Table 3. Pharmacological Therapy for Wilson's Disease

Drug	Mode of Action	Maintenance Dose	Side effects
Trientine	Induction of urinarycopper excretion by chelating action	750-1,000 mg/day three times a day; children, 20-25 mg/kg/day	Gastritis, in rare cases aplastic anemia and sideroblastic anemia, neurological deterioration during initial phase of treatment (about 26% [130])
D-Penicillamine	Induction of urinary copper excretion by chelating action	750-1,000 mg/day three times a day; children: 20 mg/kg/day	Fever, rash, proteinuria, lupus-like reation, aplastic anemia, leukopenia, thrombocytopenia, nephrotic syndrome, degenerative change in skin, elastosis perforans serpingosa, serous retinitis, hepatotoxicity, neurological deterioration during initial phase of treatment (about 50% [110])
Zinc	Blockage of copper absorption by inducing metallothionein in enterocytes	150 mg/day, three times a day; children: 50-75 mg/day	Gastritis, biochemical pancreatitis, zinc accumulation, possible changes in immune function
Tetrathiomolybdate	Detoxifying copper in plasma and blocking copper absorption by complexation with copper	20 mg, three times with meals and three times between meals [108]	Anemia, neutropenia, hepatotoxicity, neurologic deterioration during initial treatment (about 4% [108])

tients who receive the full treatment [100]. Urinary copper excretion increases above 1000 μg/day for a few months following penicillamine or trientine treatment (initial treatment). These levels range between 200-500 μg/day during maintenance therapy with a chelating agent [89].

Penicillamine

While penicillamine is the most effective treatment for removing copper through urine excretion, it is associated with severe side effects [101]. These side effects include immunological conditions (e.g., lupus-like reactions, nephrotic syndrome, myasthenia gravis, and Goodpasture syndrome), skin defects (e.g., degenerative changes and elastosis perforans serpiginosa), and joint disorders (e.g., arthropathy). Given these side effects, trientine is now the preferred method of treatment [89,99].

Trientine

Figure **14** shows the chemical structure of trientine. Trientine is known to remove copper from the blood compartment, and increases urinary copper excretion. Zinc and iron are also excreted with trientine, although in lesser amounts [102]. Trientine shares some of penicillamine's side effects, but appears to be significantly less toxic and as efficacious as penicillamine [103]. For this reason, trientine is the recommended chelator for treatment of patients with hepatic WD [99].

Zinc

Zinc is a recommended treatment for presymptomatic patients and for maintenance therapy of WD [99]. Zinc treatment of patients with WD results in increased levels of non-toxic zinc-bound metallothionein. The enterocyte metallothionein induced by zinc inhibits copper uptake from the intestinal tract, resulting in a negative copper balance [104]. Zinc is also thought to protect against copper toxicity in the liver by promoting sequestration of free copper in a non-toxic, metallothionein-bound form [105]. Treatment adequacy is determined by measuring non-ceruloplasmin-bound copper levels in the serum (5-15 μg/dL), 24-hour urinary copper excretion (<75 μg/day) [89], or by spot urinary copper excretion with less than 0.075 μg/mg creatinine [106]. Non-ceruloplasmin-bound copper levels in the serum can usually be calculated from serum copper and ceruloplasmin levels using the following equation:

non-ceruloplasmin-bound copper levels in the serum (μg/dL) = serum copper level (μg/dL) – 3 x serum ceruloplasmin level (mg/dL)

This is possible because approximately 3.15 μg of copper is bound to one mg of ceruloplasmin.

Tetrathiomolybdate (TTM)

TTM is an anti-copper drug with a unique mechanism of action developed for patients with neurological WD. It has 4 sulfur groups that allow it to form a tripartite and stable interaction with copper (Fig **14**). If given with food, TTM forms a stable complex with copper, rendering it unavailable for absorption. When given without food, however, it is well absorbed and complexes with free serum copper. TTM treatment does not result in serum copper spikes typically observed with penicillamine and trientine [107]. This may explain why neurological worsening is rare with TTM treatment versus other chelating agents [108], although a patient receiving TTM treatment was reported to develop status epilepticus [109]. While TTM is now preferred over other chelating agents for treatment of neurological WD, the FDA recently decided that further studies are required before it can be used in patients with neurological WD (from HP of Pipex Parmaceuticals Comp).

Patients with Neurological Symptoms

In patients with neurological symptoms, clinical worsening is observed during the first few weeks of treatment in approximately 50% and 26% of patients treated with penicillamine and trientine, respectively. In addition, 25% of patients treated with penicillamine are at risk of permanent neurological damage and may not recover to baseline levels of function [110]. Neurological worsening during initiation of anti-copper therapy is attributed to spikes in levels of serum non-ceruloplasmin-bound copper which occur during mobilization of large stores of copper in the liver [107]. Although neurological worsening is also observed in a few patients treated with zinc, which is slow-acting, zinc alone or combination therapy with zinc and trientine are now recommended in patients with neurological WD [99,111,112]. Another problem is that neurological symptoms sometimes do not completely subside with treatment. Liver transplantation in some patients with neurological disorders was reported to resolve neurological symptoms associated with WD. However, detailed neurological evaluations in these patients were not carried out [113]. Because early treatment is critical in patients exhibiting neurological disorders, medical education efforts targeting primary care physicians should be implemented in order to improve early diagnosis [81].

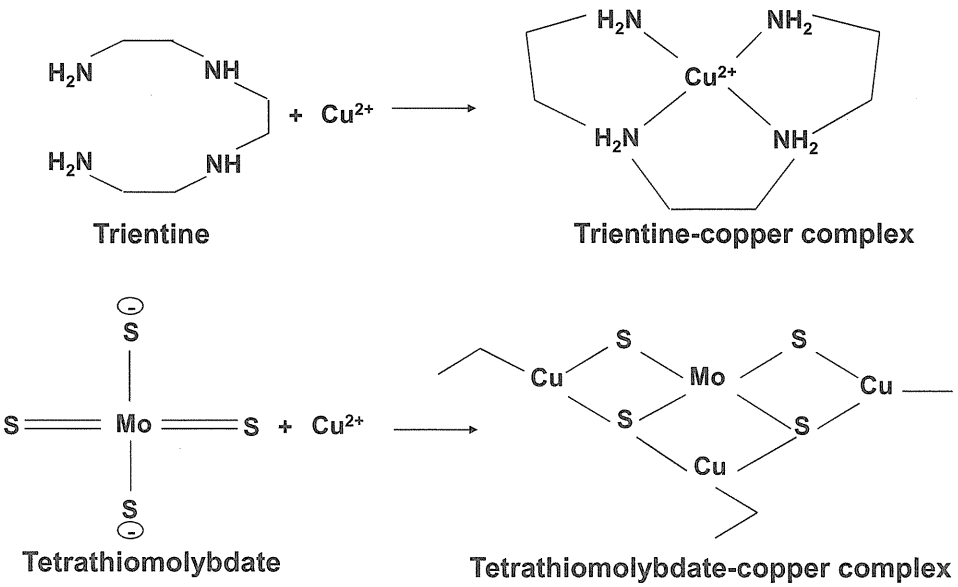

Fig. (14). Chemical reaction of chelation by trientine (upper) and tetrathiomolybdate (lower) [126].

Patients with Hepatic Symptoms

Patients with mild and moderate liver disorders are initially treated with chelating agents (trientine preferred over penicillamine) [89,99]. Serum levels of aminotransferases and non-ceruloplasmin-bound copper are normalized a few months after initial treatment, reaching adequate urinary copper excretion levels that range between 200-500 μg/day. Once this occurs, maintenance therapy is initiated with zinc alone or with a lower dose of chelating agents (i.e., trientine). In patients with fulminant hepatitis or hemolysis, liver transplantation is the most likely solution [114].

One major obstacle regarding long-term treatment of patients with WD is poor drug compliance. A recent report showed that 25% of patients were not persistently taking their medication, resulting in deterioration and occasionally fatal outcomes [115]. Accordingly, it is important for physicians to make an effort to promote compliance during therapy.

Hepatocellular carcinoma (HCC) has become an important issue for patients with WD as current treatments have improved life expectancy. In a previous study, we examined the characteristics of 25 WD patients with HCC and compared them to non-WD patients with HCC in a cohort from the Liver Cancer Study group in Japan, 1994-2003 (LCS-J) [17]. The average age at diagnosis of HCC in WD patients was considerably lower compared to non-WD patients. In addition, male to female ratios were high in WD patients. Taken together, these results show that patients with WD (mainly males) are in danger of developing HCC despite treatment. The mechanism that leads to carcinogenesis in WD remains unknown and is currently under investigation. LEC rats harboring a deletion in *ATP7B* develop HCC [76]. Tsubota *et al* reported that mRNA expression of tumorigenic proteins, Ras GTPase-activating-like protein (IQGAP1) and vimentin, was induced by persistent oxidative stress in the liver of LEC rats, making these proteins important clinical targets for HCC [116]. Production of oxygen and nitrogen reactive species, and unsaturated aldehydes that arise from copper overload in patients with WD has been reported to cause mutations in the p53 tumor suppressor gene [117]. These findings suggest that oxidative stress is associated with HCC. Vitamin E may act as an antioxidant adjunct for WD therapy [118]. The copper chelating agent, TTM, inhibits angiogenesis, fibrosis, and inflammation [119,120]. However, how these affect HCC development is unclear. Elucidation of these mechanisms will help devise strategies aimed at preventing HCC in patients with long-term WD.

ACKNOWLEDGEMENTS

This work was in part by a Grant of Research on Intractable Diseases from Ministry of Health, Labour and Welfare of Japan (22-271) and a memorial fund for Naoki, a former patient with Menkes disease.

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Received: December 22, 2010 **Revised: March 7, 2011** **Accepted: May 16, 2011**

ISTERH International Society For Trace Element Research in Humans

PROGRAM

IX ISTERH 2011

TRACE ELEMENTS IN HEALTH AND DISEASE: ESSENTIALITY, TOXICITY

RIXOS PREMIUM HOTEL, BELEK, TURKEY

16-21 OCTOBER 2011

IN COLLABORATION WITH NORDIC TRACE ELEMENT SOCIETY

Table 1. Program Schedule. Please see the detailed program for exact times

TIME	Sunday Oct. 16	Monday Oct. 17	Tuesday Oct. 18	Wednesday Oct. 19	Thursday Oct. 20	Friday Oct. 21, 2011
7:30		Registration	Registration	Registration	Registration	
8:00	Arrival	**Plenary 1** Welcome Regulation of Zinc Transporters: Robert Cousins **Symposium A:** Manganese: Neurotoxic Effects: Michael Aschner **Symposium B:** Priority of Micronutrients in Achieving MDG4: SK Roy	**Plenary 2** Copper/Zinc & Alzheimer's Disease: George Brewer **Symposium E:** Treatment of Disorders of Copper Metabolism: Hiroko Kodama **Symposium F:** Iron Physiology and Pathology: James McClung	**Plenary 3** New Developments in Iron Metabolism and its Disorders: Prem Ponka **Symposium G:** Osteoporosis: Prevention & Treatment: Erik Eriksen **Symposium H:** Impact of Arsenic on Human Health: Countering Toxicity: Parvez Haris **Minisymposium 3:** Trace Elements in Blood	**Plenary 4** 50 Years of the Miracle Element Zinc: Ananda Prasad **Symposium K:** European Micronutrient Recommendations: Susan Fairweather Tait **Symposium L :** Copper Homeostasis in Health & Disease: James Collins **Minisymposium 6:** Trace Elements Poster #3: Ana Pejović-Milić	**Plenary 5** Cancer Prevention Vs. Promotion: Jan Alexander **Symposium M :** Functional and Neurotoxic Aspects of Zinc in the Brain: Atsushi Takeda **Minisymposium 7:** Trace Elements : Food and Environment: Marie Kana Sop Business Meeting
12:00		**Lunch**	**Lunch**	**Lunch**	**Lunch**	
1:00	**Register**	**Minisymposium 1:** Basic and Animal Nutrition Poster #1: Jessee Bertinato **Symposium C:** Health Effects of Low-Dose Exposure to Toxic Metals: Monica Nordberg **Symposium D:** Boron: Indispensable Element of Our Lives: Sukru Ozturk **Minisymposium 2:** Zinc and Selenium: Josef Köhrle	**Excursion to Historical City and Harbor of Antalya**	**Minisymposium 4:** Trace Element Applications Poster#2: Barry Sampson **Symposium I:** Iodine Supplementation in Pregnancy: Sheila Skeaff **Symposium J:** Metal Carcinogenesis: Max Costa **Minisymposium 5:** Applied Nutrition: Kavitha Menon	**Excursion to Archeological Ruins and Historical City of Side**	
6:00	**Welcome Reception**	Planning Committee Meeting		Council Meeting		
7:00					**Awards Dinner**	

16:50 **Mechanisms of acetyl-CoA-mediated differential cytotoxicity of zinc in brain septal cholinergic neuronal cells.** A Szutowicz, PhD, *Medical University of Gdansk, Poland*

17:00 **Zinc transporter genes are coordinately expressed in men and women.** Meika Foster, PhD, *University of Sydney, Australia*

17:10 **Effects of low dietary zinc on zinc status, carbohydrate metabolism, and progesterone levels in pregnant rats.** Zine Kechrid, PhD, *University of Annaba, Algeria*

17:20 **Interactions between Se, I and Fe in thyroid hormone synthesis.** Josef Köhrle, PhD, *Charite-Universitatsmedizin Berlin, Germany*

17:30 **Involvement of neuroleptics in selenium deficiency and sudden death of cardiac origin.** L Hamdan and Q Timour, PhD, *INSERM, France*

17:40 **Selenium-induced senescence by oxidative DNA damage as an early barrier of tumorigenesis.** Wen-Hsing Cheng, PhD, *University of Maryland, USA*

17:00-18:30 Blue
Planning Committee Meeting

TUESDAY MORNING, October 18

08:00-09:00 Diamond 3
Plenary Session #2: Distinguished Scientist of the World: George M Brewer, MD

Chair: **Ole Andersen**

Purpose of Session: Dr. Brewer, Morton S. and Henrietta K. Sellner Emeritus Professor, Human Genetics and Emeritus Professor of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA, has worked for many years on copper, zinc, and molybdenum metabolism. He developed zinc as a therapy for Wilson's disease, which was approved by the Food and Drug Administration based on his data in 1997. He has made recent contributions which have advanced the knowledge of copper and zinc status and metabolism in Alzheimer's disease. This presentation results from an epiphany of understanding of the role of ingestion of inorganic copper on cognitive loss and its contributions to the current epidemic of Alzheimer's disease.

08:00 Announcements

08:10 **Copper and zinc in Alzheimer's Disease: Cognitive loss from ingestion of inorganic copper.** George M Brewer, MD, *University of Michigan Medical School, USA*

09:00-9:20 Coffee Break

9:20-10:40 Green
Symposium E: Treatments of Disorders of Copper Metabolism

Chair: **Hiroko Kodama**
Co-Chair: **Norikazu Shimizu**

Purpose of Session: Wilsons disease (WD), Menkes disease (MD) and occipital horn syndrome (OHS) are genetic disorders of copper metabolism. WD is characterized by copper toxicity, as presumably is AD. Chelating agents and zinc are effective for the treatment of WD. However, patients with neurologic WD show poor response to these treatments. In addition, the neurologic symptoms become temporarily worse just after treatment with chelating agents, causing serious problems in these patients. Moreover, patients with Wilsons disease may have a risk of hepatocellular carcinoma despite receiving treatment. These problems in patients with neurologic WD will be discussed in this symposium. MD and OHS are characterized by a copper deficiency, with the current-standard-of care being parenteral administrations of copper-histidine. However, the treatment is less effective for neurologic and connective tissue disorders. A novel

treatment for MD and OHS will be discussed in this symposium. Finally, the role of copper in AD and the potential for the free form as a marker for the disease will be discussed.

9:20 **Treatment of patients with neurologic Wilson's disease.** Norikazu Shimizu, MD, PhD, *Toho University School of Medicine, Japan*

9:40 **Early signs at birth or in the neonatal period before typical symptomatic onset of Menkes disease.** Yan-Hong Gu, MD, PhD, *National Research Institute for Child Health and Development, Japan*

10:00 **Combination therapy with injections of copper and oral administrations of disulfiram in the macular mouse, an animal model of Menkes disease.** Hiroko Kodama, MD, PhD, *Teikyo University School of Medicine, Japan*

10:20 **A novel treatment of Menkes disease and occipital horn syndrome**. Eishin Ogawa, MD, PhD, *Teikyo University School of Medicine, Japan*

10:40-12:00 Pink
Symposium F: Iron Physiology and Pathology

Chair: **James McClung**
Co-Chair: **Ismail Cakmak**

Purpose of Session: Recently, a better understanding of how iron absorption plays a key feedback role in influencing both its metabolism and requirements has increased. The mechanism by which iron, especially non-heme iron, transits the intestinal epithelia is known to involve a variety of proteins, which serve to sequester or facilitate its passage into systemic circulation and tissues. Thus, iron stores respond in kind. An individual's absorptive and storage profile influence signaling and the activity of metabolic pathways involved in iron utilization, which, in turn, affect requirements.

10:40 **Iron and zinc malnutrition: a neglected public health issue in developing countries**. Ismail Cakmak, PhD, *Sabanci University, Turkey*

11:00 **Iron transport and regulation of ZIP metal-ion transport proteins**. Mitchell Knutson, PhD, *University of Florida, USA*

11:20 **Nutritional countermeasures for the prevention of diminished iron status in the female athlete.** James McClung, PhD, *US Army Research Institute of Environmental Medicine, USA*

11:40 **Iron mobilization using chelation and phlebotomy.** Trond Flaten, PhD, *Norwegian University of Science and Technology, Norway*

12:00 -13:00 Lunch at Food Court

TUESDAY AFTERNOON, October 18

13:15 Excursion to Historical City and Harbor of Antalya

WEDNESDAY MORNING, October 19

09:00-10:00 Diamond 3
Plenary Session #3: New Developments in Iron Metabolism and it's Disorders

Chair: **Prem Ponka**
Co-Chair: **Gregory Anderson**

Purpose of Session: Iron is essential for oxidation-reduction catalysis and bioenergetics, but unless appropriately shielded, this metal plays a key role in the formation of toxic oxygen radicals that can attack all biological molecules. Hence, specialized molecules for the acquisition, transport, and storage of iron in a soluble nontoxic form have evolved. This symposium will discuss recently discovered

Journal of Trace Elements in Medicine and Biology, 26S1 (2011)

Early signs at birth or in the neonatal period before typical symptomatic onset of Menkes disease.

[1,2]Yan-Hong Gu*, [2]Chie Fujisawa, [2]Hiroko Kodama

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Menkes disease (MNK) is caused by mutation of the gene that encodes a copper-transporting ATPase. Subcutaneous injections of copper-histidine complex initiated soon after birth is the currently accepted mode of treatment. In this study, we summarized the early signs during the neonatal period before typical symptomatic onset of MNK and attempted to provide clues for early diagnosis. A total of 41 Japanese MNK patients were investigated. Clinical data for all patients were obtained from medical records or medical record summaries by a pediatrician's retrospective review. Prior signs at birth or in the neonatal period before typical symptomatic onset of MNK included congenital malformations (41.2%), hair abnormalities (15.2%), feeding difficulty (15.2%), hypothermia (13.0%), respiratory distress (10.7%), hypotonia (2.2%), skin abnormalities (2.2%), and hyperbilirubinemia (in two patients). There were totally 21 types of congenital malformations. Higher arched palate was most commonly found (35.7%).

Journal of Trace Elements in Medicine and Biology, 26S1 (2011)

A novel treatment of Menkes disease and occipital horn syndrome.

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[1]*Department of Pediatrics, Teikyo University School of Medicine, Tokyo, Japan.* * eogawaster@gmailcom

The current standard therapy for Menkes disease (MD) and occipital horn syndrome (OHS) is a parenteral administration of copper-histidine. However, the treatment is less effective for neurologic symptoms, because administered copper is not transported to neurons. Diethyldithiocarbamate (DEDTC), a lipophilic chelater, has shown beneficial effects in macular mice, an animal model of MD, on copper concentrations and cytochrome c oxidase activity in brain. These results led us to treat MD and OHS patients with disulfiram, a dimer of DEDTC, in combination with copper-histidine. Disulfiram was orally administered in two MD patients and an OHS patient after approval of the ethical committee of Teikyo University Hospital, with maintenance dosage of 100 mg/d, QD. Supplementation of copper-histidine was unaltered during the study period. Serum levels of copper and ceruloplasmin tended to increase in a MD patient, and in addition, he showed enriched emotional expression and behavior more often after disulfiram administration. We speculate that copper-disulfiram complex was transported to neurons resulting in neurological improvement.

Journal of Trace Elements in Medicine and Biology, 26S1 (2011)

Combination therapy with injections of copper and oral administrations of disulfiram in the macular mouse, an animal model of Menkes disease.

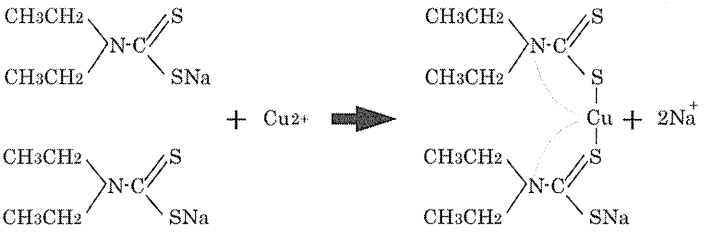
[1]Hiroko Kodama*, [1]Wattanapon Bhadhprasit,

[1]Chie Fujisawa. [1]*Teikyo University, Tokyo, Japan.*
*hkodama@med.teikyo-u.ac

Menkes disease (MD) is a neuro degenerative disorder characterized by copper deficiency caused by a defect in ATP7A. Treatment for this disease is parenteral administration of copper-histidine. When the treatment is initiated in newborn infants, neurological degeneration is prevented. Delayed treatment, however, is not effective, because copper accumulates at the blood-brain barrier (BBB) and is not transported to neurons after the BBB matures. We investigated the effects of a combination therapy of copper and disulfiram, a lipophilic chelator, in macular mice. Mice were given subcutaneous injections of $CuCl_2$ and oral administrations of disulfiram twice a week for 60 days. Copper concentrations in the brain, serum and liver of treated macular mice were significantly higher than those of control macular mice which received $CuCl_2$ and distilled water instead of disulfiram. Cytochrome C oxidase activity and catecholamine metabolism in the brains of treated mice were also improved by the combination therapy, suggesting that the administered copper is transported to the neurons.

ISTERH2011 (17,10,2011)

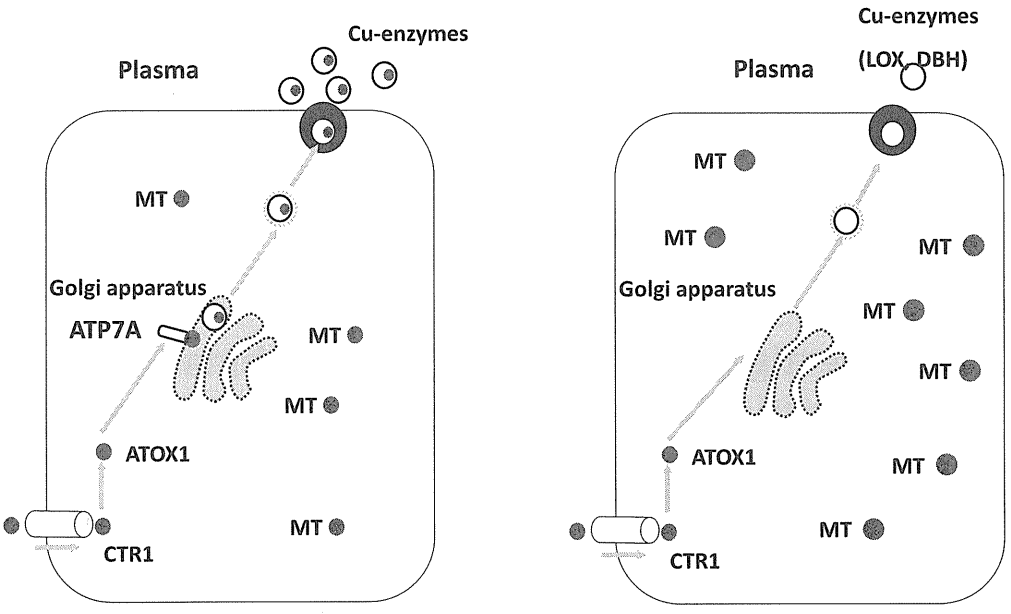
Combination therapy with copper injections and oral administrations of disulfiram in the macular mouse, an animal model of Menkes disease



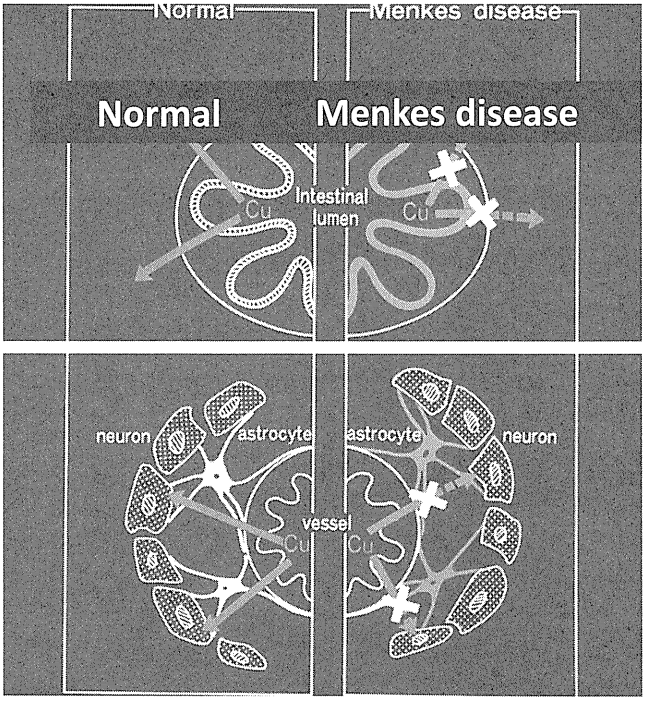
disulfiram

Hiroko Kodama*, Wattanapon Bhadhprasit, Chie Fujisawa, Tomoko Hiroki, Eishin Ogawa,

Teikyo University, Tokyo, Japan. *kodamah2018@gmail.com



Intracellular copper metabolism in Menkes disease



Injected copper will reach to neurons when the BBB is immature.
However, after the BBB becomes mature, the copper can not reach to neurons

Disturbances of Cu transport in the intestine and brain of Menkes disease

(Murata Y, Kodama H et al Pediatr Res 1997)

Object of this study

The object is to create treatment strategy of Menkes disease by transporting copper into the Golgi apparatus, and then to the neurons.

We investigated the effects of a combination therapy with injections of copper and oral administration of disulfiram, a lypophilic chelator, in the macular mouse, an animal model of Menkes disease by two methods.

One method is biochemical analysis, and another is *in vivo* molecular imaging.

Materials & Methods I

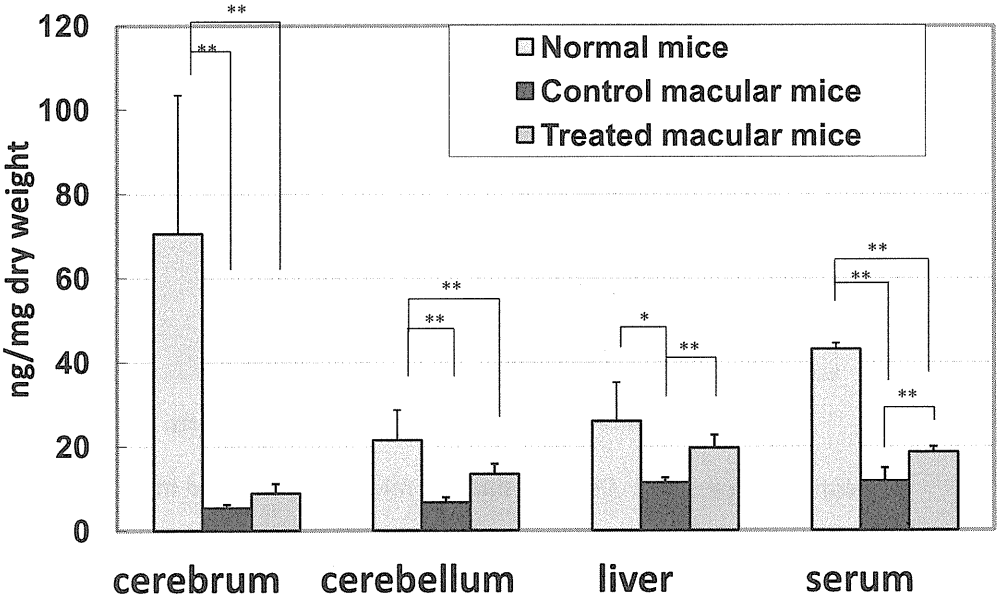
Biochemical analysis

Macular mice were given subcutaneous injections (10μg of $CuCl_2$/time) and oral administrations of disulfiram (0.3 mg/g body weight) twice a week for 8weeks.

Controls: normal littermates and macular mice treated with only copper injections

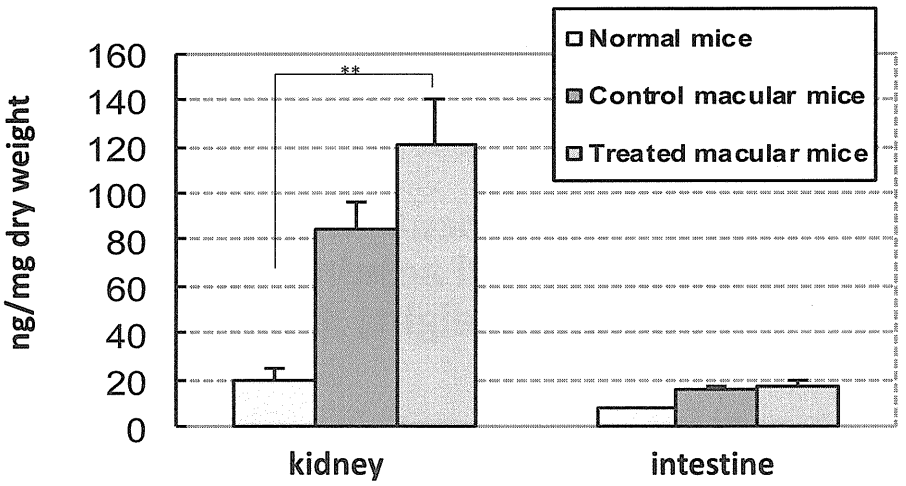
Copper concentrations in the brain, serum, liver, intestine and kidney were analyzed .

Cytochrome c oxidase activity and adrenaline/dopamine were also analyzed in the brain.



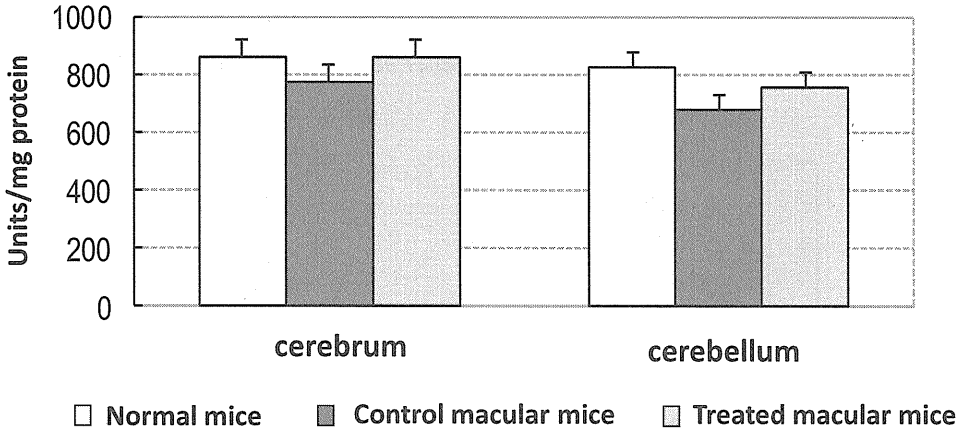
Copper concentration

Copper concentrations in the cerebellum, liver and serum of treated macular mice were significantly higher than those of control macular mice.



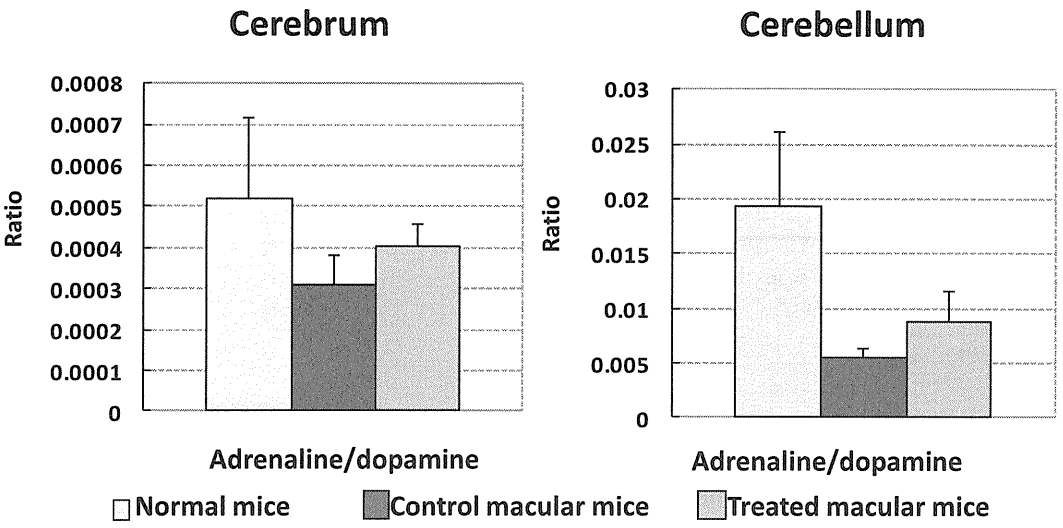
Copper concentration

The copper concentrations in the kidney and intestine of macular mice were significantly higher than those of normal mice. These concentrations in the treated macular mice were higher than those in control macular mice, but not significantly.



Cytochrome c oxidase (CCO) activity

The CCO activity in the cerebrum and the cerebellum of treated macular mice was increased by combination treatment.



Catecholamine analysis

Adrenarine/dopamine was also improved by the combination therapy

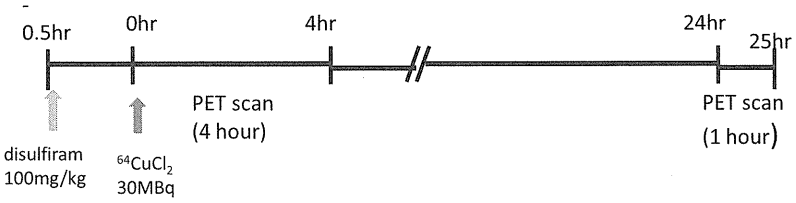
Summary of biochemical analysis

- The serum copper level was increased by the combination therapy with $CuCl_2$ and disulfiram, indicating that copper absorption is improved by the treatment.
- The copper concentration, cytochrome c oxidase and catecholamine metabolism in the brain were improved by the combination therapy, indicating that the combination therapy is effective for Menkes disease.
- The copper concentration also increased in the kidneys and intestine. However, no adverse effects were observed.

Materials & Methods II

in vivo molecular imaging in mice

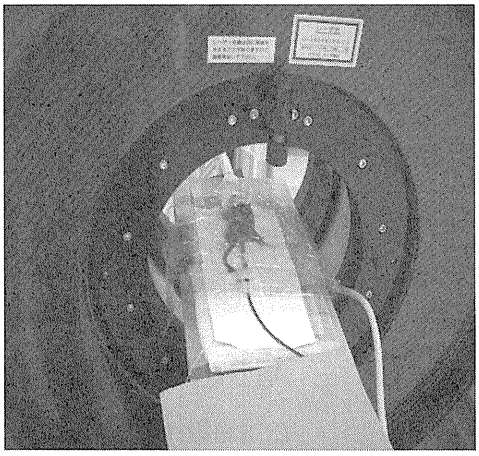
【Protocol of microPET imaging】



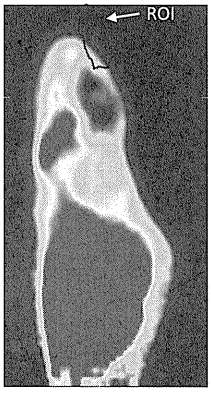
Materials: macular mice
Controls: C3H/He mice
Imaging: microPET, Focus220 (Siemens,USA)

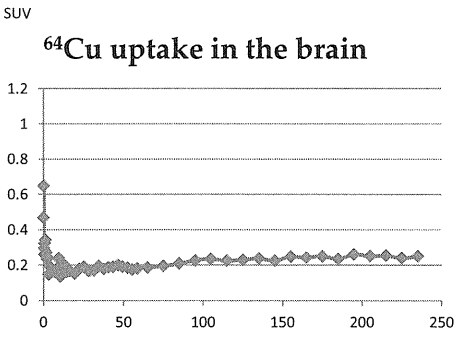
Disulfiram (100 mg/g) was intraperitoneally injected and then 30 min after $^{64}CuCl_2$ was injected intraveously.
Controls were injected only $^{64}CuCl_2$.

In collaboration with
Drs. T. Takeda, H. Shintaku
of Osaka City Univ.

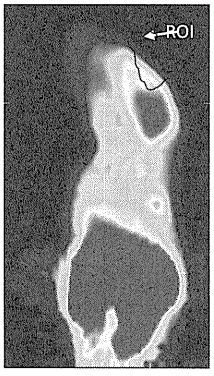


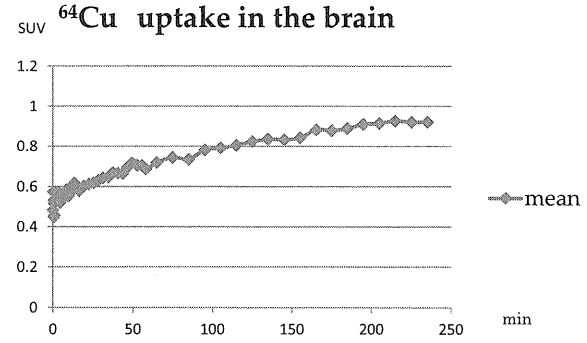
Control
$^{64}CuCl2$



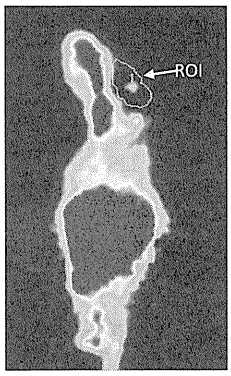


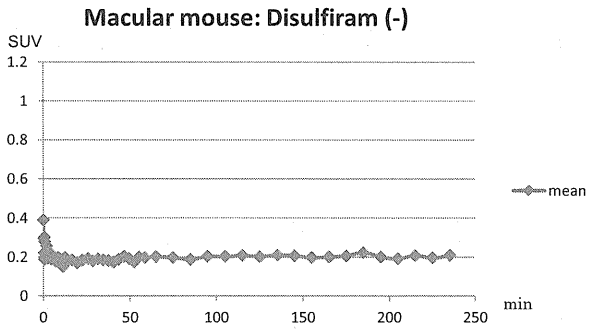
+ Disulfiram
100 mg/kg ip
30 min prior
to $^{64}CuCl2$

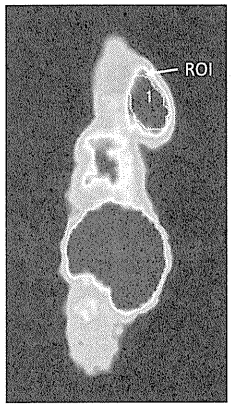


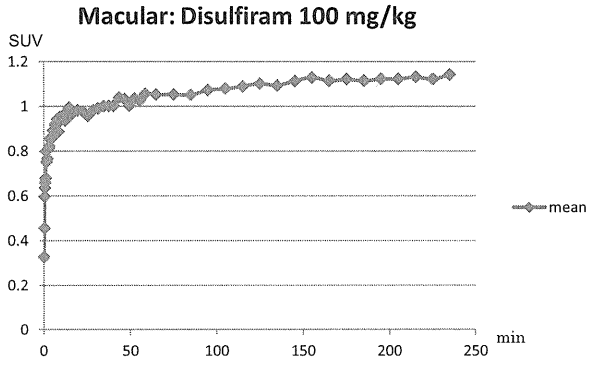


Results of molecular imaging

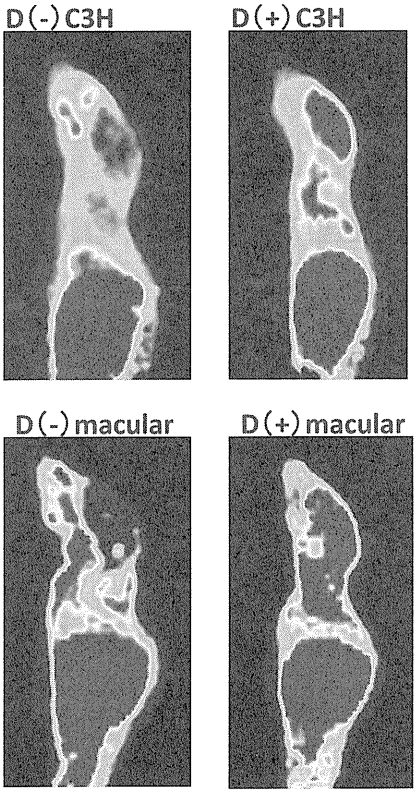


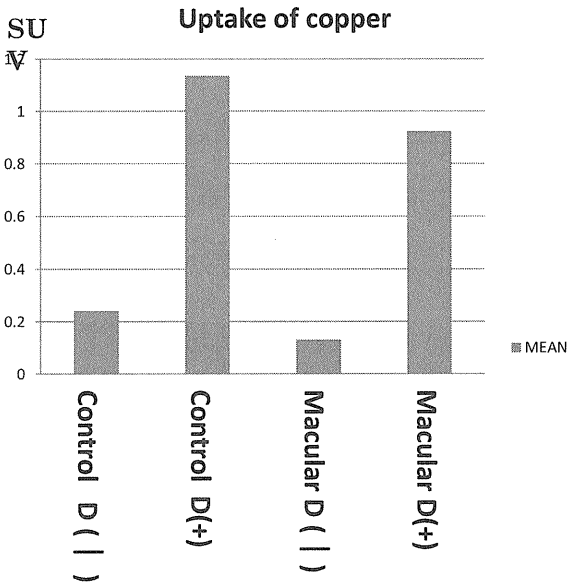






Brain uptake of $^{64}CuCl_2$ in macular mouse by using PET





D (-) copper only: D (+) copper +disulfiram

Copper uptake in the brain at 24 hours after injection

Summery of imaging by using PET

- **Injected copper was mostly taken in the liver.**
- **Copper was accumulated in the brain by the combination treatment of copper injections and disulfiram until 24 hours after injections.**

Conclusion

- **Copper absorption is improved by the combination treatment of copper injections and oral administrations of disulfiram.**
- **These results suggest that copper is able to penetrate to the Golgi apparatus and blood brain barrier, and then is used for copper-dependent enzymes.**
- **The combination therapy of the present study could be effective for Menkes disease.**