sodium polyphosphate, phytic acid, EDTA, etc.), (4) drugs taken for therapeutic purposes (e.g., antihypertensive drugs, psychotropic drugs, etc.). In this symposium, we would like to discuss the relationship between people who work and lifestyle-related diseases in the context of these zinc deficiency risks (1) to (4) based on the latest findings.

S-02: Issues and current status of occupational health management for beryllium and its compounds in Japan

Tatsushi TOYOOKA

National Institute of Occupational Safety and Health, Japan

Beryllium is a type of rare metal that is produced from ores such as beryl. Due to its excellent physical and chemical properties, including being lightweight, high strength, heat resistance, and corrosion resistance, metallic beryllium and beryllium alloys are used in a wide range of industries worldwide, including machinery, communications, computers, aerospace, and nuclear industries. On the other hand, beryllium is known to have sensitizing and carcinogenic properties (classified as Group 1 carcinogen by the IARC). In the past, around the 1970s, when exposure to beryllium was at relatively high concentrations, acute beryllium disease (chemical pneumonitis) and lung cancer were significant concerns. However, in modern times, certain exposure control measures have been implemented, leading to a considerable improvement in the incidence of these diseases. Nevertheless, it is known that even minimal exposure can cause beryllium sensitization, an immune response. Chronic Beryllium Disease (CBD), characterized by granulomatous lesions of the lungs that can develop following

sensitization, is now globally recognized as a major health issue associated with beryllium exposure.

In recent years, following the strengthening of various health management regulations concerning beryllium by the Occupational Safety and Health Administration (OSHA) in the United States, including the permissible exposure limits, we have been conducting an investigation into the state of beryllium health management in Japan, incorporating information from the field. In this presentation, we will introduce the issues related to beryllium health management in Japan that have emerged from our investigation, as well as the actual conditions of workplaces handling beryllium.

References:

[1] Toyooka T, Koda S. Management of occupational health for adverse health effects of beryllium and its compounds in workplaces - Recent trends and issues in Japan. *Sangyo Eiseigaku Zasshi*. 2021; 63(2): 31-42.

S-03: Effect of Fluoride on Neurobehavior

Mayuko HOSOKAWA[1], Akimasa SOMEYA[2], Takeshi TANIGAWA[3]

[1]Department of Epidemiology and Environmental Health Juntendo University Faculty of Medicine, [2]Juntendo University Faculty of Pharmacy Laboratory of Molecular Biology, [3]Department of Public Health Graduate School of Medicine Juntendo University

In recent years, there has been concern about the neurotoxicity of fluoride, and it has been suggested that chronic fluoride intake during development can affect the development of brain functions, including IQ decline and autism spectrum disorder (ASD) in children. ASD is thought to be caused by the interaction of “environmental factors” and “genetic factors”. Exposure to “environmental factors” during fetal life has been reported to affect germ cells during maturation, but the molecular mechanism has not been elucidated. The neurotoxic effects of fluoride exposure (on neurodevelopment and cognitive function) are also unclear.

This symposium will provide an overview and perspective on the general biological effects of fluoride exposure and the neurotoxicity that has recently been suggested. Immunotoxicity, which remains to be elucidated, will also be presented.

S-04: Toward the elucidation of carcinogenic mechanisms in the liver induced by exposure to inorganic arsenic

Kazuyuki OKAMURA, Takehiro SUZUKI, Keiko NOHARA
Health and Environmental Risk Division, National Institute for Environmental Studies

Exposure to arsenic through drinking groundwater or mining activities has been reported to induce cancer in organs such as the skin, lungs, bladder, and liver, although the exact mechanisms remain unclear. A notable feature of arsenic-induced carcinogenesis is the long latency period, even after exposure has ceased. Our laboratory focuses on cellular senescence and the senescence-associated secretory phenotype (SASP) as mechanisms by which arsenic induces hepatic tumors. Our previous study showed that exposure of the human hepatocyte cell line Huh-7 and the human hepatic stellate cell line LX-2 to sodium arsenite induces premature senescence and increases the production of SASP factors, such as MMP1 and MMP3. Furthermore, the increased production of SASP factors involved in tumorigenesis was maintained even after the cessation of arsenic exposure. On the other hand, in an animal model of arsenic exposure, we reported that arsenic intake during pregnancy increased the incidence of liver cancer in subsequent generations, including the offspring and grandchildren. This finding reveals that the effects of arsenic exposure are not limited to the directly exposed generation but can also affect future generations. Moreover, we showed that these effects are transmitted through males, and research is being conducted on changes in epigenetic modifications in sperm. Additionally, it has been reported that other environmental factors and chemical exposures can have multigenerational impacts. In this presentation, I would like to discuss the carcinogenic mechanisms associated with arsenic exposure, focusing on the cancer-promoting aspects of cellular senescence, as well as the concepts of multigenerational and transgenerational effects and their related epigenetic modifications.

Main Symposium
The study of trace elements in society -Today's status, countermeasures and issues-

S-05: Toxicity and Today's Issues of Asbestos and Man-made Mineral Fibers

Yoshiharu AIZAWA
Kitasato University, Emeritus Professor

[Introduction]

The inhalation of asbestos fibers has been found to cause lung fibrosis and other malignant diseases in humans. The production of asbestos materials has been prohibited in Japan since 2006. Therefore, man-made mineral fibers (MMMFs) have been used as substitutes for asbestos fibers. On going safety evaluations are necessary to determine the adverse effects of existing fibers as well as new MMMFs.

[Types, routes of entry and adverse effects of MMMFs]

Six types of fibrous silicate fibers including chrysotile are known as asbestos fibers. Three major groups of MMMFs are glass fibers, rock/slag wool fibers and ceramic fibers. The carcinogenicity depends on the shape of fibers, the amount inhaled and the duration of retention in the lungs. The targets of adverse effects of MMMFs are same as those of asbestos fibers, i.e., lung fibrosis, lung cancer and mesothelioma.

[Evaluations of toxicity]

1) Epidemiological studies: population studies on non-malignant diseases, lung cancer and mesothelioma. 2) Animal studies: biopersistency, fibrogenicity and carcinogenicity. 3) Cytotoxicity studies: release of LDH or cell magnetometry.

[Evaluation of IARC in 2001]

Carcinogenicity of special-purpose glass fibers and ceramic fibers as Group 2B and insulation glass wool, continuous glass filament, rock wool and slag wool as Group 3.

[Cell magnetometry]

Lung magnetometry was developed by Cohen in 1973 and the author evaluated cell toxicity when exposed to chemicals and MMMFs by using such instrument.

References:

[1] Cohen D. Ferrimagnetic contamination in the lungs and other organs of the human body. *Science*. 1973; 180: 745-748.

[2] Aizawa Y, Kudo Y. Magnetometric evaluation of toxicities of chemicals to the lungs and cells. *Environ Health Prev Med* 2010 Jul; 15(4):197-202. (Epub 2020 Apr 6.)

S-06: “Trace elements” involved in the development and metabolism of ovarian clear cell carcinoma

Masashi TAKANO
Department of Obstetrics and Gynecology, National Defense Medical College

[Background] Ovarian cancers, often found in an advanced stage, is known as a silent killer, leading to poor prognoses. Ovarian cancers are divided into four main histological types: serous carcinoma, endometrioid carcinoma, clear cell carcinoma, and mucinous carcinoma. Each type has a significantly different characteristics, in terms of sensitivity to anticancer drugs, and prognosis, and carcinogenesis. Among them, “trace elements” that are involved in its development and metabolism in clear cell carcinoma, which is common in Japan, and shows resistance to treatment, are summarized and discussed in the session.

[Clinical history and characteristics of clear cell carcinoma] Clinical characteristics of clear cell carcinoma include the fact that more than half of cases are diagnosed at advanced stage I, which is confined to the ovaries, that positive ascites cytology at the initial operation is a significant factor in prognosis, and that it is resistant to anticancer drugs. It has also been pointed out that it originates from ovarian endometriosis, and that it is prone to coexisting with thrombosis and Trousseau syndrome.

[Factors involved in the development and metabolism of clear cell carcinoma] Genetic characteristics of clear cell carcinoma include HNF1B, which is also known to cause juvenile diabetes, and ARID1A, one of the genes that make up the SWI/SNF chromatin remodeling complex. In addition, it has been shown that the iron ion concentration in cysts is high and DNA damage markers due to oxidative stress are high in endometriosis-derived carcinomas, suggesting that they develop in an oxidative stress environment. I would like to discuss with you how chronic oxidative stress reactions lead to carcinogenesis and future treatment strategies, focusing on the relationship with “trace elements.”

S-07: Trace Element Exposure and Protective Equipment in the Industrial Workplace

Satoko IWASAWA
Department of Preventive Medicine and Public Health, National Defense Medical College

The new regulations mainly target hazardous chemical substances that have been exempted from the regulations up to now and introduce a system in which businesses can take appropriate measures to prevent exposure based on the results of risk assessments, on the premise that the government will

establish upper exposure limits (concentration limits) and improve and expand communication of hazard and toxicity information (GHS classification, SDS). The system is to introduce a system in which business operators take appropriate measures to prevent exposure based on the results of risk assessments. Even for trace elements, it is necessary to confirm the hazard and toxicity by GHS classification and SDS and conduct risk assessment by paying attention to the concentration standard values to be established in the future. Even if a substance is not subject to risk assessment, the obligation to make efforts to minimize exposure concentrations will come into effect on April 1, 2023.

In addition, regarding the use of protective equipment, the Director of the Labor Standards Bureau of the Ministry of Health, Labour and Welfare issued a notice on July 4, 2023 regarding chemical substances that fall under the category of chemical substances that are harmful to the skin, etc., and selected 296 substances as skin-absorbing harmful substances, excluding those for which the use of impermeable protective clothing, etc. is obligatory under special regulations. Skin-absorbing hazardous substances are chemical substances that are clearly known to cause health problems when absorbed through or invaded by the skin. As health hazards, they are mainly based on human findings or animal findings regarding systemic effects. For these substances, it is obligatory to wear impermeable protective equipment, and appropriate protective equipment should be selected in accordance with the manual for selecting protective equipment to prevent skin damage published by the Ministry of Health, Labor and Welfare in February 2024. The person responsible for managing the wearing of protective equipment is to select effective protective equipment, maintain and control protective equipment, and perform other tasks related to protective equipment.

S-08: The beneficial effect of zinc supplementation therapy on immune function decline caused by zinc deficiency

Takamasa KIDO[1], Machi SUKA[1], Hiroyuki YANAGISAWA[2]

[1]Department of Public Health and Environmental Medicine, The Jikei University School of Medicine, [2]The Jikei University School of Medicine

Zinc deficiency causes dysfunction of the immune system. We have demonstrated that zinc deficiency causes thymic atrophy/fatty degeneration, reduction in hematopoietic stem cells or B cells in the bone marrow, and a splenic-derived inflammatory response. Also, we investigated the beneficial effects of zinc supplementation on zinc deficiency. It was suggested that atrophy / fatty degeneration in the thymic cortex region of zinc deficiency rats impairs the maturation process of T cells. In the bone marrow, hematopoietic stem cell and B cell numbers were reduced due to apoptosis and decreased transcription factors (Zn finger protein). In the spleen, the activity of the anti-inflammatory 'Th2

lymphocyte-M2 macrophage' pathway was reduced and the inflammatory response was enhanced. Importantly, zinc supplementation can reverse the effects of zinc deficiency on immune function.

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[1] Kido T, Ishiwata K, Suka M, Yanagisawa H. Inflammatory response under zinc deficiency is exacerbated by dysfunction of the Th2 lymphocyte – M2 macrophage pathway. *Immunology*. 2019;156(4): 356-72.

[2] Kido T, Suka M, Yanagisawa H. Effectiveness of interleukin-4 administration or zinc supplementation in improving zinc deficiency-associated thymic atrophy and fatty degeneration and in normalizing T cell maturation process. *Immunology*. 2022; 165: 445-459.

Oral Presentations

O-01: Kinetic analysis of TmsA, a novel trimethylselenonium methyltransferase involved in environmental selenium cycling

Kanako SOWA[1], Anna OCHI[1], Masao INOUE[1,2], Riku AONO[1], Soichi SATO[3], Yasumitsu OGRA[4], Hisaaki MIHARA[1]

[1]Coll. Life Sci., Ritsumeikan Univ., [2]R-GIRO, Ritsumeikan Univ., [3]Fac. Sci. Eng., Toyo Univ., [4]Grad. Sch. Pharm., Chiba Univ.

Aminobacter sp. m3 is a soil bacterium that degrades trimethylselenonium (TMSe), a detoxified metabolite of selenium excreted from animals. We have identified a novel methyltransferase, TmsA, which uses TMSe as a substrate. It shows 27% amino acid sequence identity to the dimethylsulfoniopropionate (DMSP)-tetrahydrofolate (THF) methyltransferase DmdA. Here, we performed the kinetic analysis of TmsA to reveal its substrate specificity and catalytic mechanism. TmsA exhibited the highest turnover rate for TMSe, whereas it showed low activity toward DMSP. Structural prediction of TmsA revealed putative catalytic and substrate-binding residues. Mutations in some of these residues decreased substrate affinity or turnover rate. These results suggest that TmsA has evolved to have a similar catalytic mechanism but a distinct substrate recognition mode to DmdA.

O-02: Mechanism of degradation and aggregation of *S*-selanylated glyceraldehyde-3-phosphate dehydrogenase

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Previous studies have revealed that post-translationally modified cytoplasmic NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPC), such as nitrosylation or glutathionylation, is inactivated, whereas the activity of selanylated GAPC in broccoli is increased by 1.4-1.6 times It has also been suggested that post-translationally modified GAPC is partially degraded and the degradation products have functions other than glycolysis, but the details are unknown. In this study, we investigated the degradation and aggregation mechanisms of selanylated GAPC in broccoli to elucidate its novel physiological functions other than glycolysis.

The degradation of selanylated GAPC was examined using broccoli sprouts 12 days after sowing that were treated with oxidative stress (10 μM paraquat) for 48 h. The aggregation of selanylated GAPC was examined using recombinant GAPC (rGAPC) expressed in *E. coli* 10G strain after inserting the broccoli-derived GAPC gene (Bo5g017500) into the pRam N-His Kan vectors.

GAPC activity in broccoli sprouts grown in a medium containing 1 μM sodium selenate was increased by 1.5-fold. When these plants were treated with 10 μM paraquat for 48 h, GAPC activity in the control and selenium-suplemented plants (+Se) was inhibited by 30 and 58%, respectively. However, the chlorophyll content of the paraquat-treated +Se plants remained significantly higher than that of the control. A degradation product of GAPC (27 kDa GAPC) was detected in both the control and +Se plants after paraquat treatment. These results suggest that the degradation product of selanylated GAPC functions in the oxidative stress defense system. On the other hand, glutathionylated GAPC was significantly aggregated after 2 h of reaction with H_2O_2 treatment, whereas selanylated GAPC did not aggregate until 6 h after reaction. This suggests that the selanylated GAPC produced is resistant to oxidative stress and functions stably within cells.

O-03: Cerium Chloride Promotes Cell Differentiation by Altering the Calcium Signaling in Normal Human Epidermal Keratinocytes

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Epidermal keratinocytes undergo morphological and functional changes during differentiation, being enucleated to become corneocytes. Calcium (Ca) has been shown to be involved in various cellular functions of epidermal cells, including proliferation, differentiation, and apoptosis. Cerium is a lanthanide-series element and rare earth metal, is used as an abrasive and a catalyst in chemical reactions. For organisms, cerium oxalate is used as a digestive drug. For skin, cerium oxide has been investigated for use in absorbing UV and promoting wound healing. However, the functions and physiological effects of inorganic cerium on the skin have rarely been investigated. In this study, we focused on the function of cerium in epidermal keratinocytes and its interaction with calcium. We investigated their effects on cell differentiation and intracellular calcium concentration. This study suggests that cerium may be involved in calcium signaling in epidermal keratinocytes.

O-04: Zinc and its transporter ZIP10 regulate the anticancer activity of tamoxifen in human breast cancer cells

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Approximately 80% of breast cancer patients are estrogen receptor (ER) positive. They benefit from hormone therapy, represented by tamoxifen (Tam); however, Tam has been reported to acquire drug resistance and some cases in which Tam is not successful. Underlying this, the regulatory mechanism of Tam activity is partially unknown. Here we demonstrated that the effect of Tam on breast cancer is strictly regulated by cellular zinc ion (Zn^{2+}) transported by zinc transporter ZIP10, which requires excessive autophagy. The anti-cancer activity of Tam is also enhanced by zinc preparation which is currently in clinical use for hypozincemia and gastric ulcers. In addition, Zn^{2+} administration enhanced the growth inhibition of tumors *in vivo*. These results showed that the anticancer activity of Tam is effectively mediated through Zn^{2+} and ZIP10 in breast cancer, and we propose the combined use of Zn^{2+} as a powerful tool to optimize breast cancer treatment with Tam.

O-05: Elucidation of the metabolic pathways of tellurium to form volatile and insoluble compounds in mammals

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Objective: This study explores two metabolic mechanisms of tellurium (Te) in animals: (1) the involvement of the enzymes thiopurine *S*-methyltransferase (TPMT) and indolethylamine *N*-methyltransferase (INMT) in Te methylation and (2) the formation of elemental Te nanoparticles in mammalian cells.
Methods: (1) Recombinant human TPMT and INMT were purified and reacted with Te compounds. Reaction products were analyzed by using LC-ICP-MS and GC-MS. (2) Human hepatoma HepG2 cells were exposed to potassium tellurite. Nanoparticle formation was confirmed by transmission electron microscopy (TEM). Then, HepG2 cells were treated with propargylglycine (PPG) to inhibit cystathionine γ-lyase followed by the reduction of sulfane sulfur species, and then exposed to potassium tellurite. The amounts of tellurium in the insoluble fraction of the cells were measured by ICP-MS.

Results: (1) Monomethylated and dimethylated forms of Te were detected in the reaction solution with TPMT, but not with INMT alone. When tellurite was reacted with TPMT and INMT at the same time, trimethylated form (trimethyltelluronium ion) was detected. (2) TEM observation showed electron-dense rod-like particles (i.e., nanorods) in the lysosomal-like structures. PPG treatment significantly reduced the production of biogenic Te nanorods.
Discussion: From the results obtained, it is demonstrated that TPMT and INMT are cooperatively responsible for the detoxification of Te oxyanions through methylation to form trimethyltelluronium ions. In addition, intracellular sulfane sulfur species play a critical role in the formation of biogenic Te nanorods. These two metabolic pathways to form volatile and insoluble Te compounds are responsible for reducing tellurite toxicity.

O-06: Use of the postal service for biological sample collection in human biomonitoring

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In Japan, there is no large-scale human biomonitoring (HBM) survey like those conducted in the United States and European countries. This study aimed to establish a simple and easy method for collecting human biological samples using the postal service. We conducted a mail-based sample collection to explore this approach. According to the Japan National Health and Nutrition Survey, Japan was divided into 12 blocks, and participants were randomly selected from internet survey company registrants residing in each block, considering gender and age. HBM and biochemical test kits were sent to participants who agreed to the study protocol. Two surveys were conducted with 300 participants each. The collected samples included blood, first-morning void urine, tap water (and purified water), and hair. HBM kits were developed specifically for the collection of samples by mail. The target substances for monitoring included elements such as lead, which were analyzed using ICP-MS. In the first survey, 264 out of 300 participants submitted samples, while 321 out of 350 participants submitted samples in the second survey (collection rates of 88% and 92%, respectively). Self-collected blood samples obtained through finger pricks encountered issues such as poor quality and insufficient sample volume. The median blood and urinary lead concentrations in analyzable samples were 9 and 0.3 ng/g, respectively. Additionally, a significant decrease in lead concentration was observed in filtered water compared to tap water. Improving collection kits and instructions would make it easier to grasp chemical exposure levels using human biological samples.

O-07: A Glucose metabolism related-mRNA expression of pancreas in zinc deficient rat

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[Objective] Zinc, an essential trace metal, is necessary for the formation of four-zinc insulin crystal structure in the β cells of the pancreatic islets of Langerhans. Zinc deficiency has long been suggested to be involved in the onset and progression of diabetes, but the relationship between zinc deficiency and diabetes is unclear. In this study, we investigated gene expression of insulin, glucagon, somatostatin, and zinc transport protein (Zip13) in the pancreas of zinc-deficient rats.

[Method] Twenty-four male SD rats aged 5 weeks were purchased. After acclimation for one week after purchase, the rats were divided into three groups: a group that freely consumed a standard zinc diet (group F), a zinc-deficient diet group (group ZnD), and a control group that was fed the same calorie diet as the zinc-deficient group (group Pf), and were kept in individual cages for four weeks. After four weeks, blood and pancreas were removed under isoflurane anesthesia, and gene expression of insulin, glucagon, and somatostatin was measured using real-time PCR based on β-actin.

[Results] ZnD group showed decreased serum zinc concentration, hair loss, dermatitis, and growth inhibition, all of which are characteristic of zinc deficiency. Four weeks after zinc-deficient dietary administration, serum insulin concentration was 100 μg/dl in the F and Pf groups, but decreased to 40 μg/dl in the ZnD group. No significant differences were observed in gene expression levels for insulin (1.0 ± 0.2 in the F group, 0.6 ± 0.5 in the Pf group, 0.9 ± 0.7 in the ZnD group) and glucagon (1.0 ± 0.2 in the F group, 0.9 ± 0.6 in the Pf group, 0.9 ± 0.4 in the ZnD group). Zip13 gene expression was 1.0 ± 0.2 in the F group, 1.0 ± 0.5 in the Pf group, and 2.6 ± 0.9 in the ZnD group.

[Discussion] Gene expression levels of insulin and glucagon in the pancreas of zinc-deficient rats showed a tendency to decrease in the ZnD and Pf groups compared to the F group. On the other hand, expression of the zinc transport protein Zip13 gene increased in the zinc-deficient group. Blood glucose regulation due to zinc deficiency may be related to glucose metabolic mechanisms in the liver, kidneys, muscles, and adipose tissue rather than blood glucose regulating hormones from the pancreas.

O-08: Changes in blood pressure-related gene expression in moderate zinc deficiency in rats

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[Objectives] We reported that hypertension occurred by moderate zinc deficiency, especially in diastolic blood pressure. Zinc deficiency reduces diet intake, but the restriction of zinc-sufficient diet causes hypotension. Reduced dietary intake cannot explain the development of hypertension in moderate zinc deficiency. Many aspects of its mechanism remain unknown. We report changes in mRNA levels of blood pressure regulation-related genes in moderately zinc-deficient rats.

[Methods] Thirty 3-week-old male Sprague-Dawley rats were randomly and equally divided to control, pair-fed (PF), and zinc-deficient (ZD) groups and fed for 4 weeks. Quantitative real time RT-PCR using TaqMan proves was performed to determine expression of Nos3, Hmox1 and Hmox2 genes. Differences between groups were statistically analyzed by the Brown-Forsythe ANOVA and *post hoc* Welch's t-test. P value less than 0.05 was considered significant.

[Results and conclusions] Blood pressure of ZD were the highest and those of PF were the lowest among all treatment groups. Diastolic blood pressure of ZD was significantly higher than PF. Systolic blood pressure of PF was significantly lower than ZD and Control. Kidney cortex Nos3 and Hmox1 mRNA expression of PF were significantly higher than ZD and Control. Kidney cortex Hmox2 mRNA expression of PF was significantly higher than Control. Kidney medulla Nos3, Hmox1 and Hmox2 mRNA expression of PF were significantly higher than ZD and Control. These results suggest that these three genes are not involved the development of hypertension caused by moderate zinc deficiency.

O-09: Association between zinc and insulin related measurements in Japanese adults

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This study examined the association between zinc and insulin related measurements in Japanese adults (n=293). Serum zinc (μg/dl) showed a significant correlation with adiponectin (γ=-0.26). Urinary zinc (μg/gCr) showed a significant correlation with fasting blood glucose (γ=0.26). These results suggested that with low serum zinc, insulin secretion decreased and adiponectin secretion increased compensatory, resulting in increased blood glucose and urinary excretion of zinc.

O-10: Dissociative identity disorder cotreated with zinc and L-carnosine

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Little is known about the effectiveness of pharmacotherapy in dissociative identity disorder (DID). Glutamatergic hyperactivity in the brain has been linked to dissociation. Zinc deficiency can lead to mental symptoms commonly observed in individuals with dissociation, possibly contributing to dissociation. Zinc and L-carnosine have anti-glutamatergic properties. We report the case of a 30-year-old woman with DID and comorbid bipolar I disorder who had zinc deficiency and was successfully cotreated with 50 mg/d of zinc and 2 g/d of L-carnosine. The patient's alternate identities disappeared, with improvements in emotional/mood instability, flashbacks, binge eating, and self-harm. Furthermore, physical symptoms of zinc deficiency were resolved. The Dissociative Experiences Scale and the 16-item Quick Inventory of Depressive Symptomatology (self-report) scores improved from 46.1 to 25.4 and from 24 to 13, respectively. Further investigation of this treatment regimen for DID and related conditions, particularly the contribution of zinc deficiency to dissociation, is necessary.

O-11: Effect of zinc supplementation on hyperammonemia induced by sodium valproate in patients with severe motor and intellectual disabilities

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[Objective] Sodium valproate (VPA) is one of the antiepileptic drugs but frequently develops hyperammonemia as a side effect. Zinc supplementation is known to decrease blood ammonia levels in patients with liver cirrhosis [1]. We investigated the changes in blood ammonia levels when zinc was administered to the patients with severe motor and intellectual disabilities (SMID) taking VPA.
[Subjects and Methods] A total of 33 patients with SMID participated in the study, taking both VPA and zinc supplementation because of zinc deficiency. Blood ammonia levels were examined before and after zinc supplementation.
[Results] The mean blood ammonia level decreased significantly from 70.9±29.0μg/dL to 46.9±21.6μg/dL ($p<0.001$). In particular, all 13 patients with hyperammonemia over 80μg/dL showed a large decrease after the supplementation.
[Discussion] VPA-induced hyperammonemia is thought to occur due to the inhibition of carbamoyl phosphate synthetase I in urea cycle by its metabolites. Ornithine transcarbamylase (OTC) is another

enzyme in urea cycle, and requires zinc for its activation. Zinc supplementation may improve urea cycle function by elevating OTC activity.

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O-12: Two cases of severe hypercupremia and hypozincemia while taking low-dose contraceptive pills

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[Objective] Hypercupremia is seen when the inflammatory response is high or during pregnancy. When the inflammatory response is high, serum copper levels rarely exceed 200 μg/dl (normal range 66-130). During pregnancy, hypercupremia becomes particularly severe due to the influence of estrogen, and serum copper levels exceed 200μg/dl. In this study, we observed two adult women who presented with severe hypercupremia and hypozincemia while taking low-dose contraceptive pills. We report and discuss these cases.

[Cases and Results] Case 1 is a woman in her 20s who started taking low-dose contraceptive pills (Tri-Cyclen: Bayer Pharmaceuticals) due to severe menstrual pain. She contracted COVID-19 immediately after taking the pills. She visited our hospital because her poor health persisted for about two months even after the end of her treatment period. Her serum copper level was 230 and serum zinc level was 63μg/dl (normal range 72-108), indicating severe hypercupremia and hypozincemia. Considering the effects of the low-dose birth control pill, a suspension of the medication was suggested. The patient was re-examined 9 days later. Blood tests showed a decrease in serum copper level to 166, a slight increase in serum zinc level to 66, and normalization of CRP to 0.16.

Case 2 was a woman in her 20s who had been taking low-dose birth control pills for the past year due to menorrhagia. She developed bacterial pyelonephritis and was admitted to our hospital. Her serum copper level was 205, serum zinc level 60, and CRP level 10.2, indicating severe hypercupremia, hypozincemia, and elevated inflammatory response. The patient improved with intravenous antibiotic therapy, and 7 days later, serum copper levels dropped to 182, serum zinc levels rose to 107, and CRP dropped to 0.26.

[Discussion and Conclusion] In case 1, the serum copper levels dropped after the low-dose pill was suspended. In case 2, the pill was not suspended, and the pyelonephritis improved and serum copper levels dropped. In addition, the inflammatory response normalized in both cases, so this may have been a factor. Low-dose pills contain estrogen, which is thought to be the cause of hypercupremia.

O-13: A cross-sectional study on the relationship between nutrients intake and gut microbiota in frailty among elderly community residents: The Kyotango Study

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[Aim] The purpose of this study was to analyze the current state and clinical risk factors of frailty among community-dwelling older to conduct a cross-sectional analysis of the individuals, correlation between frailty and nutrient intake, dietary diversity, and dietary patterns, and to elucidate the correlation between frailty-related dietary factors and the gut microbiota.

[Methods] The study included 786 participants aged ≥65 years from the Kyotango Multipurpose Cohort Study who had available data on their gut microbiota. Frailty was quantitatively assessed by selecting 32 items from the previously reported frailty index, with those scoring ≥0.21 classified as frailty (n=119) and those with scores <0.21 as non-frailty (n=667), followed by group comparisons.

[Results] The frailty group had significantly higher values and rates than the non-frailty group for the following items: age, obesity (in females only), diabetes, hypertension, history of cancer treatment, polypharmacy, disturbed sleep quality, low physical activity, serum insulin levels, and high-sensitivity C-reactive protein. The frailty group had significantly lower levels of nutrients, including plant proteins, potassium (K), magnesium (Mg), iron (Fe), copper (Cu), vitamins B and C, folic acid, and total, soluble, and insoluble dietary fiber. When analyzed by food groups of dietary fiber, the frailty group had significantly lower intakes of soy products and non-green-yellow vegetables, specifically. The Japanese Diet Index score (rJDI12) was significantly lower in the frailty group, with significant deficiencies in soy products and mushrooms included in the rJDI12.

[Conclusion] Our findings clarify the current state of frailty among older community residents and suggest the importance of a diverse range of plant-based foods, including soy products and non-green yellow vegetables, through correlation analysis with nutrients and food groups.

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O-14: ZIP13 regulates lipid metabolism by changing intracellular iron and zinc balance

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Metabolic diseases are caused by a prolonged energy imbalance, and adipose tissue is known to be the main contributor. We previously reported that ZIP13, an Slc39a transporter whose deficiency causes Ehlers-Danlos syndrome spondylocheirodysplastic type 3 associated with lipoatrophy, inhibits the adipocyte browning pathway by modulating intracellular zinc status. The precise mechanisms of how ZIP13 regulates the homeostasis of adipose tissue remain unclear and therefore, we investigated the role of ZIP13 in mature adipocytes using adipocyte-specific *Zip13*-deficient mice. We herein demonstrate that these mice show accelerated lipolysis and reduced respiratory exchange ratio. In addition, abundance of iron and zinc balance were altered during differentiation in normal adipocytes, whereas iron distribution was substantially affected in *Zip13*-deficient adipocytes, which downregulated PDE activity and enhanced β-adrenergic receptor signaling pathways. Importantly, we confirmed that ZIP13 could transport both zinc and iron, using the *Xenopus* oocyte transport system and *in silico* structural dynamics simulations, and that the defect in iron distribution perturbs proper lipolysis. Together, these results illustrate that ZIP13 acts as a key regulator for lipolysis in adipocytes via the proper use of metals, and that the ZIP13-iron axis plays an important role in regulation of lipid metabolism.

O-15: Effect of specific antibody production in the zinc deficiency

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Aim: Zinc deficiency has been reported to reduce immune function. Our previous studies showed that the number of B cells of bone marrow and spleen on zinc deficiency was decreased. In other words, zinc deficiency can reduce antibody production. We investigated whether or not ovalbumin (OVA) which was administered intraperitoneally to zinc-deficiency rats affected OVA-specific antibody production.

Materials and Methods: SD rats were intraperitoneally administered OVA twice and then fed a zinc-deficient or standard diet for 6 weeks. We determined the concentration or levels of OVA specific- total IgG, IgG1, and IgG2 in the serum using ELISA methods. The number of B cells distributed in the bone marrow and spleen was counted using FACS.
Results: The number of B cells in the bone marrow and spleen, serum total-IgG concentration, OVA specific -total IgG, -IgG1, and -IgG2 levels of the zinc deficiency group were significantly lower than those in the standard group.
Discussion: It was suggested that zinc deficiency reduced the number of B cells in the bone marrow and spleen, thereby reducing OVA-specific IgG production.

O-16: Growth promotion by proline analogues of deoxymugineic acid, a phytosiderophore secreted from rice roots

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Rice plants secrete deoxymugineic acid (DMA) to absorb iron from the soil and take it up as DMA-iron complexes, but the procurement of L-azetidine-2-carboxylic acid, which corresponds to the 4-membered ring portion of DMA, through synthesis has been a major barrier to its practical application as a fertilizer. Therefore, we synthesized a variety of analogues substituting stable and inexpensive amino acids and investigated their iron uptake activity.

The analogues were chemically synthesized by replacing the four-membered ring moiety of DMA with glycine, methylglycine, or proline, and 50 μM of the ^{55}Fe(III) complex of the analogues was added to Sf9 insect cells (1×10^7 cells) expressing the barley transporter HvYS1, and the ^{55} Fe uptake was counted. Proline deoxymugineic acid (PDMA) substituted for L-proline was found to have the same level of iron complex transport activity as DMA by ^{55}Fe measurement. However, glycine and methylglycine types showed little transport activity.

In parallel, cryo-EM analysis of HvYS1 and Fe(III)-DMA was performed. In addition, DMA analogues were evaluated for growth of rice plants in alkali fields. Cryo-EM structures of HvYS1 in complex with either Fe(III)-PDMA or Fe(III)-DMA revealed that they bind to exactly the same location, indicating that PDMA is 10 times more effective in restoring iron deficiency than existing iron chelating agents. effect

compared to existing iron chelators. The fact that PDMA could actually yield rice in alkaline poor soils shows promise as a next-generation fertilizer.

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O-17: Calculation of confidence interval of relative biological values with an illustration of iron in cocoa

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Relative biological value of iron in foods and iron salts is defined as the ratio of the biological response to the test substance and that to the standard substance. We newly developed a method to accurately calculate the confidence interval in the slope-ratio method. The 95% confidence interval of the relative biological value of iron in cocoa powder based on the slope-ratio method [1] was determined by our exact calculation method and the existing approximation method of Littell. They agreed well, because the relative standard error of the slope of the standard substance was relatively small. When the relative standard error of the slope of the standard substance is greater than 0.3, the use of the exact calculation method is recommended. The omission of dummy variable to distinguish un-supplemented diet and iron-supplemented diet in multiple regression analysis markedly narrowed the 95% confidence interval. The resampling method underestimated the confidence interval. The erroneous use of Wald interval for calculation of the 95% confidence interval should be avoided.

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O-18: Potential of isotopic analysis of ^{63}Cu and ^{65}Cu in the mouse model of DSS-induced ulcerative colitis for diagnosis of pathological conditions

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Metallomics has demonstrated isotopic analysis of biometals, where isotopes of Fe and Ca have been used to evaluate metabolism in animals and humans[1-3]. Therefore, isotopic analysis of Cu in the mouse model of ulcerative colitis was used to investigate a part of its pathogenesis and to examine diagnostic methods based on the metabolic mechanism of biometals.

A quadrupole ICP-MS instrument (Agilent 7700X) was used for the analysis. As this instrument differs from a multi-collector mass spectrometer, Cu with two isotopes was used as the measurement target. Similarly, Rb, Ir and Tl were measured together, and validation was performed using NIST bovine liver. Total Cu concentrations and ^{65}Cu/^{63}Cu isotope ratios were measured in plasma, tissue and feces of healthy mice and DSS-induced colitis model mice.

CVs of ^{65}Cu/^{63}Cu, ^{87}Rb/^{85}Rb, ^{193}Ir /^{191}Ir, and ^{205}Tl/^{203}Tl were 0.2-0.4%. The accuracies of isotope analysis were high even for quadrupole types in these elements. In healthy mice, ^{65}Cu/^{63}Cu was almost the natural ratio. In contrast, ^{65}Cu/^{63}Cu was elevated in small intestine of DSS-mice before the onset of the disease and exceeded the natural ratio. As the disease progressed, ^{65}Cu/^{63}Cu increased together with a decrease in Cu concentration in large intestine.

^{63}Cu was suggested to preferentially decrease from the colon with inflammation. The Cu concentration in the liver increased in conjunction with this, suggesting that ^{63}Cu in the colon might be redistributed to the liver. From the results in present study, the possibility of a new diagnostic method was found using both the Cu concentration and isotope ratio as indexes.

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Poster Presentations

P-01: A monitoring method for contaminating elements in plasma by high energy SR-XRF

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High energy synchrotron radiation X-ray fluorescence analysis (SR-XRF) is a simple method of irradiating a sample with a beam and is excellent for detecting heavy elements. In this study, we examined the potential of this method as a monitoring method for uranium and cesium in plasma.

An acrylic adhesive polypropylene film was used as a support film free of contamination of the target elements. One μl of rat plasma was dropped onto the film, dried, and used as SR-XRF specimens. SR-XRF measurements were performed at the Synchrotron Radiation Facility of Japan Synchrotron Radiation Research Institute. Calibration curves were prepared using control rat plasma with drops of uranium and cesium added. The detection limit of these elements in 240-second counting was 0.05 μg/g and 0.1 μg/g, respectively, which is highly sensitive. This method, which does not require complicated pretreatment, showed promise as a method for monitoring contaminating elements in plasma. Quantification of uranium-treated rat plasma is also reported.

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P-02: Development of a simple pretreatment method for ICP-MS measurements and metallomics analysis of inflammatory condition

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Inductively Coupled Plasma Mass Spectrometry (ICP-MS) can quantitatively measure trace elements in various crude samples. However, it is not widely used in pharmacological research due to the difficulty of pretreating biological samples. Therefore, we searched for a suitable and simple processing method to establish a procedure for measuring biological samples and to explore changes of trace elements in mouse plasma at inflammatory conditions.

First to establish the sample preparation for ICP-MS, organic matters in human plasma were digested by long incubation with nitric acid and then, subjected to the measurement of the trace elements by ICP-MS. The accuracy of this preparation was confirmed by spike-recovery tests and standard plasma as well. At least 18 series of elements could be quantitatively measured by this method within 10% of error.

Next, we performed metallomic analysis using plasma from mice in which an inflammatory response was induced by intraperitoneal administration of lipopolysaccharide (LPS). As a result, essential trace elements such as selenium, iron, copper, and zinc were decreased 6 hours after administration. Many elements showed a tendency to recover throughout 24 hours after administration, but selenium continued to decrease. We measured the mRNA levels of inflammatory cytokines (TNFα and IL-1β) in the mouse liver. We found that they increased significantly throughout 6 hours after administration but had decreased to almost steady levels by 24 hours. The continuous decrease in selenium associated with inflammation suggested a change in the antioxidant system associated with the inflammatory response. Therefore, we examined the anti-inflammatory effects of the selenium-containing compound Ebselen and a novel selenium-containing compounds, which have antioxidant properties. As a result, Ebselen and the novel selenium-containing compounds suppressed the inflammatory response induced by LPS in cultured cells, and the latter in particular showed a remarkable suppression effect on the production of inflammatory cytokines. Further in vivo verification is currently underway.

P-03: Enhanced sensitivity of whole-cell As(III) sensors via the regulation of intracellular arsenic metabolism

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Arsenic (As(Ⅲ)) is a toxic substance that is widely present on Earth, and the development of whole-cell As(Ⅲ) sensors has attracted significant attention. Recently, we discovered that LuxR, a protein with multiple cysteines and a high binding affinity for As(Ⅲ), exhibits As(Ⅲ) aggregation properties and functions as an As(Ⅲ) sensor with an OFF-type response. In this study, we aimed to further increase the sensitivity of the As(Ⅲ) sensor. Increasing the contact frequency between the sensor element and the ligand is crucial for enhancing sensor sensitivity. We utilized a strain deficient in the As(Ⅲ) efflux protein ArsB to elevate the As(Ⅲ) concentration in *E. coli,* successfully increasing the sensitivity of the As(Ⅲ) response. Next, we used ArsR-LuxR, a fusion of the As(Ⅲ) metabolic regulator ArsR and LuxR, as a sensor element to easily control the intracellular As(Ⅲ) concentration, and it showed an OFF-type response similar to the LuxR-alone sensor. To further enhance sensitivity, we constructed the ArsR-LuxR High sensor by mutating the As(Ⅲ) binding domain of ArsR, which is crucial for regulating As(Ⅲ) metabolism, and suppressing the expression of ArsB, similar to the *arsB*-deficient strain. Fluorescence correlation spectroscopy revealed As(Ⅲ) aggregation properties of these sensors. ICP-MS measurements of intracellular As(Ⅲ) levels showed that the intracellular As(Ⅲ) levels of both sensor-expressing strains increased rapidly up to a medium As(Ⅲ) concentration of 1 ppm, at which the ArsR-LuxR High-expressing strain could grow. Then As(Ⅲ) levels increased slowly to 10 ppm, where the ArsR-LuxR-expressing strain could grow. The ArsR-LuxR High-expressing strain had a limiting value for intracellular As(Ⅲ) levels in the first step, where aggregation of the ArsR-LuxR High sensor element occurred, while the ArsR-LuxR-expressing strain had a limiting value in the second step, where aggregation occurred. This study demonstrates that for high sensitivity of As(Ⅲ) sensors, the sensor elements need to aggregate in the initial step, and that controlling arsenic metabolism is effective for this purpose.

P-04: Effects of gut microflora on the external excretion of selenium

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Selenium (Se) exists in diverse chemical forms in plants and animals, and they show physiological properties depending on their chemical structures. Although gut microflora were reported to metabolize Se, the influence of the Se transformation is not fully evaluated. In this study, we investigated the effects of gut microflora on the excretion of Se in a host animal. Gut microflora-suppressed model rats were prepared by administration of antibiotics. We determined administered-Se after administration of ^{82}Se-enriched selenite. A serum selenoprotein decreased in a health model compared to the gut microflora-suppressed model. On the other hand, the biosynthesis of trimethylselenonium ion (TMSe), a urinary selenometabolite, was promoted by gut microflora in rats. There was no significant difference in total Se in urine and in fecal between both models. These results suggested that Se was metabolized into an easily excretable metabolite by gut microflora, and it avoided the nutritional metabolic pathway of Se in a host animal.

P-05: Analysis of Molecular Mechanisms by which Major Components of Grape Peel Prevent Air Pollution Lung Injury

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Health hazards caused by air pollution are a major problem worldwide, with fine particulate matter (PM2.5), the main causative substance, increasing the mortality rate of lung and cardiovascular diseases via excessive production of reactive oxygen species (ROS). In contrast, oleanolic acid (OA), the main component of grape peel, is a triterpene and is known to possess various physiological activities such as antioxidant and anti-inflammatory effects. Therefore, in this study, we analysed the efficacy of OA in a mouse model of air pollution lung injury and the molecular mechanisms by which OA exerts its efficacy.

Oral administration of OA (5-80 mg/kg) to male ICR mice tended to suppress air pollutant-induced increases in the number of inflammatory cells (especially neutrophils), protein and dsDNA levels in bronchoalveolar lavage fluid (BALF). However, the effect was not pronounced, so we decided to investigate the efficacy of intravenous administration. Thus, we encapsulated OA within polyethylene

glycol-modified liposomes (OA-Lipo); OA-Lipo was prepared by a simple hydration method and its physical properties, including particle size, were evaluated. Intravenous administration of OA-Lipo (20-100 μg/kg) was found to be more effective against air pollutant-dependent lung injury at lower doses than oral OA administration. OA-Lipo also significantly suppressed air pollutant-dependent ROS production and increased expression of inflammatory cytokines such as TNF-α and IL-1β. Furthermore, we found that OA-Lipo induced increased expression of various antioxidant factors in the lungs of mice.

These results suggest that OA-Lipo may prevent the development of air pollution-induced lung injury by exerting its antioxidant effects through increased expression of various antioxidant factors.

P-06: Carbon distribution in living cells with femtosecond single pulse illumination by X-ray free electron laser

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X-ray microscopies are powerful tools for visualizing cellular elements and structures; however, radiation damage has hindered its application to living cells. We developed an X-ray microscope using femtosecond pulse illumination generated by a soft X-ray free electron laser, which enable to take images before Brownian motion; thus radiation damage is negligible. Employing Wolter mirrors we fabricated for illumination and objective optics allowed us to take images with a large field of view. We successfully captured carbon images of mammalian living cells in culture medium at both 300 and 390 eV. We found a large dark region at nucleus, which was assumed nucleolus, a well-known organelle and the primary site of ribosome subunit biogenesis, and therefore carbon is expected to be accumulated at the nucleolus. Another prominent carbon accumulation was in the nuclear membrane, which was not uniform at the membrane. We also found linear path-like structures connecting the nucleolus to the nuclear membrane, which was hardly seen in visible light microscopies. It might be a pathway of biomolecules from the nucleolus to the cytoplasm. Future examination of cells treated with chemicals related to biogenesis such as inhibitors may answer the question of what the carbon distribution signifies in living cells.

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P-07: Comparison of Trace Element Content in Dried Seaweed from Domestic, Korean, and Chinese Sources

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The content of trace elements in food may vary depending on whether it is domestically produced or imported, resulting in different health risks. This study evaluated the health risks associated with consuming farmed seaweed, commonly consumed by Japanese people, by comparing domestic products, imported products from Korea, and Chinese products [1].

From 2021 to 2022, we purchased about 90 samples of farmed dried seaweed from Japan and about 25 samples of imported Korean products in Sapporo. These samples were acid-digested in sealed Teflon containers, and heavy metal concentrations were measured using triple quadrupole ICP-MS (Agilent Technologies 8800 ICP-QQQ) at Hokkaido University.

The concentrations of seven elements (Fe, Mn, V, Co, Cu, Zn, As) in Japanese products were nearly twice as high as those in imported Korean products. The average concentrations of Cd, U, and Th in Korean seaweed were several times higher than those in Japanese products. The trace element concentrations [1] of V, Cd and Ni in Chinese seaweed were about an order of magnitude higher than those measured in Japanese products.

We estimated the carcinogenic and non-carcinogenic risks for each product using data [2] from the US Environmental Protection Agency. Cadmium contributed the most, and it was understandable that nori imported from China is sold not as dried seaweed but as mixed products like rice crackers and furikake in Japan.

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P-08: Mechanism of extracellular elemental selenium nanoparticles formation in *Escherichia coli*

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E. coli converts toxic selenite to less toxic Se nanoparticles (SeNPs) that accumulate extracellularly, but the process is poorly understood. Here, we purified the extracellular SeNPs and performed X-ray absorption fine structure analysis, X-ray photoelectron spectroscopy, and TEM observation. The results indicated the presence of a membrane covering the elemental selenium. SeNPs were bound to lipids, proteins, and cell wall components, suggesting that SeNPs were enwrapped by cell membranes. Furthermore, mutant strains lacking genes crucial for maintaining envelope structure integrity accumulated multiple SeNPs intracellularly. Comparing SeNP sizes between WT and mutant strains revealed that SeNPs underwent intracellular maturation, reaching approximately 100 nm before being excreted outside the cell. In conclusion, our results suggest that SeNPs are formed in the cytoplasm, encapsulated by the cell membrane, and subsequently released into the extracellular space.

P-09: Lineage tracing of zinc transporter ZIP10-expressing cell in the skin

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We previously reported that the zinc transporter ZIP10 is essential for the skin homeostasis[1]. However, the lineage of ZIP10-expressing cells has not been fully elucidated. In this study, we crossed Zip10-GFP-knockin (KI) mice with Rosa26-tdTomato reporter mice to generate Zip10-EGFP/tdTomato-KI mice. We analyzed the characteristics of tdTomato fluorescent protein-expressing cells (progeny of ZIP10/GFP-expressing cells) in these mice using FACS, and observed their localizations. In the skin tissues of Zip10-EGFP/tdTomato-KI mice treated with tamoxifen, tdTomato localization was partly different from GFP localization. Additionally, some of the tdTomato-positive cell population among the skin-derived cell suspensions expressed endothelial cell markers. These findings suggest that some ZIP10/GFP-expressing cells have the potential to differentiate into endothelial cells, which might be crucial for maintaining skin tissue homeostasis.

Reference:

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P-10: Occupational cadmium exposure and risk of proximal tubular dysfunction: A Survival Analysis Study

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[Objective] Cadmium is known to cause various health problems, and in recent years, especially in Europe, there has been a re-evaluation of health risks in industrial workplaces, particularly the early onset of proximal tubular dysfunction. On the other hand, there has not been sufficient evaluation using data from Japanese individuals, who have a higher baseline blood cadmium concentration (Cd-B) due to dietary intake. Therefore, we elucidate the relationship between Cd-B and proximal tubular dysfunction, using an increase in β_2-microglobulin as an indicator, utilizing the medical check-up data in Japanese industrial workplaces.

[Methods] Medical check-up data from 338 workers at two plants were collected between 1997 and 2020. Workers with at least two medical check-ups were included, excluding those with other renal dysfunctions. Proximal tubular dysfunction was defined as a β_2-microglobulin of 300 or more in two or more consecutive check-ups. A multivariate Cox proportional-hazards regression model with time-dependent covariates analyzed the relationship between Cd-B and the time to onset of proximal tubular dysfunction, adjusting for age, sex, and smoking history.

[Results] Of the 338 workers, 238 met the eligibility criteria. The geometric mean of Cd-B was 1.97 μg/L. The Cox proportional hazards model showed that higher time-dependent Cd-B levels were significantly associated with an increased risk of proximal tubular dysfunction, with a hazard ratio of 1.17 (95% CI 1.06 to 1.29).

[Discussion] In this study, it was found that workers exposed to cadmium with higher Cd-B had a significantly higher risk of developing proximal tubular dysfunction. Although it has already known that cadmium exposure causes proximal tubular dysfunction, the fact that health risk was observed even under current occupational health and safety management conditions cannot be ignored. Evaluating the current occupational exposure limit (5 μg/L) is a future challenge, and continuous monitoring is important to protect workers' health.

P-11: Mechanisms of abnormal lipid and trace element metabolism in adult rat pups induced by continuous intake of high-fat diet in mothers and pups

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Objective

The aim of this study was to elucidate the mechanisms of abnormal lipid metabolism, impaired autophagy and fluctuations in trace element metabolism in the liver of adult rat pups due to consumption of a high-fat diet by lactating mother rats and post-weanling pups, and to clarify the possibility of preventing these disorders by ingesting green tea extract (GTE) in lactating mother rats.

Methods

In the FFF and FGF groups, pregnant and lactating mother rats and post-weaning male pups were fed a high-fat diet (45% fat). In the FGF group, rats were fed a high-fat diet plus 0.24% GTE during lactation. In the CCC group, rats were fed a control diet (13% fat). Lipid metabolism and autophagy-related protein levels in each group were measured using Western blotting. Trace element concentrations in the liver were measured using ICP-MS.

Results and discussion

Accumulation of triglycerides (TG) was observed in the liver of the FFF group. Measurements of protein levels in the liver showed a significant increase in DGAT-1, which synthesizes TG using dietary fatty acids as substrates, a decreasing trend in MTTP, a protein that supplies lipids to the blood, and a significant decrease in FAS, an enzyme responsible for fatty acid synthesis in the liver, in the FFF group. Furthermore, an accumulation of phospho-p62, which carries unwanted substances to autophagosomes, was observed in the FFF group. In contrast, no significant changes in lipid metabolism and autophagy-related protein levels in the liver and no TG accumulation in the liver were observed in the FGF group. With regard to trace metals, significantly higher levels of Fe and significantly lower levels of Mn were found in the FFF group compared to the CCC group, with similar fluctuations in the FGF group.

In the FFF group, TG synthesis from dietary fatty acids was increased and the supply of lipids to the blood was reduced, resulting in TG accumulation in the liver, as well as a reduction in autophagy function. In the FGF group, on the other hand, these metabolism-related proteins were restored to normal levels, and the abnormalities in lipid metabolism were considered to have been improved. With regard to trace elements, high fat intake induced accumulation of Fe and a decrease in Mn concentration in the liver, but these trace element changes were not ameliorated by GTE intake.

P-12: Effects of intratracheal insilltaion of ITO nanoparticles with on the lungs and kidneys of female rats

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Objectives: The purpose of this study was to evaluate lung and kidney damage caused by intratracheal administration of indium-tin oxide nanoparticles (ITO NPs) in female rats.

Methods: Female Wistar rats were administered a single intratracheal dose of 20 mg In/kg body weight (BW) of ITO NPs. The control rats received only an intratracheal dose of distilled water. A subset of rats was periodically euthanized throughout the study from 1 to 48 weeks after administration. Indium concentrations in the lungs, mediastinal lymph nodes, kidneys, as well as pathological changes in the lungs and kidneys were determined.

Results: Indium concentrations in the lungs of the two ITO NP groups gradually decreased over the 48-week observation period. Indium levels in mediastinal lymph nodes and kidneys in the ITO 18 nm group increased up to week 22 after administration, but decreased slightly at 48 weeks. Conversely, in the ITO 40 nm group, indium concentrations in both organs increased until week 48 after administration. Pulmonary and renal toxicities were observed histopathologically in both the ITO groups.

Conclusions: Previously, we reported that ITO nanoparticles administered intratracheally in male rats developed pulmonary and kidney damage [1]. Our results demonstrate that intratracheal administration of 20 mg In/kg body weight of ITO NPs in female rats produces pulmonary and renal toxicities.

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P-13: Localization of Gadolinium-EDTMP on Bone Surfaces and Its Effect on Osteoclast Differentiation

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Objective: We have been developing a gadolinium (Gd) compound, Gd-EDTMP chelate, for neutron capture therapy targeting bone tumors. Our previous studies have shown that Gd-EDTMP rapidly distributes to bone after intraperitoneal injection in mice. This uptake mechanism may involve osteoclastic activity similar to bisphosphonate drugs [1], but the details remain unclear. Additionally, Gd-EDTMP can transfer Gd to calcium phosphate solid phase plates (PCa) through physical contact. In this study, we investigated the effects of Gd-EDTMP on osteoclast differentiation using RAW264 cells differentiated into osteoclasts on PCa in vitro.

Methods: PCa was fixed to the bottom of culture wells, and Gd-EDTMP (equivalent to 2 mg-Gd/kg and 20 mg-Gd/kg) was added to the culture medium. Gadoteridol was used as a control. RAW264 cells were cultured on PCa and differentiated into osteoclasts by adding receptor activator of nuclear factor-kappa B ligand (RANKL). After 7 days of culture, osteoclast differentiation was confirmed microscopically, and osteoclastic activity was assessed by the area of resorption pits formed on PCa. The Gd content in washed and recovered PCa plates and cells was quantified using ICP-MS (Agilent 8800).

Results and Discussion: Differentiation of RAW264 cells into osteoclasts on PCa was dose-dependently reduced in the Gd-EDTMP groups compared to the control group, with many cells remaining undifferentiated. The decreased pit area on PCa indicated reduced osteoclastic activity in the Gd-EDTMP groups. Gd uptake in undifferentiated cells suggested that Gd transferred to PCa affects the differentiation process of RAW264 cells into osteoclasts. These findings suggest that Gd-EDTMP not only serves as a neutron capture therapy agent but also has potential as a novel therapeutic approach for bone tumors by inhibiting osteoclast differentiation.

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P-14: Evaluation of the effects of fluoride exposure on the learning ability and memory in F1 rats

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The objective of this study was to elucidate fluoride neurotoxicity by examining exposure in the developmental stage of the fetus. The study focused on learning ability and memory evaluated using behavioral tests. Pregnant rats were exposed to fluoride via their drinking water (0 ppm, 150 ppm). After weaning, the F1 rats were divided into the Control-Control (CC) group, and the perinatal exposure Fluoride-Control (FC) group, and the continued exposure Fluoride-Fluoride (FF) group. The F1 rats were maintained until 12 weeks old and subjected to passive avoidance and Y-maze tests. After these behavioral tests, the rats were sacrificed, and the weights of each organ were measured. A significant decrease in final body weight was observed in the FF group compared to those in the CC and FC groups for males and females, respectively. In the Y-maze test, males in the FF group had significantly decreased alternation, which is related to short-term memory, compared to males in the CC group. The FF group had significantly higher relative kidney weight per final body weight compared to males in the CC group. These results suggest that the continuous exposure to fluoride suppresses growth in both males and females and, in males, impairs short-term memory as well. Males are more sensitive to the toxic effects of fluoride exposure than are females.

P-15: Human biomonitoring to evaluate exposure to toxic and essential trace elements in children: JECS pilot study

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Human biomonitoring (HBM) and cohort studies are being conducted for environmental health research in countries around the world. The Japan Environment and Children's Study (JECS), a large-

scale birth cohort study, was initiated in 2011 and involved around 100,000 parent-child pairs across the country. The purpose of this study is to determine the suitability of detection of each element in blood and urine, the concentrations of element in children's blood and urine samples, and the associations between elements in the samples.

Blood and urine samples were collected from 89 children aged 10 in the JECS Pilot Study. Each sample was pretreated using the alkaline solution dilution method. The analysis of 35 elements in the test solutions (34 elements in the blood samples) was conducted using ICP-MS, with measurement conditions applied according to each element. Pearson's correlation coefficient was used to test the correlation between elements in each sample after transforming analytical values logarithmically. IBM SPSS Statistic 29 was used to perform statistical analysis, and the significance was determined at p 0.05.

Except for elements that could not be applied due to undetected, contamination from the environment, or molecular interferences, 23 elements were statistically analyzed in the blood samples and 28 elements were analyzed in the urine samples. The main distribution of each element in blood showed that Li, B, Ca, Co, Cu, Br, Sr, Mo, Ag, Sb, and Tl were in plasma; Mg, Mn, Zn, Ge, As, Rb, Cd, Cs, Hg, and Pb were in red blood cells, and Se and Te were distributed a similar extent in plasma and red blood cells. Moderate correlations were observed between elements in each sample between 5 pairs of whole blood, 6 pairs of plasma, and 13 pairs of red blood cells. On the other hand, correlations were observed among various elements in urine, with 7 pairs exhibiting significant correlations. Moderate positive correlations were observed for Li, Co, As, Ag, Hg, and Tl in both whole blood and urine.

P-16: Application of Zn compounds on insulin resistance and DM complications derive from glycation reaction

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In this study, we investigated the effects of a zinc complex ($[Zn(hkt)_2]$), which has been suggested to improve insulin resistance, on impaired glucose tolerance and glycation stress-induced renal dysfunction using an animal model of glycation stress loaded with methylglyoxal (MGO). Five-week-old male C57BL/6JmsSlc mice were acclimated and divided into four groups: Normal, Control, $ZnSO_4$, and $[Zn(hkt)_2]$ groups. The Normal and Control groups received 1% CMC solution, and the $ZnSO_4$ and $[Zn(hkt)_2]$ groups received 0.2 mg Zn/kg BW intraperitoneally for 2 months, respectively. The area

under the time curve of blood glucose levels based on oral glucose tolerance test (OGTT) results showed a decreasing trend in the $ZnSO_4$ and $[Zn(hkt)_2]$ groups, suggesting improved glucose tolerance. Furthermore, HbA1c levels were significantly decreased in the Zn compounds groups, that is Zn compounds could have reduced the postprandial hyperglycemia. Regarding the effect on renal function, the urinary creatinine/albumin ratio recovered to the same level in the $[Zn(hkt)_2]$ group as in the Normal group. In conclusion, the Zn compound-treated groups were effective in improving in vivo glycation reactions caused by AGEs and in ameliorating renal glomerular damage.