NIMD Forum 2011

Methylmercury Toxicity:

Up-to-date Research on Mechanisms, Toxicology and Pathology

January 27-28, 2011

Venue: Minamata Disease Archives, Conference Hall

55-10 Myojin, Minamata City, Kumamoto 867-0055, Japan

Address

It is a great pleasure for me to be with you here and to have an opportunity to say a few words at the occasion of opening of NIMD forum 2011. First of all, on behalf of National Institute for Minamata Disease, I would like to extend our warmest welcome to all of you here to attend NIMD forum 2011. And also I would like to express my sincere gratitude to invited researchers from abroad who came all the way to Minamata here. Our institute was established in October 1978 and has more than 32 year's history. NIMD is a small but a unique institute; its purpose is to conduct comprehensive research on single chemical "mercury", especially "methylmercury" which caused Minamata Disease. Comprehensive means that we are conducting medical, environmental and social researches on Minamata disease and mercury. Therefore we cover very broad areas such as effects on human health, air and soil contamination, biogeochemistry and epidemiology.

NIMD forum has been held almost every year since 1997. The forums have been indeed excellent opportunities to bring many of active and experienced researchers from all over the world to Minamata and to share and to discuss the latest research findings on mercury. This is the twelfth NIMD forum. This year's forum focuses on methylmercury toxicity with special attention to up-to-date research on mechanisms, toxicology and pathology. We have fifteen presenters, seven from abroad, five from universities in Japan and three from NIMD. They are all leading scientists in this field. I hope that all participants will exchange ideas and views frankly so that everyone may be benefited from this forum. Last but not least, I sincere hope that two days meeting will be useful and fruitful for better understanding of current status of methylmercury toxicity researches. I want to once again extend our warmest welcome to all of you.

January 27, 2011

Koji Okamoto, M.D., M.P.H.
Director General
National Institute for Minamata Disease

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Hiroyuki Watanabe
Officer in International Affairs and Information Section, Department of International Affairs and Environmental Sciences,
National Institute for Minamata Disease

Invited Speakers and Chairpersons

Dr. William Rostene (Inserm, France)

Dr. Chris Newland (Auburn University, USA)

Dr. Sandra Ceccatelli (Karolinska Institute, Sweden)

Dr. Michael Aschner (Vanderbilt University Medical Center, USA)

Dr. Yoshito Kumagai (Tsukuba University, Japan)

Dr. Jean-Paul Bourdineaud (Bordeaux University, France)

Dr. Shuji Izumo (Kagoshima University, Japan)

Dr. Roshan Tofighi (Karolinska Institute, Sweden)

Dr. Ebany J. Martinez-Finley (Vanderbilt University Medical Center, USA)

Dr. Gi-Wook Hwang (Tohoku University, Japan)

Dr. Takashi Hirooka (Tokyo University of Science, Japan)

Dr. Takashi Toyama (Tsukuba University, Japan)

Dr. Akira Naganuma (Tohoku University, Japan)

Dr. Toshiyuki Kaji (Tokyo University of Science, Japan)

Program

January 27

9:20-9:25 **Opening address** Koji Okamoto (NIMD, Japan)

9:25-9:35 **Brief introduction** Fusako Usuki (NIMD, Japan)

9:35-10:55 **Session 1** Chair: Akira Yasutake (NIMD, Japan)
William Rostene (Inserm, France)

1-1. Chris Newland (Auburn University, USA)
Neuroprotection and Methylmercury: Selenium, DHA, and Nimodipine (魚由来成分のセレンや DHA およびカルシウム遮断薬であるニモジピンのメチル水銀毒性防御効果)

Follow-up remark: Mineshi Sakamoto (NIMD, Japan)
Protective effects of selenomethionine against methylmercury-induced neuronal degeneration in developing rat brain (メチル水銀により惹起されるラット発達期脳の神経変性に対するセレノメチオニンの抑制効果)

1-2. Sandra Ceccatelli (Karolinska Institute, Sweden)
Effects of methylmercury on survival and differentiation of neural stem cells (神経幹細胞の生存と分化に及ぼすメチル水銀の影響)

10:55-11:15 **Coffee break**

11:15-12:25 **Session 2** Chair: Masaaki Nakamura (NIMD, Japan)
Michael Aschner
(Vanderbilt University Medical Center, USA)

2-1. Takashi Toyama (Tsukuba University, Japan)
Keap1/Nrf2 system regulates cellular accumulation of methylmercury, thereby blocking its toxicity (メチル水銀蓄積に対する Keap1/Nrf2 システムの制御)

2-2. Yoshito Kumagai (Tsukuba University, Japan)
A unique protein mediating cellular protection against

Methylmercury (メチル水銀毒性防御に関与する特異な蛋白分子)

12:25-13:40 **Lunch**

13:40-14:50 **Session 3**

Chair: Toshiyuki Kaji (Tokyo University of Science, Japan)
Jean-Paul Bourdineaud (Bordeaux University, France)

3-1. Gi-Wook Hwang (Tohoku University, Japan)

Involvement of the post-translational modification of proteins in methylmercury toxicity (メチル水銀毒性における蛋白質の翻訳後修飾の関与)

3-2. Fusako Usuki (NIMD, Japan)

Posttranscriptional defects of antioxidant selenoenzymes cause oxidative stress under methylmercury exposure (メチル水銀曝露下における転写後変化と酸化ストレス)

14:50-16:20 **Session 4** Chair: Yoshito Kumagai (Tsukuba University, Japan)
Megumi Yamamoto (NIMD, Japan)

4-1. William Rostene (Inserm, France)

Protection by the chemokine CCL2/MCP1 of methylmercury neurotoxicity (メチル水銀神経毒性におけるケモカインの作用)

15:25-15:45 **Coffee break**

4-2. Takashi Hirooka (Tokyo University of Science, Japan)

The microvascular cells are a target of methylmercury toxicity
(メチル水銀の標的としての微小血管)

16:20-17:30 **Session 5** Chair: Gi-Wook Hwang (Tohoku University, Japan)
Chris Newland (Auburn University, USA)

5-1. Ebany J. Martinez-Finley (Vanderbilt University Medical Center, USA)

Methylmercury neurotoxicity in a *C. elegans* model system
(線虫モデルシステムにおけるメチル水銀毒性)

5-2. Jean-Paul Bourdineaud (Bordeaux University, France)

Zebrafish as a model of the mercurial contamination of the aquatic food web: histological, bioenergetical, and transcriptional issues
(メチル水銀毒性モデルとしてのゼブラフィッシュ)

19:00-20:30 **Reception**

January 28

9:00-10:10 **Session 6** Chair: Akira Naganuma (Tohoku University, Japan)

6-1. Shuji Izumo (Kagoshima University, Japan)

Early changes of astrocytes in the molecular layer of cerebellar cortex of rats with methyl mercury intoxication (メチル水銀毒性におけるアストログリアの初期変化)

6-2. Michael Aschner (Vanderbilt University Medical Center, USA)

The role of glia in modulating methylmercury neurotoxicity
(メチル水銀神経毒性におけるグリア細胞の役割)

10:10-10:30 **Coffee break**

10:30-11:40 **Session 7** Chair: Sandra Ceccatelli (Karolinska Institute, Sweden)
Shuji Izumo (Kagoshima University, Japan)

7-1. Masatake Fujimura (NIMD, Japan)

Neuritic degeneration contributes to MeHg-induced neuronal cell death (メチル水銀による神経細胞死への神経軸索変性の寄与)

7-2. Roshan Tofighi (Karolinska Institute, Sweden)

Methylmercury activates multiple cell death pathways in neuronal and glial cells (メチル水銀曝露下での神経細胞及びグリア細胞における神経細胞死経路)

11:40-11:50 **Brief summary** Fusako Usuki (NIMD, Japan)

11:50-11:55 **Closing address** Koji Okamoto (NIMD, Japan)

1-1 Neuroprotection and Methylmercury: Selenium, DHA, and Nimodipine

Christopher Newland
Auburn University, Auburn, AL.

It has been suggested that fish nutrients like DHA and selenium might confer protection against the neurotoxicty of methylmercury. Other hypotheses about neuroprotection have derived from mechanisms by which methylmercury may exert its neurotoxicity, like disruption of calcium homeostasis. We have investigated these hypotheses by combining methylmercury exposure with fish nutrients or, in ongoing studies, with the calcium channel blocker, nimodipine. Neuroprotection by fish nutrients was examined both in developmental and adult-onset methylmercury exposures. Neither DHA nor selenium protected against methylmercury's developmental neurotoxicity, which was found to occur in rats at very low daily intakes of 40 ug/kg/day. Selenium, however, significantly postponed the onset of methylmercury's neurotoxicity when exposure began in adulthood. Whether this was due to actions in the brain or to peripheral actions is uncertain. In ongoing studies using mice, dietary nimodipine delays the appearance of neuromotor deficits, including performance on a running wheel. Nimodipine also delayed the appearance of cognitive deficits that we detected using an "incremental repeated acquisition" procedure. This exposure produced no detectable nimodipine in blood but did produce a large number of detectable metabolites. Interestingly, no such protection was seen when nimodipine exposure occurred through subcutaneous implants, which produced detectable nimodipinne but fewer metabolites. One hypotheses to test would be that the protection came from a metabolite, rather than from nimodipine.

Key Words: selenium, DHA, nimodipine, neuroprotection, neuro-behavioral effects
References: 1) Heath JC et al., Neurotoxicology, 31, 169-179, 2010.
2) Reed MN et al., Neurotoxicology, 27, 721-732, 2006.
3) Newland MC et al., Animal Models of Cognitive Impairment. pp 101-146, CRC Press, 2006.

[紹介]

演者の Newland 先生は、「アメリカ合衆国・オーバーン大学」において研究を行っておられます。今回の発表は、「魚由来成分のセレンや DHA およびカルシウム遮断薬であるニモジピンのメチル水銀毒性防御効果」についてです。ラットを用いた実験で、DHA やセレンは発達期のメチル水銀毒性には効果がありませんでしたが、成人期の曝露ではセレンはその毒性発現を遅延させました。また、食餌性のニモジピンが、マウスの運動障害や認知障害などの毒性発現を遅延させることが明らかになりました。

Selenium: セレン
DHA: ドコサヘキサエン酸
Nimodipine: ニモジピン
Neuroprotection: 神経保護作用
Neuro-behavioral effects: 神経行動効果

Follow-up remark

Protective effects of selenomethionine against methylmercury-induced neuronal degeneration in developing rat brain

Mineshi Sakamoto[1], Akira Yasutake[1], Megumi Yamamoto[1], Hing Man Chan[2], Masae Ryufuku[3], Akiyoshi Kakita[3], Sanae Oumi[4], Chiho Watanabe[4]

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[2] University of Northern British Columbia, Canada

[3] Brain Research Institute, Niigata University, Niigata, Japan

[4] University of Tokyo, Japan

Although a protective effect of selenium (Se) against the toxicity of methylmercury (MeHg) has been confirmed in some animal studies, its direct effects in the brain has not been reported. Substantiation of the direct protective effects of selenomethionine (SeMet), one chemical form of Se in food, against MeHg-induced neurotoxicity would provide important information for the risk evaluation of MeHg on seafood. Our goal in this study is to demonstrate the direct protective effects of Se against the neuronal degeneration caused by MeHg in developing brains which is vulnerable to MeHg exposure. We used a model rat of fetal-type Minamata disease which causes widespread neuronal degeneration in the brain at a high dose of MeHg. Male Wistar rats of postnatal days 14 (PD14) were maintained by their dam. They were orally administered MeHg, either MeHg (8 mg Hg/kg/day in a solution containing L-cysteine and condensed milk), SeMet (2 mg/kg/day) alone or MeHg and SeMet co-exposure (MeHg was administered 30 min after SeMet) for 10 consecutive days. Twenty-four hours after the final administration, the effects of different treatment were investigated by histopathological and chemical analyses. Decreases in body and liver weights were observed in MeHg and SeMet groups, but were negated by co-exposure. Neuronal degeneration in the cerebral neocortex, decreased GSH-Px activity and increased hydroperoxide were observed in MeHg group, and co-exposure to SeMet prevented the effects. On the other hand, SeMet decreased GSH and GSH-Px activity and increased T-Bil, which indicate liver damage by SeMet, but the effect was negated by co-exposure to MeHg. Co-exposure to SeMet increased the total mercury concentrations in the cerebral cortex and cerebellum. Co-exposure to SeMet also increased the percentage of inorganic Hg in all organ tissues, which indicates increased demethylation by SeMet.

This is, to our knowledge, the first report which shows the direct protective effect of Se against MeHg-induced neuronal degeneration in the brain. We consider the neuroprotection effect will be derived by a MeHg and Se complex such as bis-methylmercury selenide, which was formed by co-exposure to MeHg and SeMet. This may be also explained by the fact that SeMet-induced liver damage was prevented by MeHg.

Key Words: methylmercury, developing rat brain, selenomethionine, neuronal degeneration, neuroprotective effects

References: 1) Sakamoto M et al., Brain Res., 784, 351-354, 1998.
2) Sakamoto M et al., Brain Res., 949, 51-59, 2002.
3) Sakamoto M et al., Dev. Brain Res., 152, 171-176, 2004.

[紹介]

演者の坂本は、「国立水俣病総合研究センター」において研究を行っています。今回は、Newland 先生の発表に対する追加発言として「メチル水銀により惹起されるラット発達期脳の神経変性に対するセレノメチオニンの抑制効果」について発表を行います。メチル水銀はラットの発達期脳の神経に傷害をあたえますが、セレノメチオニンの同時投与によってその変化は抑制されました。

Methylmercury: メチル水銀
Developing rat brain: 発達期ラット脳
Selenomethionine:
セレノメチオニン（セレンを含むアミノ酸で、食物などに含まれるセレンの摂取源）
Neuronal degeneration: 神経変性
Neuroprotection effects: 神経保護作用

1-2 Effects of methylmercury on survival and differentiation of neural stem cells

Sandra Ceccatelli

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Neural stem cells (NSCs) play an essential role in both the developing and adult nervous system. In the last few years, we have studied the mechanisms by which NSCs undergo cell death in response to neurotoxicants [1)], such as methylmercury (MeHg) [2)]. The mouse NSC line C17.2 and rat primary embryonic cortical NSCs were used as experimental models to investigate the effects of MeHg on survival and differentiation. The results show that NSCs are more vulnerable to MeHg, as compared to differentiated cells. MeHg induces apoptosis via Bax-activation, cytochrome *c* translocation and caspase activation with subsequent fodrin cleavage and formation of the 120 kD fragment. In addition, there is a significant increase of the 150 kD fodrin breakdown product indicating that calpains are also activated. The caspase and calpain proteases are concomitantly activated in NSCs undergoing apoptosis, as proven by the partial protection exerted by the caspases (zVAD-fmk) or calpains (E64d) inhibitor alone and the full protective effect of the two inhibitors together. Remarkably, exposure to MeHg at concentrations relevant to human exposure inhibits spontaneous neuronal differentiation of NSCs via Notch signaling [3)]. Conversely, other neurotoxic food contaminants, such as non-dioxin like PCBs, promote neuronal differentiation [4)]. The evaluation of NSC differentiation offers sensitive endpoints to identify substances with developmental neurotoxic potential and further points to NSCs as a relevant *in vitro* model for neurotoxicity studies.

Key words: Apoptosis, caspases, calpains, spontaneous differentiation, developmental neurotoxicity.

References: 1) Ceccatelli S. et al., Toxicol. Lett., 149, 59-66, 2004.

2) Tamm C. et al., J. Neurochem., 97, 69-78, 2006.

3) Tamm C. et al., Neuroreport, 19, 339-43, 2008.

4) Tofighi R. et al., Toxicol. Sci., submitted.

[紹介]

演者の Ceccatelli 先生は、「スウェーデン・カロリンスカ研究所」において研究を行っておられます。今回の発表は、「神経幹細胞の生存と分化に及ぼすメチル水銀の影響」についてです。神経幹細胞 (Neural stem cells) は、発生期および成体内に存在していて神経やグリア細胞に分化する重要な細胞です。培養細胞を用いた実験によって、神経幹細胞は分化した細胞よりもメチル水銀感受性が高く、アポトーシスをおこしやすいことが明らかになりました。さらに、低濃度のメチル水銀は神経幹細胞の分化を抑制することも明らかになりました。

Apoptosis: アポトーシス (プログラムされた“自殺性”の細胞死)
Caspases: カスパーゼ (アポトーシス誘導に関与する蛋白質分解酵素)
Calpains: カルパイン (カルシウム依存性の蛋白質分解酵素)
Spontaneous differentiation: 自然分化
Developmental neurotoxicity: 発達神経毒性

2-1 Keap1/Nrf2 system regulates cellular accumulation of methylmercury, thereby blocking its toxicity

Takashi Toyama
Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan.
Research Fellow of the Japan Society for the Promotion of Science, Japan.

Methylmercury (MeHg), a naturally occurring environmental electrophile, exhibits neurotoxicity through accumulation in the brain. We reported previously that MeHg activates nuclear factor-erythroid 2-related factor 2 (Nrf2), a basic-leucine zipper transcription factor, thereby up-regulating its downstream gene products and that Nrf2 plays an important role in reducing the cellular accumulation of MeHg[1)]. In our current study, we investigated the protective effect of isothiocyanates, which are known to activate Nrf2, on the accumulation of mercury and its toxicity following exposure of mice to MeHg *in vitro* and *in vivo*. Isothiocyanates 6-methylsulfinylhexyl isothiocyanate (6-HITC) and sulforaphane (SFN) activated Nrf2 and upregulated downstream proteins associated with MeHg excretion such as glutamate-cysteine ligase, glutathione *S*-transferase, and multidrug resistance–associated protein in primary mouse hepatocytes. Under these conditions, intracellular glutathione levels increased in wild-type but not Nrf2-deficient primary mouse hepatocytes. Pretreatment with these isothiocyanates before MeHg exposure suppressed cellular accumulation of mercury and cytotoxicity in wild-type but not Nrf2-deficient primary mouse hepatocytes. Compared with that in wild-type mice, oral administration of MeHg (5 mg/kg/day) to Nrf2-deficient mice for 8 days resulted in increased sensitivity to mercury concomitant with an increase in mercury accumulation in the brain and liver. Injection of SFN before administration of MeHg resulted in a decrease in mercury accumulation in the brain and liver of wild-type, but not Nrf2-deficient, mice. These data suggesting that 6-HITC and SFN can suppress mercury accumulation and intoxication caused by MeHg intake through activation of Nrf2.

Key words: Nrf2, isothiocyanate, chemoprevention.
References: 1) Toyama T et al., Biochem. Biophys. Res. Commun., 363, 645-650, 2007.

[紹介]

演者の外山先生は、「筑波大学」において研究を行っておられます。今回の発表は、「メチル水銀蓄積に対する Keap1/Nrf2 システムの制御」についてです。転写因子である Nrf2 はメチル水銀により活性化され、その結果、下流の遺伝子発現が増加してメチル水銀の細胞内蓄積を減少させます。Nrf2 を活性化することが知られているイソチオシアン酸塩を細胞または実験動物に処置することによって、メチル水銀の細胞内蓄積および細胞死を抑制できることが明らかになりました。

Nrf2: (DNA に特異的に結合して遺伝子転写を制御する転写因子の一つ)
Isothiocyanate: イソチオシアン酸塩
Chemoprevention: 化学的予防

2-2 A unique protein mediating cellular protection against methylmercury

Yoshito Kumagai
Doctoral Programs in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba.

Methylmercury (MeHg) is an environmental electrophile that reacts readily with protein thiols to form a covalent bond formation, thereby causing the functional loss and alteration in the structure. Some of unbound MeHg is also covalently bound to low molecule nucleophiles such as glutathione (GSH), by nonenzymatic and possibly enzymatic processes by the action of GSH S-transferases (GSTs), to form a MeHg–GSH adduct. This adduct is transported out to extracellular space by multidrug resistance-associated proteins (MRPs). These detoxification processes can substantially reduce intracellular MeHg. Nuclear factor-erythroid 2-related factor 2 (Nrf2), a basic-leucine zipper transcription factor, is found to coordinately regulate gene expression of glutamate cysteine ligase (GCL) catalyzing GSH synthesis, GSTs and MRPs. We have found that Nrf2 plays a role in the initial response through modification of Keap1, the negative regulator of Nrf2, leading to activation of Nrf2 and cellular protection through up-regulation of GCL, GSTs and MRPs during exposure to MeHg *in vitro* and *in vivo*[1, 2, 3]. Hydrogen sulfide (H_2S) is found to be a biological signaling molecule involved in vascular and nervous system functions. Most interestingly, H_2S is extensively dissociated into a nucleophile HS^- because of its p*K*a value = 6.88 while p*K*a value of GSH is 8.66, implying that most of GSH exists as its protonated form at physiological conditions. However, role of H_2S in chemical modification of cellular proteins and cytotoxicity during exposure to MeHg is poorly understood. In this symposium, I will introduce current our findings that not only GSH but also enzymatically produced H_2S are key nucleophiles that protect against electrophilic attack of MeHg, resulting in reduction of the cell damage.

Key words: hydrogen sulfide, Keap1/Nrf2 system, covalent modification, detoxification, thiol adduct.

References: 1) Toyama T et al., Biochem. Biophys. Res. Commun., 363, 645-650, 2007.
2) Toyama T et al., Toxicol. Appl. Pharmacol., 249, 86-90, 2010.
3) Toyama T et al., Environ. Health Perspect., submitted.

[紹介]

演者の熊谷先生は、「筑波大学」において研究を行っておられます。今回の発表は、「メチル水銀毒性防御に関与する特異な蛋白分子」についてです。メチル水銀は、蛋白質のチオール (Thiol) 基と容易にイオウ原子を介して共有結合する親電子性環境物質です。イオウ原子を含む硫化水素は、血管系や神経系における生物学的なシグナル分子であることが最近知られてきました。メチル水銀曝露下での蛋白質の化学修飾や細胞毒性における硫化水素の役割について検討し、硫化水素はメチル水銀の親電子物質としての攻撃を防御し、細胞障害を減弱させることが明らかになりました。

Hydrogen sulfide: 硫化水素
Keap1/Nrf2 system: Keap1/Nrf2システム
Covalent modification: 共有結合性の修飾
Detoxification: 解毒
Thiol adduct: チオール付加化合物

3-1 Involvement of the post-translational modification of proteins in methylmercury toxicity

Gi-Wook HWANG
Graduate School of Pharmaceutical Sciences, Tohoku University, Japan.

Methylmercury (MeHg) is a toxic environmental pollutant, causing serious neurological and developmental effects in humans. However, the mechanism underlying the MeHg toxicity is not fully understood. We used yeast cells (*S. cerevisiae*) to elucidate the defense mechanisms against MeHg toxicity, and search for novel genes involved in MeHg resistance because *S. cerevisiae* is a eukaryotic organism having many gene products that are functionally similar to those of mammals such as humans. We have previously reported that Cdc34, Hrt3, Ylr224w and Rad23, proteins related to ubiquitin-proteasome system (UP system), confer MeHg resistance to yeast cells. It seems likely that certain proteins that have not yet been identified but exist in cells, increase MeHg toxicity. The toxicity of MeHg might be reduced by enhanced degradation of these proteins through the UP system which is strongly conserved from yeast to human cells. We also found that the UP system plays an important role in lending protection against MeHg toxicity not only in yeast cells and but also in human cells. Hrt3 and Ylr224w are F-box proteins which are known to recognize and bind to substrate proteins that are then degraded by the UP system. We recently identified Dld3 and Eno2 as proteins that specifically bind to these F-box proteins. We also found that both proteins involved in reinforcement of MeHg toxicity. Dld3 and Eno2 are known to be proteins involved in synthesis of pyruvate, and overexpression of each protein might induce increase in interacellular levels of pyruvate. We found that deletion of Yil006w, a transports pyruvate into mitochondria, confers resistance to MeHg in yeast cells. These results suggest that promotion of pyruvate inflow into the mitochondria might enhance MeHg toxicity. In the future, detailed studies on the role of the pyruvate in MeHg toxicity will help elucidate the mechanism of MeHg toxicity and the corresponding biological defense mechanism.

Key words: ubiquitin, proteolysis, pyruvate, mitochondria.
References: 1) Hwang G.W. et al., FASEB J., 16, 709-711, 2002.
2) Hwang G.W. et al., FEBS Lett., 580, 6813-6818, 2006.
3) Hwang G.W. et al., J. Toxicol. Sci., 34, 413-416, 2009.

[紹介]

演者の黄先生は、「東北大学」において研究を行っておられます。今回の発表は、「メチル水銀毒性における蛋白質の翻訳後修飾の関与」についてです。蛋白質の翻訳後修飾には様々なものが知られています。その中で、細胞内で不要となった蛋白質にユビキチンで"印"を付け、これをアミノ酸へと分解してリサイクルするユビキチンープロテアソームシステム (UP システム) (Ubiquitin-proteasome system, UP system) が、メチル水銀毒性の防御メカニズムに重要な役割をもつことが明らかになりました。UP システムによって分解され、メチル水銀毒性を増強させる蛋白質について検討したところ、新たな蛋白質が同定されました。この蛋白質はピルビン酸生成に関係しており、ミトコンドリアへのピルビン酸の流入がメチル水銀毒性に関与していることが明らかになりました。

Ubiquitin: ユビキチン (細胞内で不要となったタンパク質の"印"の役割をするタン パク質)
Proteolysis: 蛋白質分解
Pyruvate: ピルビン酸 (エネルギー代謝経路酵素の一種)
Mitochondria: ミトコンドリア (細胞内に存在する酸素呼吸を行う小器官)

3-2 Posttranscriptional defects of antioxidant selenoenzymes cause oxidative stress under methylmercury exposure

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The critical role of oxidative stress in the pathogenesis of methylmercury (MeHg) cytotoxicity has been clarified, but the molecular mechanisms underlying MeHg-mediated oxidative stress remain to be elucidated. Here I demonstrate a posttranscriptional effect of MeHg on antioxidant selenoenzymes by using a myogenic model cell line[1)] showing apoptosis by exposure to MeHg. Quantitative real-time PCR analysis showed down-regulation of the major antioxidant selenoenzyme, glutathione peroxidase 1 (GPx1) mRNA in spite of up-regulation of other antioxidant enzyme mRNAs. MeHg-induced decrease in GPx1 mRNA was not suppressed by the co-addition with antioxidant Trolox but by the pretreatment with sodium selenite, suggesting that the decrease in GPx1 mRNA was due to MeHg-induced relative intracellular selenium deficiency. This notion was supported by the inhibition study of nonsense-mediated mRNA decay (NMD)[2)], which recognizes a UGA codon for selenocysteine on GPx1 as a nonsense codon and degrades GPx1 mRNA in selenium-deficient condition. In contrast, thioredoxin reductase 1 (TrxR1), another antioxidant selenoenzyme of the thioredoxin system, was likely skipped by NMD because of a UGA codon in the last exon. However, TrxR1 activity was decreased despite mRNA upregulation, which was probably due to the synthesis of aberrant TrxR1 protein without selenocysteine. Changes in selenoenzyme GPx1 and TrxR1 mRNAs were observed earlier than was the incidence of oxidative stress and upregulation of other antioxidant enzyme mRNAs. Results indicated that the MeHg-induced relative selenium-deficient condition affects the major antioxidant selenoenzymes GPx1 and TrxR1 through a posttranscriptional effect, resulting in the disturbance of cellular redox systems and the incidence of oxidative stress[3)]. Treatment with ebselen, a seleno-organic compound, effectively suppressed oxidative stress and protected cells against MeHg-induced relative selenium deficiency and cytotoxicity.

Key words: oxidative stress, antioxidant selenoenzyme, nonsense-mediated mRNA decay (NMD), posttranscriptional defect, ebselen

References: 1) Usuki F et al., Neurotoxicology, 29, 22-30, 2008.

2) Yamashita A et al., Biochim. Biophys. Acta., 1754, 305-315, 2005.
3) Usuki F et al., J. Biol. Chem., 2010 Nov 24 [Epub. Ahead of print].

[紹介]

演者の臼杵は、「国立水俣病総合研究センター」において研究を行っています。今回の発表は、「メチル水銀曝露下における転写後変化と酸化ストレス」についてです。メチル水銀の細胞毒性には酸化ストレス傷害が重要な役割をもちますが、なぜ、メチル水銀が酸化ストレスを起こすかはよくわかっていませんでした。培養細胞を用いた実験によって、メチル水銀がセレン動態への影響を介してセレン含有抗酸化酵素の遺伝子発現を制御し、酸化ストレスを引き起こすことが明らかになりました。また、セレノ有機化合物であるエブセレンが、メチル水銀による酸化ストレスと細胞毒性を抑制することも明らかになりました。

Oxidative stress: 酸化ストレス
Antioxidant selenoenzyme: セレン含有抗酸化酵素
Nonsense-mediated mRNA decay (NMD):
遺伝子配列の途中に生じたナンセンスコドンを含む変異 mRNA を排除する mRNA 監視機構
Posttranscriptional defect: 転写後の欠陥
Ebselen: エブセレン (セレノ有機化合物)

4-1 Protection by the chemokine CCL2/MCP-1 of the neurotoxic effect of methylmercury

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Industrial pollution due to heavy metals such as mercury is a major concern for the environment and public health. Mercury, in particular methylmercury (MeHg), primarily affects brain development and activity, resulting in neurotoxic effects. As chemokines are considered to be involved in neuroinflammatory and neurodegenerative diseases [1)], we tested the possibility that one of these chemokines, CCL2/MCP-1, can be implicated in the neurotoxic effects of MeHg. Using *in vitro* experiments on rat pure cortical neurons in culture and *in vivo* by means of a fish-based diet to mice [2)], we demonstrate that the chemokine CCL2/MCP-1, *via* its cognate receptor CCR2, is able to protect cortical neurons from MeHg-induced cell death through a mechanism involving the glutathione pathway. Furthermore, MeHg induced a decrease in mice brain CCL2/MCP-1 concentrations and a significant cortical microglial recruitment. Finally, the protective effect of CCL2/MCP-1 was confirmed in CCL2 KO mice which showed a more pronounced neuronal cell death than wild-type mice in specific layers of the cortex in response to MeHg. We suggest that during the early phase of MeHg contamination, an increased release of CCL2/MCP-1 by neurons acting as an autocrine feedback loop and by glial cells such as microglia, could partially protect from neuronal apoptotic cell death. The dying neurons thus inform the microenvironment in order to produce more CCL2/MCP-1. When the contamination lasts, CCL2 cannot anymore prevent the neurotoxic effect of MeHg and the neuronal cell death spreeds up. These original findings open new avenues on the implication of the chemokine systems in brain deficits due to MeHg intoxication.

Key words: chemokines, CCL2/MCP-1, neuronal cell death, microglia, methylmercury.

References: 1) Rostene W et al., Nature Rev. Neurosci., 8, 895-903, 2007.
2) Bourdineaud JP et al., Environ. Health, 7, 53-66, 2008.

[紹介]

演者の Rostène 先生は、「フランス・インサーム」において研究を行っておられます。今回の発表は、「メチル水銀神経毒性におけるケモカインの作用」についてです。培養神経細胞およびケモカインを遺伝子操作で喪失させたケモカインノックアウトマウスを用いた実験によって、生体内のケモカインがメチル水銀毒性に対して抑制的に働いていることが明らかになりました。

Chemokines: ケモカイン (神経炎症や神経変性に関係する蛋白質)
CCL2/MCP-1: (ケモカインの一種)
Neuronal cell death: 神経細胞死
Microglia: ミクログリア (グリア細胞の一種、食作用がある)
Methylmercury: メチル水銀

4-2 The microvascular cells are a target of methylmercury toxicity

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Methylmercury is a toxic metal compound that induces severe neuropathy in the brain of exposed humans and animals, as is observed in patients with Minamata disease. The neuropathological lesions are localized in the cerebral cortices along deep sulci and fissures including the calcarine, and the granular cell layers in cerebellum. Although the mechanisms of the selective vulnerability have been incompletely understood, it appears to be possible that vascular toxicity of methylmercury is important for the selective vulnerability in the brain. We have investigated the cytotoxic effects of methylmercury on human brain microvascular pericytes and endothelial cells in a culture system. The pericytes at low cell densities were highly susceptible to methylmercury cytotoxicity. This susceptibility was due to the not only constitutively high but also methylmercury-induced expression of LAT-1, which transports methylmercury into cells[1)]. On the other hand, the endothelial cells were resistant to methylmercury, which was due to higher expression of glutathione and metallothionein[2)]. Their proliferation was inhibited by suppression of FGF-2 expression[3)]. Vascular permeability is regulated by the VEGF system, which is consisted of VEGF family proteins and receptors. In endothelial cells, methylmercury induced the expression of VEGFR-1 and VEGFR-2; in addition, methylmercury increased in the secretion of PlGF, a VEGF family protein that enhances the binding of VEGF-A to VEGFR-2 by preventing the binding of VEGF-A to VEGFR-1. In pericytes, methylmercury increased the VEGF-A_{165} secretion. Furthermore, methylmercury promoted the synthesis of hyaluronan in both endothelial cells and pericytes. These results suggest that methylmercury induces the vasogenic edema by stimulation the autocrine/paracrine signaling of VEGF and hyaluronan secretion in microvessels. We propose the possible mechanisms of the selective localization of the cerebral lesion caused by methylmercury as follows: 1) Methylmercury induces the damage of perocyes. 2) methylmercury inhibits the repair of damaged endothelial cell layers. 3) methylmercury increases the permeability of brain microvascular tissue by activation of the VEGF system. 4) methylmercury increases hyaluronan that maintains water in the extracellular matrix of brain microvascular tissue.

Key words: brain microvascular cells, VEGF system, vasogenic edema.

References: 1) Hirooka T et al., Toxicol. In Vitro, 24, 835-41, 2010.
2) Hirooka T et al., J. Toxicol. Sci., 35, 287-94, 2010.
3) Hirooka T et al., J. Toxicol. Sci., 34, 433-39, 2010.

[紹介]

演者の廣岡先生は、「東京理科大学」において研究を行っておられます。今回の発表は、「メチル水銀の標的としての微小血管」についてです。ヒト脳の微小血管細胞を用いた実験によって、メチル水銀が血管周囲細胞の障害や血管内皮細胞の再生障害、血管内皮細胞増殖因子システムの活性化による血管透過性の増大、および血管構造の水分量を調整するヒアルロナン(Hyaluronan) 量の増大を引き起こすことが明らかになり、メチル水銀が血管性浮腫を誘発する可能性が示唆されました。

Brain microvascular cells: 脳微小血管細胞
VEGF system: 血管内皮細胞増殖因子システム
Vasogenic edema: 血管性浮腫

5-1 Role of Skn-1 in methylmercury-induced dopaminergic neurotoxicity in *Caenorhabditis elegans*

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Mercury (Hg) is a persistent environmental contaminant that exerts its toxic effects on the nervous system through molecular mechanisms that remain unknown. Epidemiological studies have pointed to the contribution of methylmercury (MeHg) to dopamine neuron vulnerability and the predisposition to Parkinson's disease (PD). Nrf2, a phase II antioxidant transcription factor, has been shown to be involved in MeHg neurotoxicity. Overexpression of Nrf2 inhibits MeHg-mediated cell death and deficits in Nrf2 leaves cells vulnerable to MeHg. We hypothesize that skn-1, the *Caenorhabditis elegans (C. elegans)* orthologue of mammalian Nrf2, is an important factor in MeHg-induced dopaminergic neurodegeneration. We knocked down skn-1 (skn-1 KO) in *C. elegans* and exposed both N2 (control) and skn-1 KO worms to 0, 10, 20 and 30µM MeHgCl for 30 minutes or 4 hours following synchronization. Our data suggests that skn-1 KO nematodes (LC_{50}=16µM MeHg) are more sensitive to MeHg than N2 controls (LC_{50}=25µM MeHg). Dopaminergic neuronal morphology was subsequently observed via fluorescent analysis at L1, L4 and adult life stages. Presence of puncta was observed at 1µM MeHg. Longevity and brood size were also assessed. Decreased longevity was observed at high doses in both N2 and skn-1KO nematodes. MeHg, at 20µM, had a significant effect on brood size, and decreased nematode size immediately following exposure indicates a developmental delay. We are currently testing the vulnerability of parkin, a juvenile onset PD gene, to MeHg to delineate the contribution of MeHg to PD. Our data suggest that *C. elegans* is a valuable model for studying the effects of MeHg on the nervous system, delineating molecular mechanisms of toxicity and determining genetic susceptibility. Acknowledgements: ES R01 07331.

Key Words: *C. elegans*, skn-1, Parkinson's Disease.

[紹介]

演者の Martinez-Finley 先生は、「アメリカ合衆国・バンダービルド大学」で研究を行っておられます。今回の発表は、「線虫モデルシステムにおけるメチル水銀毒性」についてです。線虫のシー・エレガンスは全遺伝子の解析が終了しており、遺伝子操作 (特定の遺伝子の欠損や付加) が容易で、毒性メカニズム研究に有用です。哺乳類における転写因子 Nrf2 に相当する線虫の Skn-1 を遺伝子操作で欠損させたシー・エレガンスでは、メチル水銀毒性に対する感受性が野生株よりも高くなることが明らかになりました。シー・エレガンスは、メチル水銀の神経系への影響を研究し、毒性や遺伝子感受性を明らかにするのに有用な実験モデルです。現在、メチル水銀とパーキンソン病への寄与について明らかにするために、若年性パーキンソン病の原因遺伝子であるパーキン (Parkin) のメチル水銀に対する脆弱性について検討しています。

C. elegans: シー・エレガンス (線虫の一種, モデル生物として広く利用されている)
Skn-1: (哺乳類における転写因子、Nrf2 に相当)
Parkinson's Disease: パーキンソン病

5-2 Zebrafish as a model of the mercurial contamination of the aquatic food web: histological, bioenergetical, and transcriptional issues.

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Methylmercury (MeHg) is a potent neurotoxic involved in the Minamata tragedy. In the Amazonian basin, gold mining leads to MeHg biomagnification all along the food web, culminating in piscivorous fish, ultimately responsible for contamination of human beings through fish consumption. In order to assess the biological impact of dietary MeHg on fish, we contaminated zebrafish with MeHg-contaminated food (13.5 □g of Hg/g of food, an environmentally relevant dose). A genome scale analysis of gene expression was conducted on the skeletal muscle because this tissue does not perform MeHg demethylation, and among 5280 different transcripts, 60 appeared up-regulated and 15 down-regulated by more than 2 times. A net impact of MeHg was noticed on 14 ribosomal protein genes, indicating a perturbation of protein synthesis. Several genes involved in mitochondrial metabolism, the electron transport chain, endoplasmic reticulum (ER) function, detoxification, and general stress responses were differentially regulated, suggesting an onset of oxidative stress and ER stress. Several other genes for which expression varied with MeHg contamination could be clustered in various compartments of the cell's life, such as lipid metabolism, calcium homeostasis, iron metabolism, muscle contraction, and cell cycle regulation. Mitochondria from contaminated zebrafish muscles presented structural abnormalities under electron microscopy observation such as cristae disorganization. In permeabilized muscle fibers, we observed a strong inhibition of state 3 mitochondrial respiration, a decrease of cytochrome *c* oxidase activity and in the rate of ATP release after 49 days of exposure. Contrarily to muscles, brain mitochondrial respiration was not modified by MeHg exposure. GABA transaminase, glial fibrillary acidic protein, and glutathione S-transferase genes (*abat*, *gfap*, and *gst*) were up regulated, whereas the glutathione peroxidase gene *gpx1* was repressed, suggesting an impact of MeHg on GABA concentration, and the onset of an oxidative stress and inflammation. A transmission electron microscopic observation revealed a disorganization of the optical tectum.

Key words: mitochondrial impairment, transcriptional response, zebrafish, muscle ATP depletion, optical tectum.
References: 1) Cambier S et al., Environ. Sci. Technol., 44, 469-475, 2010.
2) Cambier S et al., Int. J. Biochem. Cell Biol., 41, 791-799, 2009.
3) Oliveira Ribeiro CA et al., Environ. Toxicol. Pharmacol., 25, 304-309, 2008.
4) Gonzalez P et al., Environ. Sci. Technol., 39, 3972-3980, 2005.

[紹介]

演者の Bourdineaud 先生は、「フランス・ボルドー大学」において研究を行っておられます。今回の発表は、「メチル水銀毒性モデルとしてのゼブラフィッシュ」についてです。ゼブラフィッシュは全遺伝子の解析が終了していて、毒性研究に有用なモデルです。ゼブラフィッシュにメチル水銀を含む餌を与え、筋肉の 5280 種類の遺伝子発現解析の結果から、酸化ストレスや小胞体ストレスが誘導されていることが示唆されました。筋肉ではミトコンドリアの構造変化と機能異常が明らかになりましたが、脳ではミトコンドリア呼吸の変化は認められませんでした。また、電子顕微鏡による検討で、視覚に関係する視蓋の異常が認められました。

Mitochondrial impairment: ミトコンドリア障害
Transcriptional response: 転写応答
Zebrafish: ゼブラフィッシュ (熱帯淡水魚の一種)
Muscle ATP depletion: 筋肉 ATP 枯渇
Optical tectum: 視蓋

6-1 Early changes of atrocities in the molecular layer of cerebellar cortex of rats with methylmercury intoxication.

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Selective involvement of granule neurons in the cerebellar cortex is one of the characteristic histopathology of methylmercury (MeHg) intoxication. However, its pathogenic process is still unclear and little has been described about changes of molecular layer of the cerebellar cortex. Recent studies have described roles of astrocytes in pathogenesis of the neuronal damage in MeHg intoxication, most of them were done as *in vitro* studies and histopathologic changes of astrocytes are not reported. In this study we performed histopathologic analysis of cerebellar lesions of MeHg-poisoned rats in order to clarify early phase of pathogenic process of cerebellar lesions in MeHg intoxication. We administered MeHg to adult rats by exposure of drink-water containing 20 ppm of MeHg for 21 or 28 days, and sacrificed on 2-4 days after termination of MeHg exposure. Cerebellar vermis were processed for histopathologic examination and performed routine neuropathological, immunohistochemical, and electron microscopic studies. Sagittal sections of cerebellar vermis showed some pyknotic neurons in the granule layer indicating presence of cerebellar lesions. In the molecular layer, increase of Iba1-positive activated microglia and intense staining of GFAP immunohistochemistry in Bergmann's glia were observed. In addition, many small vacuoles were noticed in the neuropil of the molecular layer. Purkinje cells were well preserved in number. EM study revealed swollen astrocytic processes in which formation of vacuoles and accumulation of dense lamellar bodies were observed. Surrounding structures such as Purkinje cell dendrites and pre- and post synapses did not show any morphological abnormality. These glial cell changes seen in the molecular layer of the cerebellum suggest that primary involvement of astrocytes may occur in acute phase of MeHg intoxication.

Key words: methylmercury intoxication, cerebellar cortex, astrocytes, neuropathology
electron microscopy

[紹介]

演者の出雲先生は、「鹿児島大学」において神経病理学的研究を行っておられます。今回の発表は、「メチル水銀毒性におけるアストロサイトの初期変化」についてです。メチル水銀障害の病理像の特徴として小脳皮質の顆粒細胞が選択的に障害されることが知られていますが、その病理過程および分子層の病理変化についてはなお明らかではありません。今回、メチル水銀中毒モデルラットの小脳を用いた神経病理学的研究で、ミクログリアの活性化やアストロサイトの空胞変性が明らかになりました。プルキンエ細胞の樹状突起やニューロンシナプス構造の形態変化は認められないことから、グリアの初期障害が示唆されました。

Methylmercury intoxication: メチル水銀中毒
Cerebellar cortex: 小脳皮質
Astrocytes アストロサイト
Neuropathology: 神経病理
Electron microscopy 電子顕微鏡

6-2 Comparative Study on the Response of Rat Primary Astrocytes and Microglia to Methylmercury Toxicity

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As two major glial cell types in the brain, astrocytes and microglia play pivotal and yet different roles in maintaining optimal brain function. Even though both cell types have been implicated as major targets of methylmercury (MeHg), their unique sensitivities and adaptive responses to this metal can vary given their distinctive properties and physiological functions. This study was carried out to compare the respective responses of neonatal rat primary cortical astrocytes and microglia following treatment with MeHg, addressing the effects of MeHg on cell viability, reactive oxygen species (ROS) generation and glutathione (GSH) levels, as well as mercury (Hg) uptake and the expression of NF-E2-related factor 2 (Nrf2) (a major regulator of intracellular antioxidant response). Results showed that microglia are more sensitive to MeHg than astrocytes, consistent with their higher Hg uptake and lower basal GSH levels. Microglia also demonstrated higher ROS generation upon MeHg treatment compared to astrocytes. Nrf2 and its downstream genes were upregulated in both cell types, but with different kinetics (much faster in microglia). In summary, microglia and astrocytes each exhibit a distinct sensitivity to MeHg, resulting in their differential temporal adaptive responses. These unique sensitivities appear to be dependent upon the cellular thiol status of the particular cell type. Collectively, these studies suggest that microglia and astrocytes assume different roles on a protracted time-scale, with microglia representing the early responders and astrocytes taking on a similar role at a later stage following MeHg treatment (This work was supported by grant from the National Institute of Environmental Health Sciences R01 ES07331).

Keywords: methylmercury, microglia, astrocytes, reactive oxygen species, glutathione, Nrf2.

[紹介]

演者の Aschner 先生は、「アメリカ合衆国・バンダービルド大学」において研究を行っておられます。今回の発表は、「メチル水銀神経毒性におけるグリア細胞の役割」についてです。脳神経系に存在する 2 種類のグリア細胞、すなわちミクログリアとアストロサイトは脳機能を維持する上で重要な細胞です。培養細胞を用いた実験によって、アストロサイトよりもミクログリアの方がよりメチル水銀に感受性が高いこと、ミクログリアはより水銀の取り込みが多くグルタチオン含量が低いこと、さらに活性酸素種産生が多いことが明らかになりました。以上のことから、メチル水銀毒性におけるミクログリアの初期応答の関与が示唆されました。

Methylmercury: メチル水銀
Microglia: ミクログリア (グリア細胞の一種)
Astrocytes: アストロサイト (グリア細胞の一種)
Reactive oxygen species: 活性酸素種
Glutathione: グルタチオン (生体内に存在する抗酸化物質の一種)
Nrf2: (DNA に特異的に結合して遺伝子転写を制御する転写因子の一種)

7-1 Neuritic degeneration contributes to methylmercury-induced neuronal cell death

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Methylmercury (MeHg) is an environmental neurotoxicant which induces neuropathological changes in both central nervous and peripheral sensory nervous systems. The molecular mechanism underlying MeHg-induced neuronal cell death was investigated using cultured cortical neuronal cells. We demonstrated that neuritic degeneration precedes MeHg-induced apoptotic death in neurons exposed to 100 nM MeHg. Immunocytochemical and ELISA analyses for neurite-specific proteins namely, tau and MAP2, showed that injury to tau-positive axons was first induced followed by damage to the dendrites and cellular bodies. To further investigate the factors responsible for neuronal death, we investigated the expression levels of Rho-family proteins (Rac1, Cdc42, and RhoA), which regulate neuritic functions and apoptosis in neurons. Western blot analysis demonstrated that MeHg downregulated the expression levels of Rac1 and Cdc42 but did not affect RhoA. The results indicate that neuritic degeneration, in particular axonal degeneration triggered by the downregulation of Rac1 expression, contributes to MeHg-induced apoptotic cell death in cultured cerebrocortical neurons[1)]. So we hypothesized that MeHg-induced axonal degeneration might be caused by neuritic extension/retraction incoordination. This idea brought our attention to the Rho/ROCK pathway because it has been known to be associated with the development of axon and apoptotic neuronal cell death. Next we show that inhibition of Rho/ROCK pathway prevents MeHg-intoxication in cultured cortical neuronal cells. A Rho inhibitor, C3 toxin, and two ROCK inhibitors, Fasudil and Y-27632, significantly protected MeHg-induced axonal degeneration and apoptotic neuronal cell death in cultured cortical neuronal cells exposed to 100 nM MeHg. The results suggest that inhibition of Rho/ROCK pathway rescues MeHg-mediated neuritic extension/retraction incoordination and is effective for the prevention of MeHg-induced axonal degeneration and apoptotic neuronal cell death[2)].

Key words: neuritic degeneration, neuronal cell death, Rac1, ROCK inhibitor.
References: 1) Fujimura M et al., Neurotoxicology, 30, 16-22, 2009.
2) Fujimura M et al., Toxicol. Appl. Pharmacol., 250, 1-9, 2011.

[紹介]

演者の藤村は、「国立水俣病総合研究センター」において研究を行っています。今回の発表は、「メチル水銀神経細胞死への神経軸索変性の寄与」についてです。神経細胞は細胞体、軸索、樹状突起から形成されているのですが、その中でも神経軸索は神経間の情報伝達に重要な役割を果たしています。培養神経細胞を用いた実験で、メチル水銀が最初に Rac1 という神経軸索を伸展させる蛋白質の発現を抑制することによって神経軸索を変性させた後、神経細胞死を引き起こすことが明らかになりました。また、メチル水銀による軸索変性と神経細胞死は薬剤 (ROCK 阻害剤等) によって防御できることも明らかになりました。

Neuritic degeneration: 神経軸索変性
Neuronal cell death: 神経細胞死
Rac1: (神経軸索を伸展させる蛋白質)
ROCK inhibitor: ROCK 阻害剤

7-2 Methylmercury activates multiple cell death pathways in neuronal and glial cells

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The identification of the mechanisms that drive methylmercury (MeHg)-induced neurotoxicity has been a main research topic in neurotoxicology. In the nervous system MeHg can induce necrotic or apoptotic cell death depending on the level and duration of exposure. While necrosis is an ATP-independent process characterized by cell and organelle swelling, loss of plasma membrane integrity and lysis, apoptosis is ATP-driven with hallmarks including cell shrinkage, chromatin condensation, plasma membrane blebbing, activation of specific proteases (e.g. caspases and calpains), and DNA fragmentation at specific sites. We have investigated the signaling cascades leading to cell death induced by MeHg in different cell types including primary rat cerebellar granule cells (CGC), the mouse HT22 hippocampal neuron cell line, the human D384 astrocytoma cell line as well as the rodent pituitary cell lines AtT20 and GH3[1,2)]. As a general feature, all these cells when exposed to MeHg undergo apoptosis in a caspase-independent manner. Instead, the Ca^{2+}-activated proteases calpains are activated[1,2)]. Also, translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus with subsequent chromatin condensation and large-scale DNA fragmentation occurs in CGC[1)]. The Lysosomal proteases as executor factors in MeHg-induced cell death appears to be critical in the astrocytoma, hippocampal and pituitary cell lines. Pretreatment with antioxidants prevent MeHg-induced apoptosis pointing to oxidative stress playing a major role in MeHg-induced toxicity in our experimental models[1,2)]. Interesting, the plasma membrane voltage-dependent anion channels (VDAC) that play a role in the redox homeostasis of normal cells, are open and active in MeHg- exposed HT22 cells, suggesting that these channels also play a role in anion efflux in apoptotic cells[3)]. In conclusion, it is evident that the response to MeHg depends on the cell type and that multiple cell death pathways can be activated concomitantly. Multiple cell models should be used for *in vitro* studies aimed at identifying neurotoxic effects and their underlying molecular mechanisms.

Key words: apoptosis, necrosis, caspases, calpains, lysosomes, oxidative stress, VDAC.
References: 1) Ceccatelli S. et al., Chem. Biol. Interact., 188, 301-308, 2010. Review.

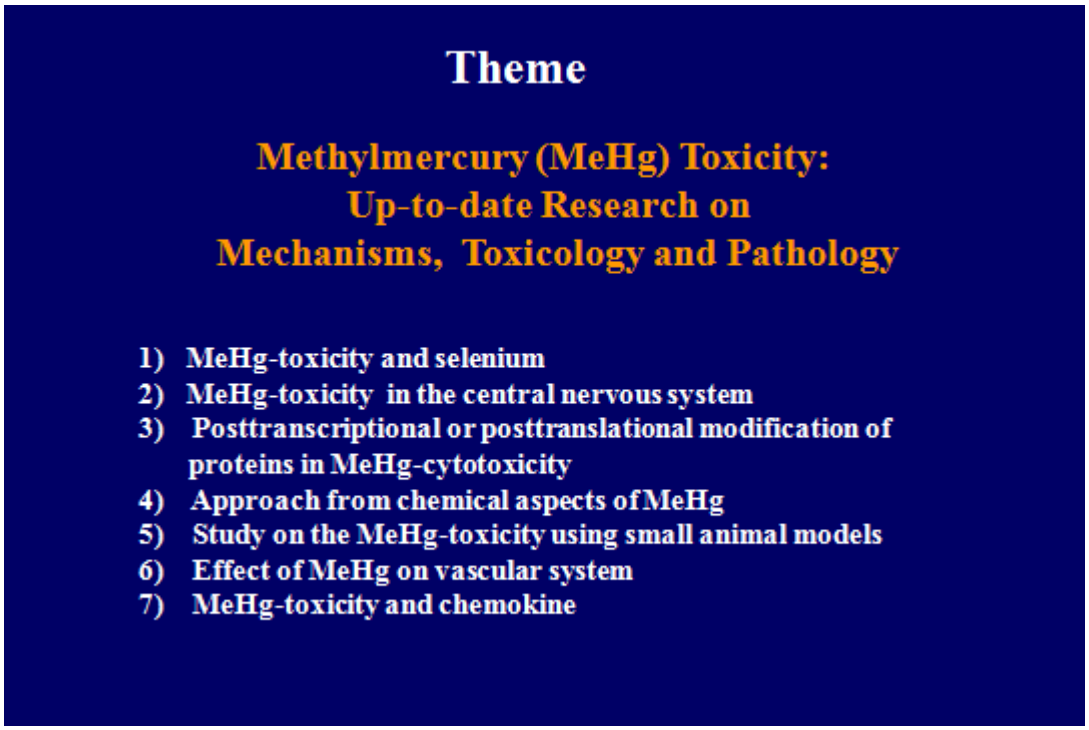
2) Tofighi R. et al., Neurotox. Res., 19, 183-194, 2011.
3) Elinder F. et al., Cell Death Differ., 12, 1134-1140, 2005.

[紹介]

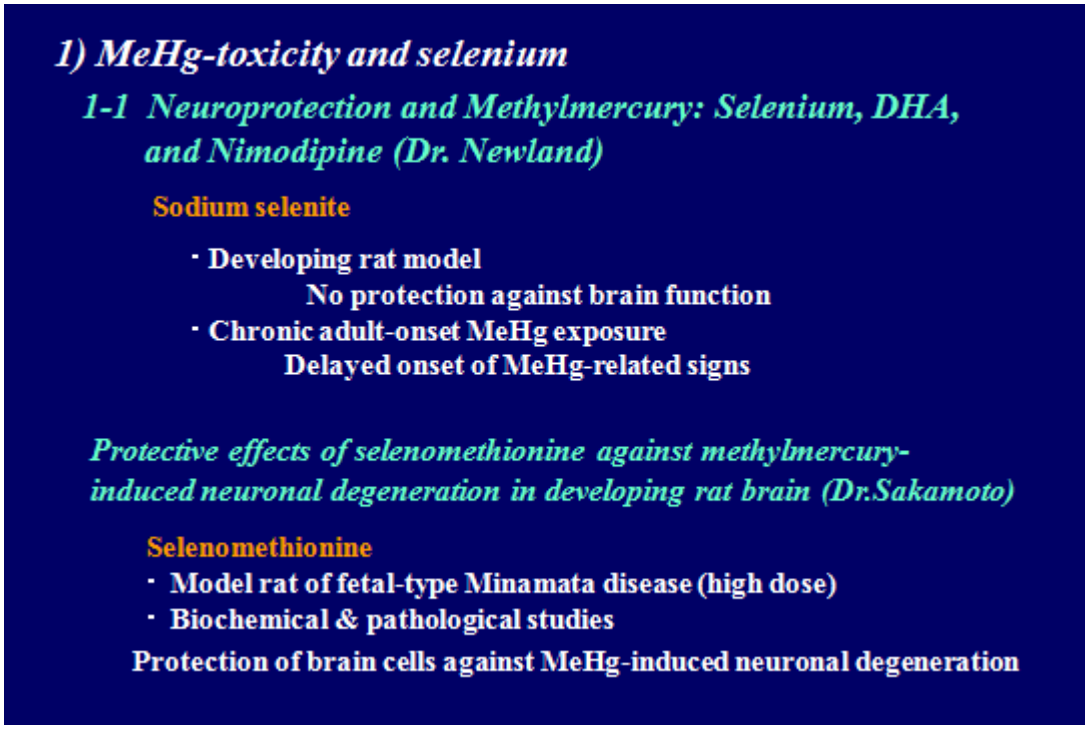
演者の Tofighi 先生は、「スウェーデン・カロリンスカ研究所」において研究を行っておられます。今回の発表は、「メチル水銀曝露下での神経細胞及びグリア細胞における神経細胞死経路」についてです。メチル水銀は脳神経系において曝露量や曝露期間に応じてアポトーシスあるいはネクローシスという異なる細胞死を引き起こします。5 種類の培養細胞を用いた実験によって、メチル水銀に対する応答は細胞種によって異なり、様々な細胞死経路が活性化されることが明らかになりました。

Apoptosis: アポトーシス（プログラムされた“自殺性”の細胞死)
Necrosis: ネクローシス (壊死、“他殺性”の細胞死)
Caspases: カスパーゼ (アポトーシス誘導に関与する蛋白質分解酵素)
Calpains: カルパイン (カルシウム依存性の蛋白質分解酵素)
Lysosomes: リソゾーム (細胞内小器官)
Oxidative stress: 酸化ストレス
VDAC (voltage-dependent anion channels): 電位依存性陰イオンチャネル

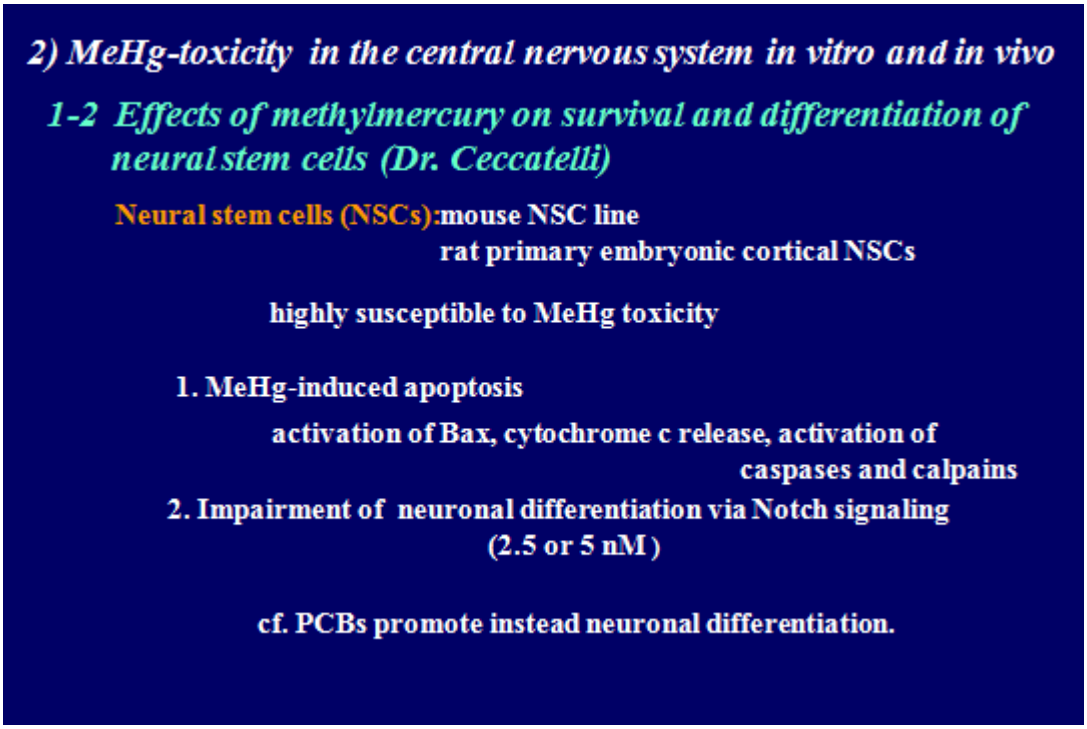
Summary



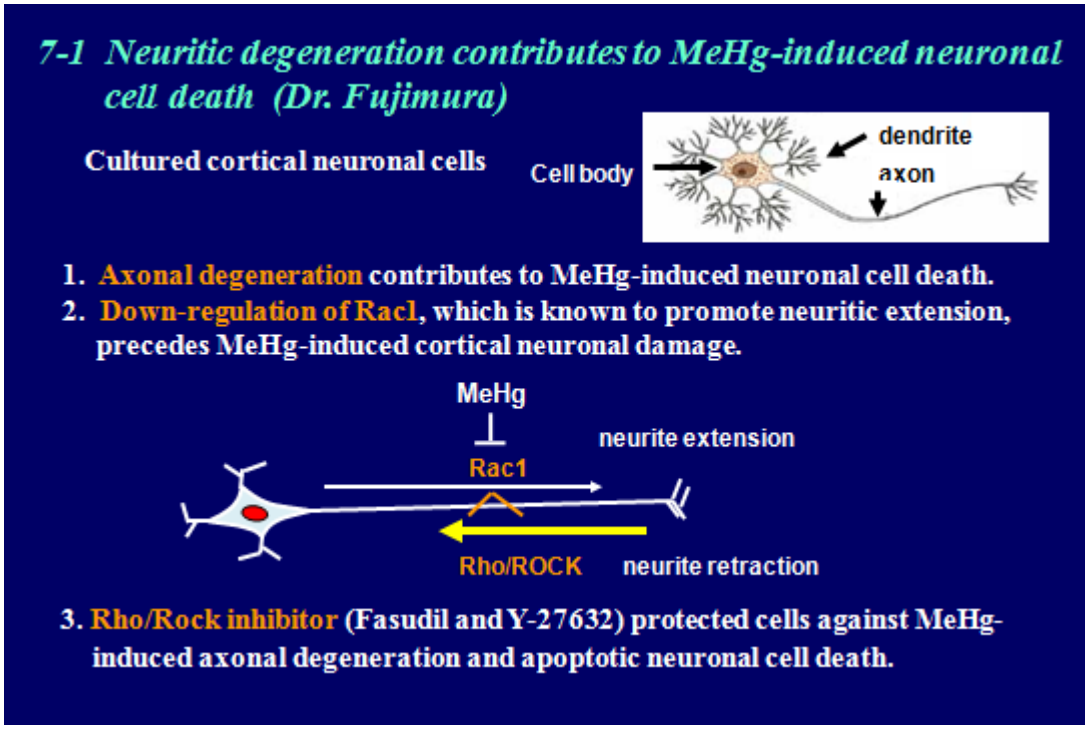
In this FORUM, we had seven topics on MeHg toxicity: up-to-date research on mechanisms, toxicology, and pathology.



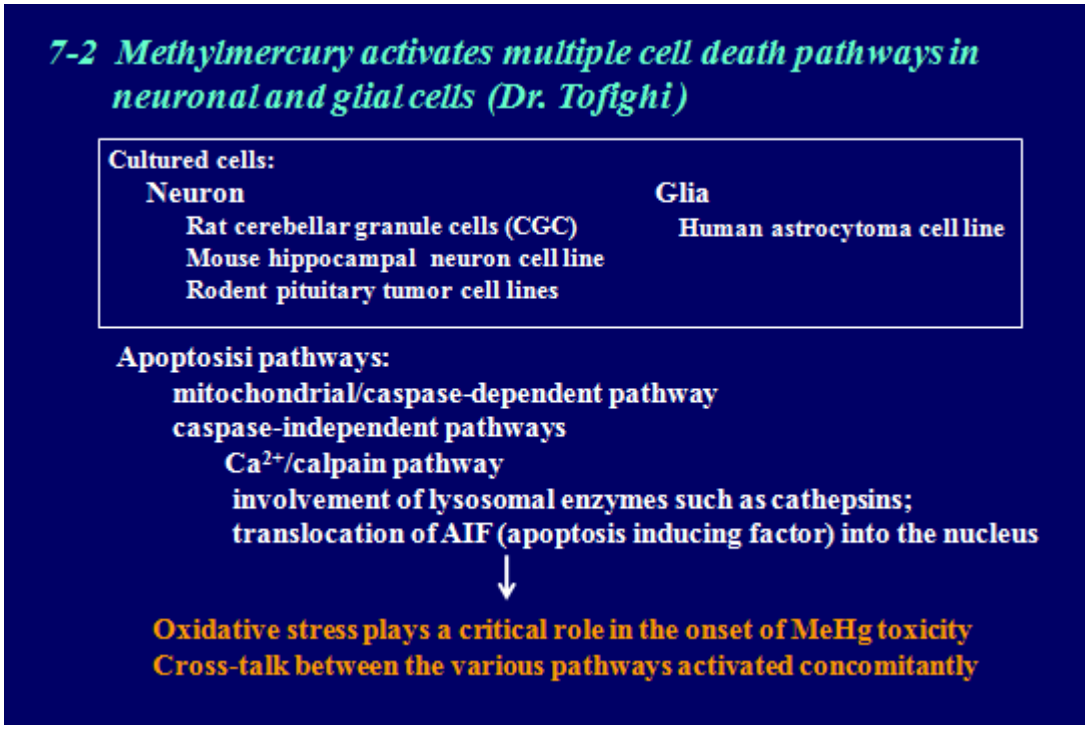
The first topic was MeHg-toxicity and selenium. Dr. Newland and Dr. Sakamoto showed the protective effect of selenium component against MeHg cytotoxicity, although their reagents, rat models and assessment methods were different. Dr. Newland showed that sodium selenite was useful to delay the onset of MeHg-related signs in chronic adult-onset MeHg exposure. And Dr. Sakamoto showed that selenomethionine was useful to protect brain cells against MeHg-induced neuronal degeneration in fetal-type Minamata disease rat model.



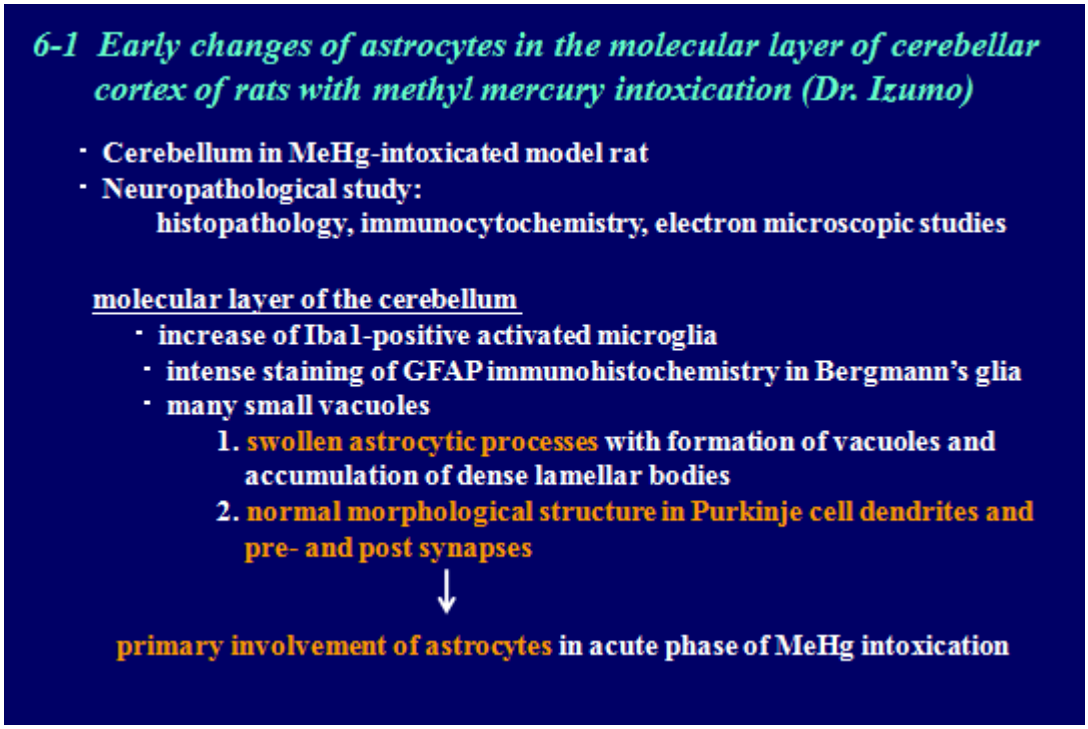
The second topic was MeHg-toxicity in the central nervous system *in vitro* and *in vivo*. Dr. Ceccatelli mentioned the effect of MeHg on survival and differentiation of mouse neural stem cell line and rat primary embryonic cortical neural stem cells. Both neural stem cells were highly susceptible to MeHg and showed apoptosis. In addition, at the concentrations that were not cytotoxic but still were relevant for human exposure, impairment of neuronal differentiation via Notch signaling was recognized in rat primary cortical neural stem cells.



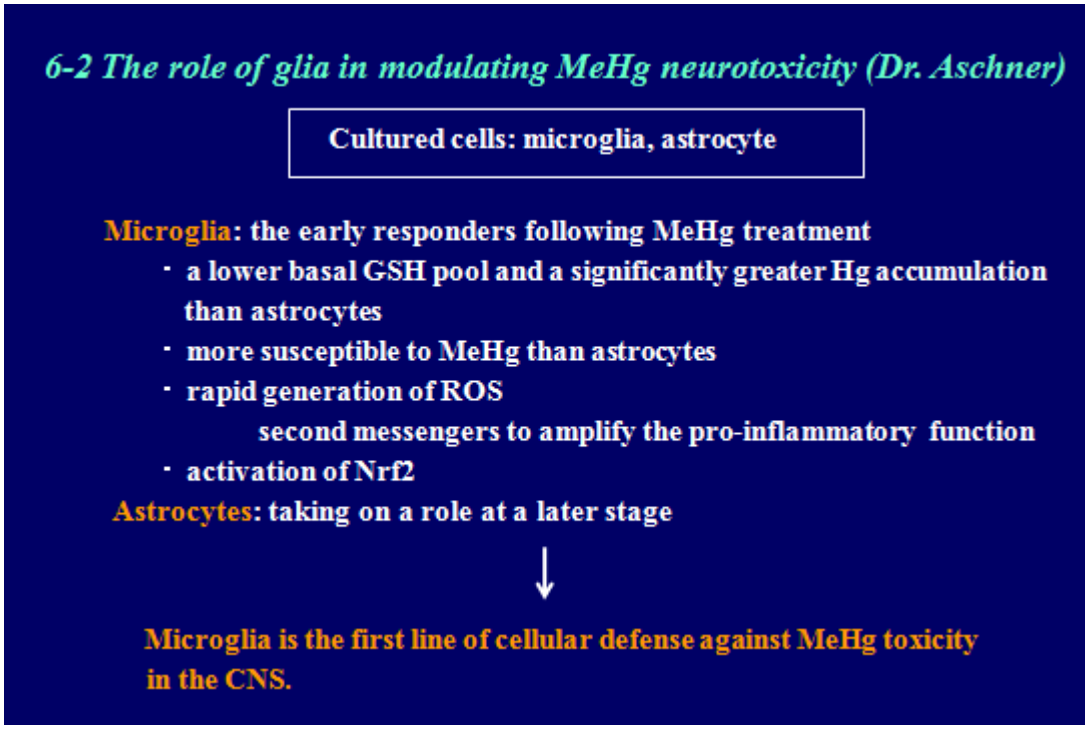
Dr. Fujimura showed the process of MeHg-induced cerebrocortical neuronal cell death. MeHg suppressed the expression of Rac1, which is known to promote neuritic extension, during the early stage of MeHg-induced cytotoxicity, resulting in axonal degeneration and ultimately neuronal apoptosis. Inhibition of the Rho/ROCK pathway protected cells against MeHg-induced axonal degeneration and apoptotic neuronal cell death.



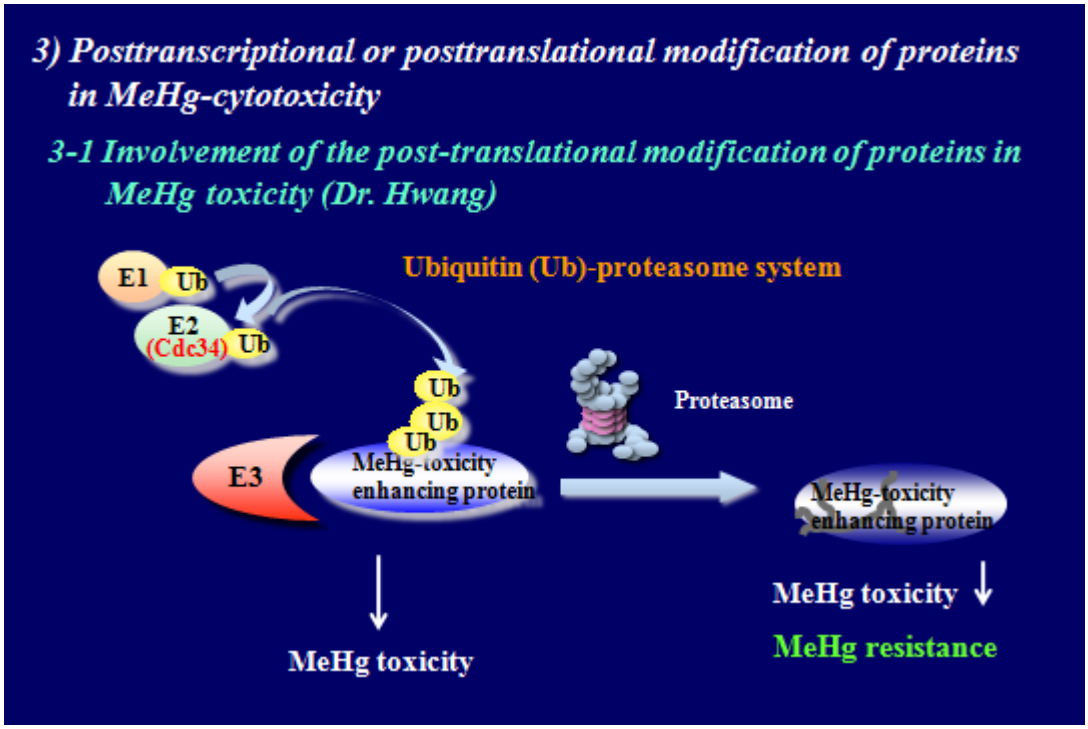
Dr. Tofighi showed various signaling cascade leading to cell death under MeHg exposure in 5 kinds of neuronal and glia cells. In these cells oxidative stress played a critical role in the onset of MeHg toxicity and apoptosis was induced via mitochondrial/ caspase-dependent and -independent pathways. The results suggest that cross-talk between the various pathways were activated concomitantly under MeHg exposure.



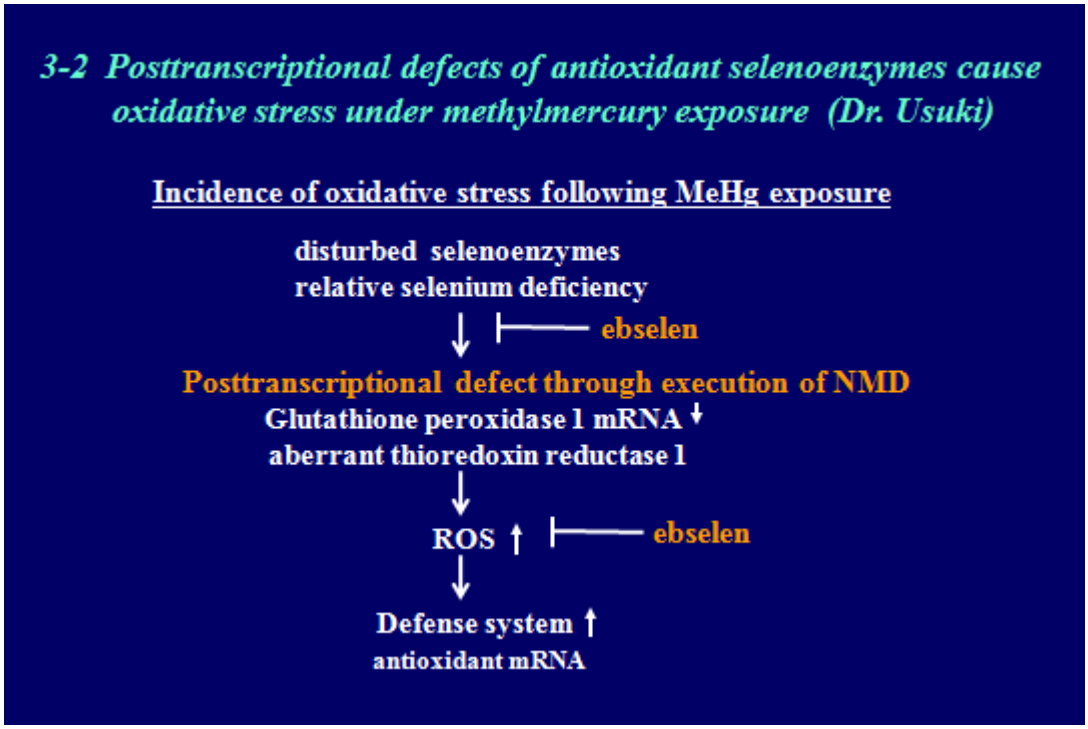
The important roles of glia cells in MeHg-neurotoxicity were demonstrated by Dr. Izumo and Dr. Aschner. Dr. Izumo showed neuropathological findings on molecular layer of the cerebellum in MeHg-intoxicated model rat. Electron microscopy demonstrated morphological changes in astrocyte processes with normal Purkinje cell dendrites, and normal pre- and postsynapses. The results suggest that astrocytes are primarily involved in acute phase of MeHg intoxication.



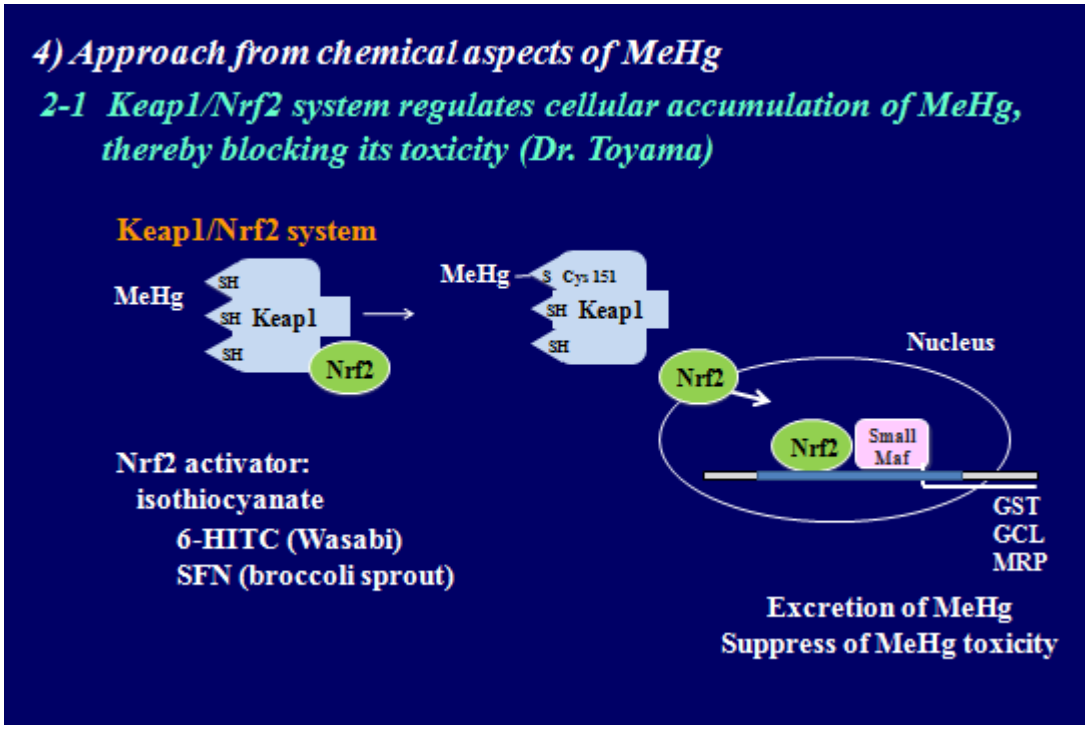
Dr. Aschner showed comparative studies of the effect of MeHg on microglia and astrocytes. Microglia showed a lower basal glutathione pool and a significantly greater mercury accumulation than astrocytes. Microglia was more susceptible to MeHg than astrocytes. The results suggest the possibility that microglia is the first line of cellular defense against MeHg toxicity in the central nervous system.



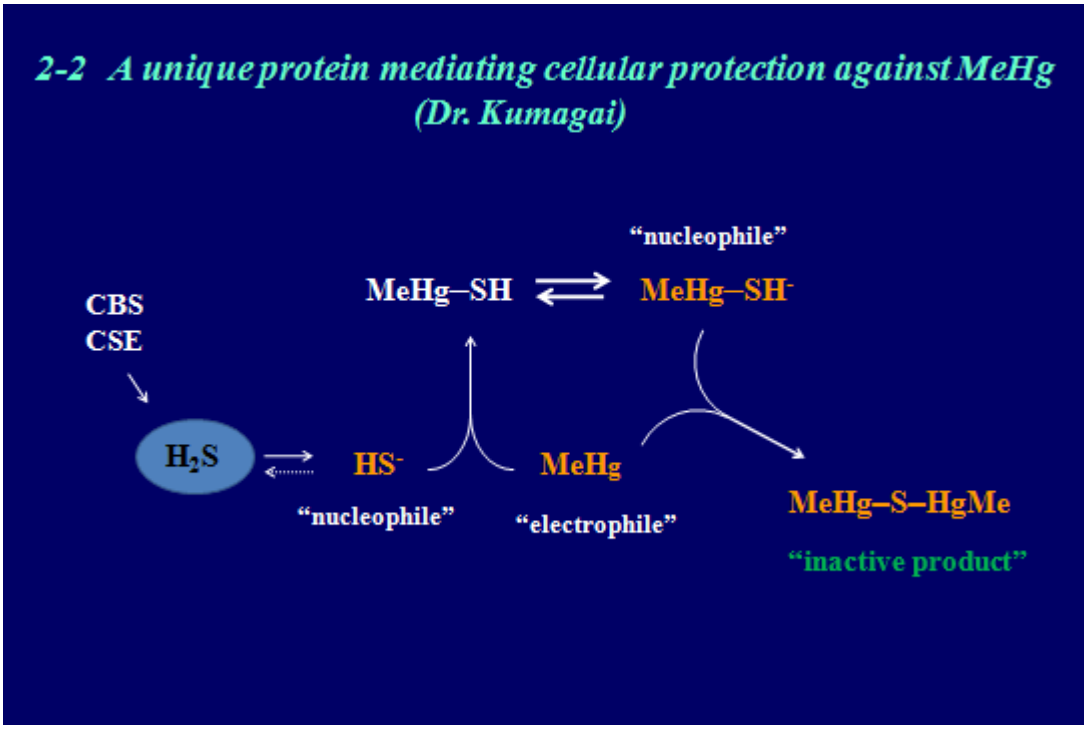
The third topic was talked by two speakers. Dr. Hwang described the search for novel genes involved in methylmercury resistance using yeast cells. Many of the identified gene products were related to ubiquitin-proteasome systems. Cdc34 was one of them. He also identified other proteins which enhanced methylmercury toxicity. They are decreased by ubiquitin-proteasome system. The results suggest that ubiquitin-proteasome system may play an important role as defense mechanisms against methylmercury toxicity.



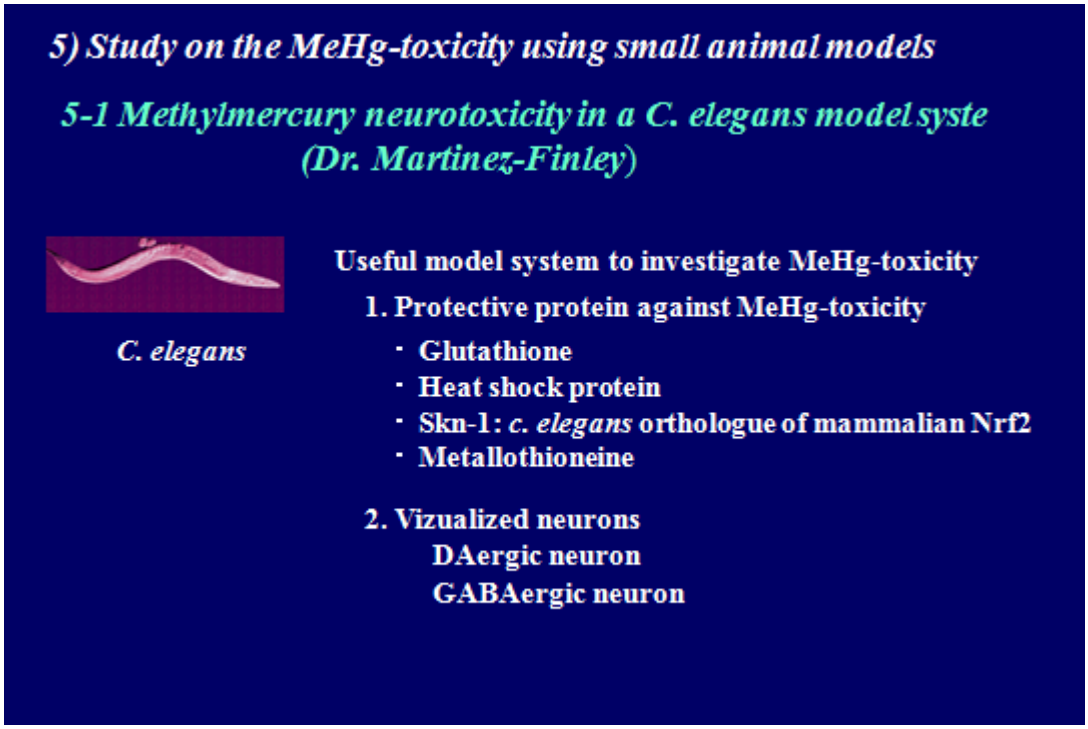
Dr. Usuki showed the role of post-transcriptional defect of antioxidant selenoenzymes in the incidence of MeHg-mediated oxidative stress. MeHg-induced relative selenium-deficient condition affects the major antioxidant selenoenzymes through a posttranscriptional effect, resulting in the disturbance of cellular redox systems and the incidence of oxidative stress. Treatment with ebselen protected cells against MeHg-induced relative selenium deficiency and cytotoxicity.



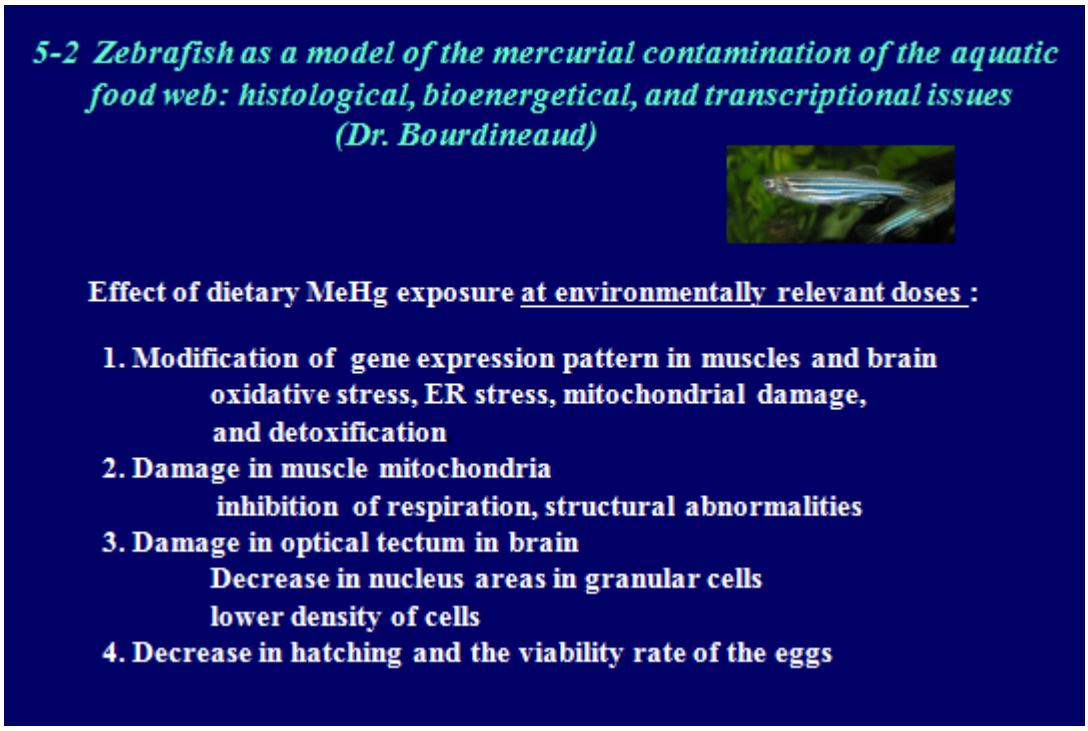
The fourth topic on an approach from chemical aspects of MeHg was talked by two speakers. Dr. Toyama showed the protective role of Keap1/Nrf2 system in MeHg-cytotoxicity. The transcription factor Nrf2 is activated through the modification of cysteines of Keap1 by electrophiles and oxidative stress. Nrf2 activators, isothiocyanates, protect cells against MeHg-cytotoxicity. It was interesting that Wasabi and broccoli sprout contain components of Nrf2 activator, isothiocyanate.



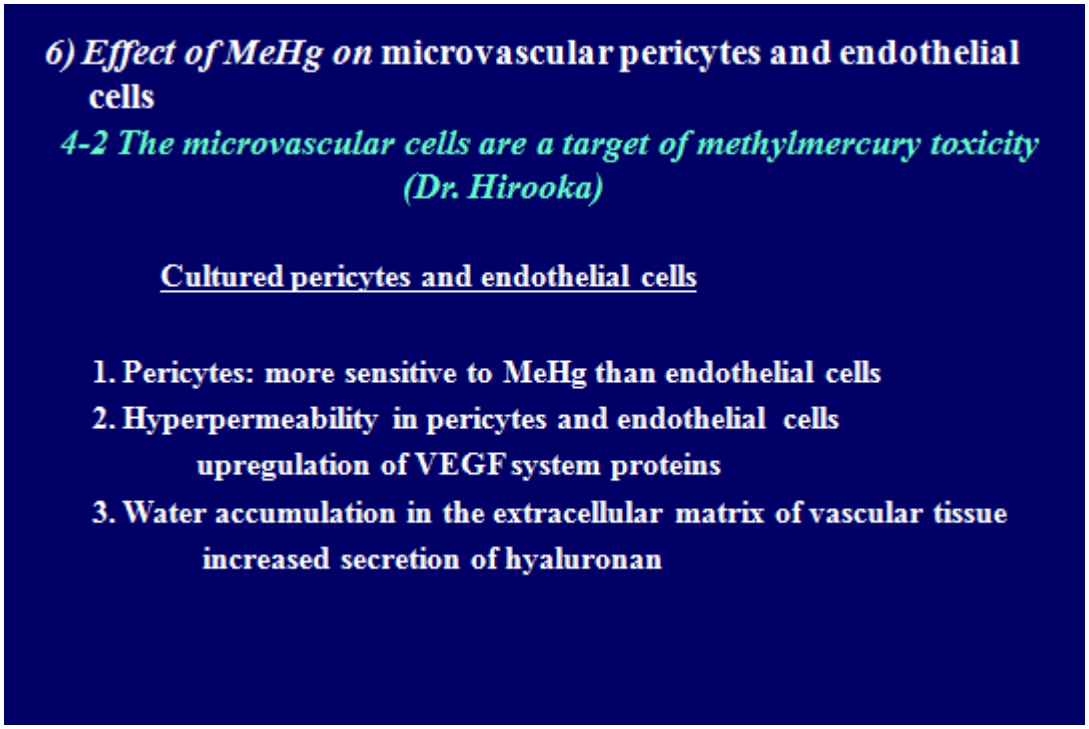
MeHg is an electrophile and the conjugation of MeHg by nucleophiles is essential for its detoxification. Recently hydrogen sulfide is found to be a biological signaling molecule. CBS and CSE are known to be hydrogen sulfide producing enzymes. From the results of the experiments on the treatment with materials to generate hydrogen sulfide, overexpression or knockdown of CBS, and CSE-knockout mice, Dr Kumagai showed the possible protective role of hydrogen sulfide in MeHg-toxicity.



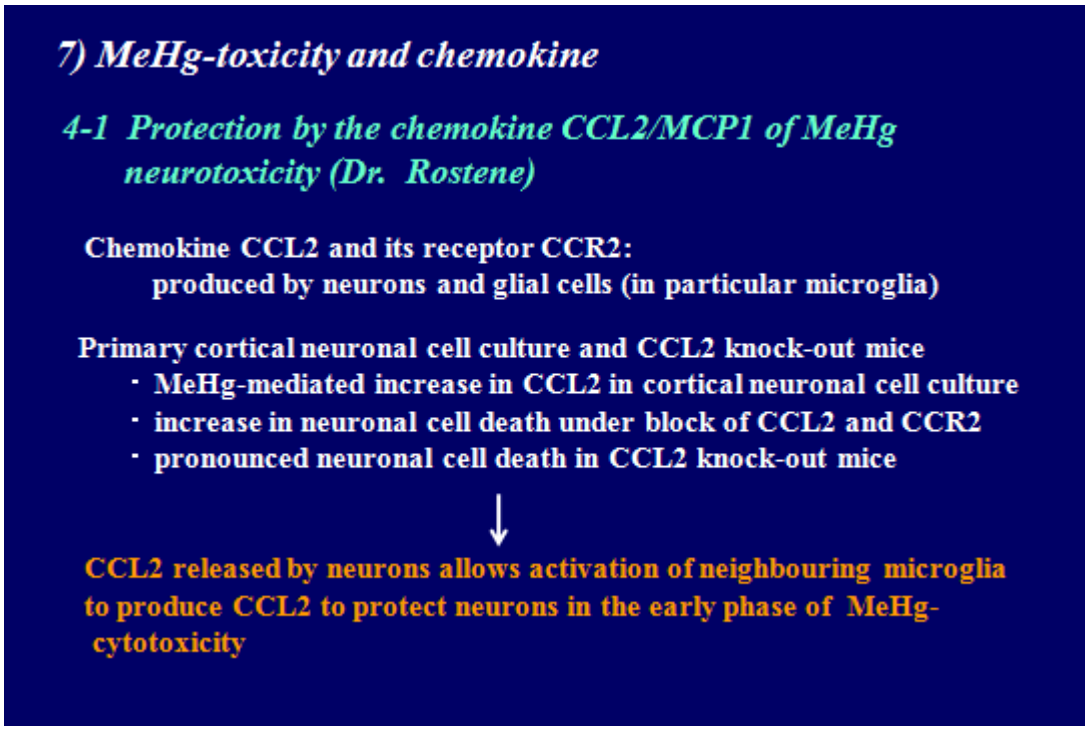
The fifth topic was “Study on the MeHg-toxicity using small animal models”. Dr. Martinez-Finley showed that C. elegans is a useful animal model system to investigate MeHg-toxicity. C. elegans has the protective proteins against MeHg-toxicity such as glutathione, heat shock protein, Skn-1 (an orthologue of mammalian Nrf2), and metallothioneine, which are the same as mammals. And c. elegans allows us to visualize individual neurons *in vivo.*



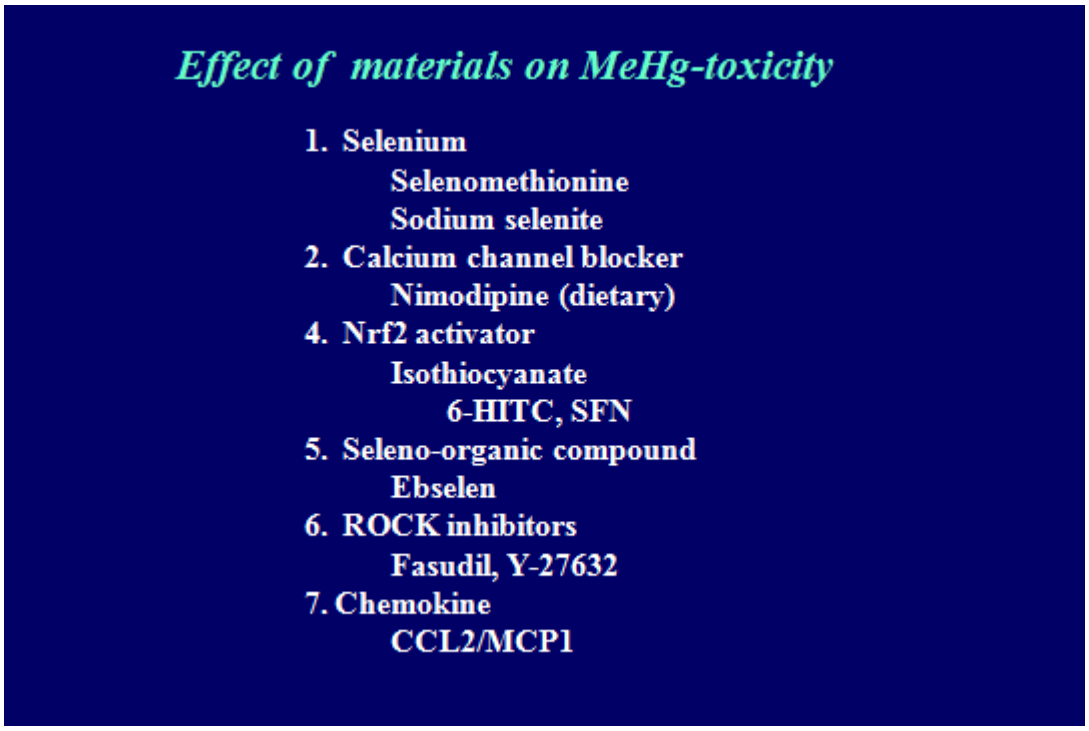
Zebrafish is another useful animal model for scientific research. Dr. Bourdineaud showed the result of his research on the effect of dietary MeHg exposure at environmentally relevant doses. MeHg exposure mediated the modification of gene expression pattern related to oxidative stress, ER stress, mitochondrial damage, and detoxification. Morphological changes in muscle mitochondria and optical tectum in brain were also observed. In addition, decreases in hatching and viability rates of the eggs were observed under MeHg exposure.



The sixth topic on the effect of MeHg on microvascular systems was talked by Dr. Hirooka. Study on cultured pericytes and endothelial cells showed that MeHg exposure upregulated VEGF system proteins in these cells and increased the secretion of hyaluronan from both cells. The results suggest hyperpermeability in these cells and the possible induction of water accumulation in the extracellular matrix of vascular tissue.



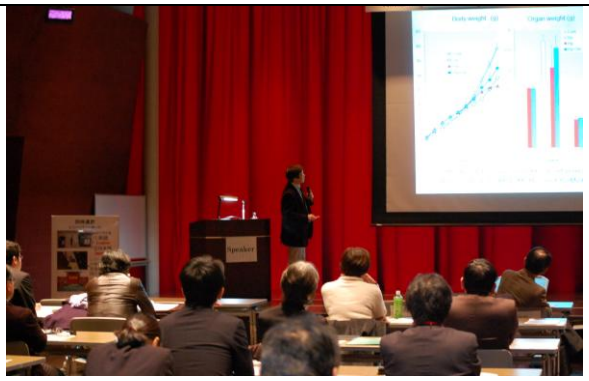
The seventh topic was MeHg-toxicity and chemokine. Chemokine CCL2 and its receptor CCR2 are produced by neurons and glial cells. Dr. Rostene showed the protective effect of CCL2 against MeHg-neurotoxicity using neuronal cell culture and CCL2 knock-out mice. MeHg increased CCL2 release in cortical neuronal cell culture. Block of CCL2 and CCR2 in cortical neuronal cell culture and CCL2 knock-out mice caused significant increase in neuronal cell death under MeHg exposure.



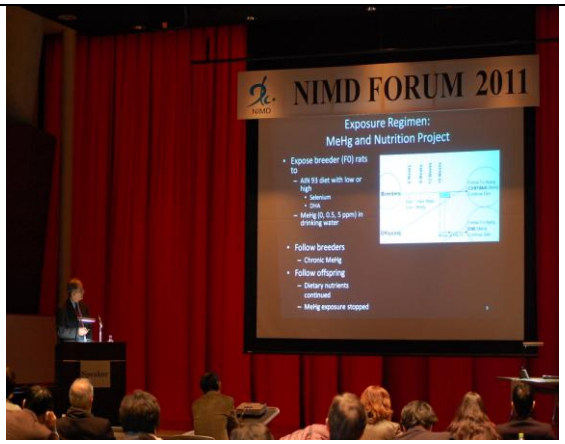
Lastly, in this FORUM, materials listed here were shown to protect cells against MeHg-toxicity. We hope that continued investigation and discussion on MeHg research will lead to the development of useful drugs to treat with MeHg-intoxication.

Photographs

Meeting Place (1)



Meeting Place (2)



Meeting Place (3)

Meeting Place (4)

Meeting Place (5)

Group Photograph

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