Characteristic Effects of L-Methionine on Tissue Distribution of Methylmercury in Mice

Tatsumi Adachi*[a, b]

[a]*Department of Basic Medical Sciences, National Institute for Minamata Disease, 4058–18 Hama, Minamata, Kumamoto 867–0008, Japan and* [b]*Faculty of Pharmaceutical Sciences, Chiba Institute of Science, 15–8 Shiomi-cho, Choshi, Chiba 288–0025, Japan*

(Received September 6, 2005; Accepted January 5, 2006)

The effects of L-methionine (Met) on tissue distribution of methylmercury (MeHg) in mice were investigated and compared to those of other amino acids and amino acid analogues to determine whether Met could play special roles in distribution of MeHg. Three hr after the injection with MeHg-L-cysteine (Cys) (10 μmol/kg each), brain Hg concentration was suppressed by a co-injection with Met, L-phenylalanine (Phe), L-leucine (Leu) or 2-amino-2-norbornanecarboxylic acid (BCH), although the concentration was not affected by that with L-serine, α-aminoisobutyric acid or α-(methylamino)isobutyric acid. Hg concentration in liver was enhanced but that in the kidney was suppressed only by the co-injection with Met. Hg concentrations in blood and plasma were suppressed only by Met at least within 2 hr, but these differences disappeared at 3 hr. These results suggest that Met plays important and special roles in tissue distribution of MeHg in mice, at least in the liver and kidney, although MeHg distribution in the brain is similarly affected by not only Met but also other compounds such as Phe, Leu and BCH.

Key words —— methylmercury, L-methionine, amino acid transport system

INTRODUCTION

Methylmercury (MeHg) is easily absorbed from intestine, and passes through almost all barriers including blood-brain and blood-placenta barriers.[1,2] These properties of MeHg result in its long half-life and toxicity for the central nervous system and fetus. MeHg is known to have a high affinity for the thiol group.[3,4] Therefore, in plasma, most MeHg binds to albumin,[5,6] and MeHg conjugates with L-cysteine (Cys) or glutathione (GSH) are also observed.[7] In addition, these low molecular weight (LMW) thiol compounds accelerate the intestine absorption[8] and tissue uptake of MeHg,[9,10] suggesting that MeHg conjugates with these compounds appear to be a driving force in the MeHg transport into various tissues. Interestingly, GSH is important for the MeHg secretion from tissues, particularly in liver and kidney, for the following reasons.[5,11] MeHg-GSH secreted from the liver to circulation is transported to the kidney,[5,11,12] and renal accumulation (including urinary excretion) of MeHg reflects the efflux rate of GSH from the liver.[5,11] In addition, a similar relationship between urinary excretion of MeHg and the efflux rate of GSH from the kidney is also investigated.[5,11] Thus, LMW thiol compounds can modulate tissue accumulation of MeHg in several ways.

It has been revealed that *in vivo* MeHg transport into the brain[9,13–15] and fetus[16] is markedly suppressed by neutral amino acids such as L-methionine (Met) and L-phenylalanine (Phe), since the constitution of MeHg-Cys is similar to that of Met. In addition, an *in vitro* study using epithelial cells demonstrates that uptake of MeHg-Cys into the cells is also inhibited by Met.[17] Therefore, MeHg-Cys has been considered to pass through the neutral amino acid transport system,[9,10,13–15] especially the Na^+-independent system L that prefers the large neutral amino acids.[18–20] Although Met is a high affinity substrate for this system,[18–20] it is converted to Cys[21] that can enhance the tissue uptake of MeHg.[9,10] Accordingly, Met might play complex and special roles in the fate of MeHg compared to other amino acids. It is reported that an inhibitory level on placenta transport of MeHg by Met is lower than by Phe, due to the Met-induced increase in a plasma Cys concentration.[16] However, only a few reports reveal the differences in the effects of Met and other amino acids on MeHg accumulation in tissues without brain such as liver and kidney.

In the present study, the effects of Met on tissue distribution of MeHg were examined after a co-injection with MeHg-Cys, and were compared to those of other neutral amino acids and amino acid analogues (non-metabolisable substrates for the amino acid transport system). In addition, MeHg distribu-

*To whom correspondence should be addressed: Faculty of Pharmaceutical Sciences, Chiba Institute of Science, 15–8 Shiomi-cho, Choshi, Chiba 288–0025, Japan. Tel.: +81-479-30-4684; Fax: +81-479-30-4740; E-mail: tadachi@cis.ac.jp

tion in plasma was also investigated to estimate the contribution of Met to alterations in the levels of plasma LMW thiol compounds.

MATERIALS AND METHODS

Reagents —— Methylmercuric chloride was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). Amino acids [Cys, Met, Phe, L-leucine (Leu), and L-serine (Ser)] and α-aminoisobutyric acid (AIB) were purchased from Wako Pure Chemical Industries (Osaka, Japan). 2-amino-2-norbornanecarboxylic acid (BCH) and α-(methylamino)isobutyric acid (MeAIB) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animals —— C57BL/6N male mice were obtained from CLEA Japan Co. (Osaka, Japan), and maintained at 23 ± 2°C and 50–60% relative humidity and exposed to a 12-hr light cycle from 7:00 a.m. Mice were maintained on standard laboratory chow (CE-2, CLEA Japan Co.) and tap water *ad libitum*. All experimental procedures were approved by the Ethics Committee on Animal Experiments of the National Institute for Minamata Disease (NIMD).

MeHg Injection and Mercury Determination —— MeHg and Cys (1 : 1) were dissolved in saline, and some of the divided solutions were mixed with an amino acid or amino acid analogue, with a final concentration 100 times higher than MeHg. MeHg solution was injected into the tail vein of mice (age 8 weeks) at a dose of 10 μmol Hg/kg. Three hr after the injection, each mouse was anesthetized using pentobarbital. Blood was collected from the inferior caval vein in a heparinized syringe, and an aliquot of blood was centrifuged at 3000 rpm for 3 min to separate plasma. Each mouse was perfused *via* the heart with ice-cold saline to remove blood from tissues, and kidney, liver and brain were then excised. Hg content in each sample was determined by the oxygen combustion-gold amalgamation method[22] using a Rigaku Mercury Analyzer SP-3 or MA-2 (Nippon Instruments Co., Tokyo, Japan) and expressed as total Hg. The effects of Met on the fate of MeHg were also examined 1 hr after the injection.

Other mice intravenously injected with MeHg-Cys solution with or without Met mentioned above were anesthetized using ether 2 hr after the injection. Blood was collected from the femoral artery using a heparinized glass pipette, and from its aliquot, plasma was obtained as described above. An aliquot of the fresh plasma was immediately subjected to ultrafiltration using a Kurabo Centricut W-50 membrane filter at 5000 × g for 4 min to obtain less than 50000 molecular weight fraction. Total Hg concentrations in the filtrates (plasma LMW fraction), whole plasma, and blood were determined as described above.

Statistical Analysis —— Significant differences between individual means were determined by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test or Student's t-test. Differences were considered significant at $p < 0.05$.

RESULTS

Figure 1 shows Hg concentrations (expressed as percentages in relation to Hg levels in the respective control mice injected with MeHg-Cys alone) in various tissues after simultaneous injection together with MeHg-Cys and one of the amino acids or amino acid analogues. Three hr after the injection, brain Hg concentration was significantly suppressed by Met, Phe, Leu and BCH (Fig. 1A, nos. 1–4, 6), but was not affected by Ser, AIB and MeAIB (Fig. 1A, nos. 1, 5, 7, 8). At that time, Hg concentration in liver was enhanced but that in kidney was suppressed by Met, whereas the concentrations were not affected by other compounds (Fig. 1B and 1C, nos. 1–8). Met similarly affected Hg concentrations in these tissues even at 1 hr, as were the cases at 3 hr (Fig. 1A–1C, nos. 1, 2, 9, 10). Hg concentrations in blood and plasma were significantly suppressed only by Met at 1 hr (Fig. 1D and 1E, nos. 9, 10), although there was no significant difference in these concentrations at 3 hr (Fig. 1D and 1E, nos. 1–8). Thus, Met alone could affect the MeHg distribution in all tissues examined.

To clarify the reason for the alterations in tissue accumulation of MeHg caused by the co-injection with Met, the effects of Met on MeHg distribution were examined in circulation including Hg concentration in plasma LMW fraction, which reflects a driving force in tissue uptake of MeHg.[7,10] Although Hg concentrations in both blood and whole plasma were suppressed by Met 2 hr after the injection of MeHg-Cys, the percentage of Hg concentration in the whole plasma in relation to that in the blood was enhanced (Table 1). In addition, Met significantly enhanced the percentage of Hg concentration in the plasma LMW fraction to that in the whole plasma (Table 1). However, Hg concentration in the LMW

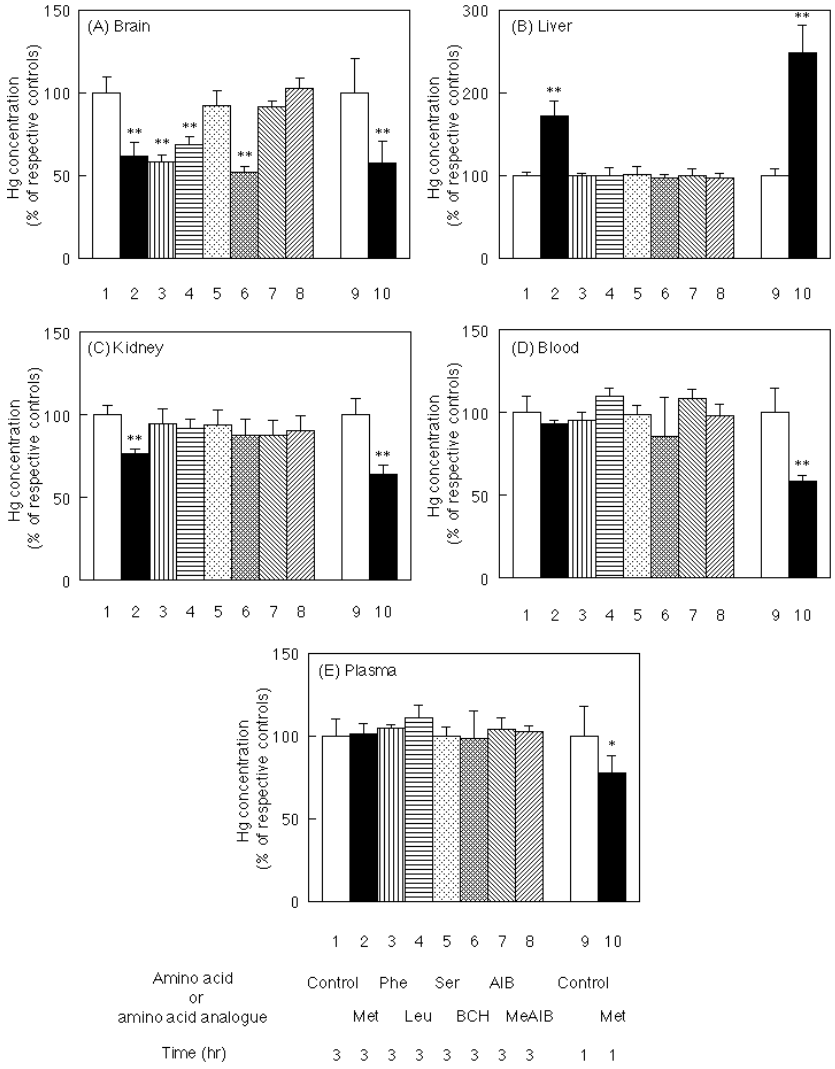

Fig. 1. Effects of Amino Acids and Amino Acid Analogues on Tissue Hg Distribution 1 or 3 hr after Injection of MeHg-Cys in Mice

Mice were intravenously injected with MeHg-Cys (10 μmol/kg each) alone (Control) or co-injected with MeHg-Cys (10 μmol/kg each) and each compound (Met, Phe, Leu, Ser, BCH, AIB or MeAIB, 1 mmol/kg each). Values (percentages of tissue Hg concentrations in relation to those in the respective control mice) represent the mean ± S.D. obtained from 3 to 6 mice. Hg concentrations in the control mice at 1 and 3 hr were 0.24 ± 0.05 and 0.25 ± 0.01 μg/g in the brain, 1.21 ± 0.10 and 4.49 ± 0.19 μg/g in the liver, 27.39 ± 2.73 and 41.25 ± 2.51 μg/g in the kidney, 3.92 ± 0.55 and 1.96 ± 0.19 μg/ml in the blood and 1.17 ± 0.21 and 0.64 ± 0.06 μg/ml in the plasma, respectively. (*) and (**) indicate significant differences from the respective control mice at the specified times at $p < 0.05$ and $p < 0.01$, respectively.

fraction tended to be enhanced by Met, but not significantly so (Table 1).

DISCUSSION

In the present study, MeHg accumulation in the brain was suppressed by a co-injection with some compounds including Met (Fig. 1A). It has been suggested that MeHg is transported into the brain as its Cys conjugate through the neutral amino acid transport system L.[9,10,13–15,17)] This hypothesis has recently been confirmed by the study using *Xenopus laevis* oocytes that reveals the uptake of MeHg-Cys is enhanced by a co-expression of LAT-1 and 4F2 heavy chain,[23)] by which Na^+-independent uptake of neutral amino acids with branched or bulky chains is reported to be increased.[18)] Met, Phe, Leu and BCH (a selective inhibitor for the system L)[19,20)] are high affinity substrates for the system L,[18–20)] whereas Ser (a high affinity substrate for the system ASC and asc)[18–20,24)] and MeAIB (a selective inhibitor for the system A)[19)] are not. In this study, brain uptake of MeHg-Cys would competitively be inhibited by the former but not by the latter (Fig. 1A), which coincides with the hypothesis mentioned above. In con-

Table 1. Effects of Met on Hg Distribution in Blood and Plasma 2 hr after Injection of MeHg-Cys in Mice

		Hg distribution	
		Treatment	
		MeHg-Cys alone (Control)	MeHg-Cys with Met
Blood	(μg/ml)	2.82 ± 0.28	$2.03 \pm 0.16^{b)}$
Whole plasma	(μg/ml)	0.87 ± 0.09	$0.73 \pm 0.08^{a)}$
Plasma LMW fraction	(ng/ml)	22.84 ± 7.98	28.51 ± 1.27
Whole plasma/blood	(%)	30.96 ± 0.86	$36.09 \pm 1.61^{b)}$
Plasma LMW fraction/whole plasma	(%)	2.59 ± 0.79	$3.93 \pm 0.62^{a)}$

Mice were intravenously injected with MeHg-Cys (10 μmol/kg each) with or without Met (1 mmol/kg). Values represent the mean $\pm$ S.D. obtained from 4 to 5 mice. *a*) and *b*) indicate significant differences from control mice at $p < 0.05$ and $p < 0.01$, respectively.

trast, AIB, which is a high affinity substrate for the system A and asc but also transported through the system L,[19,24,25] could not inhibit the uptake (Fig. 1A). Therefore, further study would be necessary in regard to the role of AIB for *in vivo* transport of MeHg into the brain. The previous study indicated that Met increased a plasma Cys concentration in pregnant rats, and this increase would cause that Met showed a lower inhibitory level than Phe in placenta transport of MeHg.[16] Contrary to this finding, in this study using mice, Met did not affect the Hg concentration in plasma LMW fraction (Table 1), which reflects a driving force in tissue uptake of MeHg.[7,10] Therefore, the inhibitory level of brain uptake of MeHg by Met might be identical to the levels by other compounds including Phe (Fig. 1A).

Different from in the brain, Hg concentrations in other tissues such as liver and kidney were affected only by a co-injection with Met (Fig. 1B and 1C), suggesting that these alterations would not be caused by the effects through the amino acid transport system. It has been reported that Met inhibits GSH efflux from liver, and this leads to an increase in the hepatic GSH concentration.[26,27] Since MeHg-GSH secreted from the liver to circulation is transported to the kidney,[5,11,12] this might be a major reason that co-injected Met with MeHg-Cys causes not only the increased hepatic Hg level but also the decreased levels in the kidney, blood and plasma (Fig. 1B–1E).

It has been demonstrated that the fate of MeHg is affected by dietary levels of protein and sulfur amino acids,[28–33] through the alterations in the metabolism of thiol compounds such as GSH[28,29,34,35] and in the amino acid transport.[29,31,32,34] Interestingly, a dietary Met supplement to the refined diet increased Hg accumulation in the liver and decreased that in the kidney regardless of dietary protein levels.[31] Since similar effects of co-injected Met with MeHg-Cys were observed in this study (Fig. 1B and 1C), such alterations might be a special effect of Met on tissue distribution of MeHg. However, there might be marked species differences in the effects of Met at least between mice and rats, since infusion of Met did not affect the Hg concentrations in liver and kidney in rats injected with MeHg-Cys.[14]

In conclusion, Met plays important and special roles in tissue distribution of MeHg in mice, especially in the liver and kidney, except for competitively inhibiting tissue uptake of MeHg through the amino acid transport system as observed in the brain.

Acknowledgements The author is grateful to Ms. Ryuko Takenoshita-Shimoda (NIMD) and Ms. Rieko Ochiai (NIMD) for their technical assistance.

REFERENCES

1) World Health Organization (WHO) (1979) *Environmental Health Criteria 1, Mercury*, WHO, Geneva.
2) World Health Organization (WHO) (1990) *Environmental Health Criteria 101, Methylmercury*, WHO, Geneva.
3) Bach, R. D. and Weibel, A. T. (1976) Nuclear magnetic resonance studies on anion-exchange reactions of alkylmercury mercaptides. *J. Am. Chem. Soc*., **98**, 6241–6249.
4) Simpson, R. B. (1961) Association constants of methylmercury with sulfhydryl and other bases. *J. Am. Chem. Soc*., **83**, 4711–4717.
5) Hirayama, K., Yasutake, A. and Adachi, T. (1991) Mechanism for renal handling of methylmercury. In *Advances in Mercury Toxicology* (Suzuki, T., Imura, N. and Clarkson, T. W., Eds.), Plenum Press, New York, pp. 121–134.

6) Yasutake, A., Hirayama, K. and Inoue, M. (1989) Mechanism of urinary excretion of methylmercury in mice. *Arch. Toxicol*., **63**, 479–483.
7) Yasutake, A., Adachi, T., Hirayama, K. and Inouye, M. (1991) Integrity of the blood-brain barrier system against methylmercury acute toxicity. *Jpn. J. Toxicol. Environ. Health*, **37**, 355–362.
8) Hirayama, K. (1975) Transport mechanism of methyl mercury — intestinal absorption, biliary excretion and distribution of methyl mercury. *Kumamoto Med. J*., **28**, 151–163.
9) Hirayama, K. (1980) Effect of amino acids on brain uptake of methyl mercury. *Toxicol. Appl. Pharmacol*., **55**, 318–323.
10) Hirayama, K. (1985) Effects of combined administration of thiol compounds and methylmercury chloride on mercury distribution in rats. *Biochem. Pharmacol*., **34**, 2030–2032.
11) Hirayama, K., Yasutake, A. and Inoue, M. (1987) Effect of sex hormones on the fate of methylmercury and on glutathione metabolism in mice. *Biochem. Pharmacol*., **36**, 1919–1924.
12) Naganuma, A., Oda-Urano, N., Tanaka, T. and Imura, N. (1988) Possible role of hepatic glutathione in transport of methylmercury into mouse kidney. *Biochem. Pharmacol*., **37**, 291–296.
13) Aschner, M. and Clarkson, T. W. (1988) Uptake of methylmercury in the rat brain: effects of amino acids. *Brain Res*., **462**, 31–39.
14) Aschner, M. (1989) Brain, kidney and liver ^{203}Hg-methyl mercury uptake in the rat: relationship to the neutral amino acid carrier. *Pharmacol. Toxicol*., **65**, 17–20.
15) Kerper, L. E., Ballatori, N. and Clarkson, T. W. (1992) Methylmercury transport across the blood-brain barrier by an amino acid carrier. *Am. J. Physiol*., **262**, R761–R765.
16) Kajiwara, Y., Yasutake, A., Adachi, T. and Hirayama, K. (1996) Methylmercury transport across the placenta via neutral amino acid carrier. *Arch. Toxicol*., **70**, 310–314.
17) Aschner, M. and Clarkson, T. W. (1989) Methyl mercury uptake across bovine brain capillary endothelial cells *in vitro*: the role of amino acids. *Pharmacol. Toxicol*., **64**, 293–297.
18) Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E. and Endou, H. (1998) Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J. Biol. Chem*., **273**, 23629–23632.
19) Christensen, H. N. (1990) Role of amino acid transport and countertransport in nutrition and metabolism. *Phys. Rev*., **70**, 43–77.
20) Kanai, Y. and Endou, H. (2003) Functional properties of multispecific amino acid transporters and their implications to transporter-mediated toxicity. *J. Toxicol. Sci*., **28**, 1–17.
21) Bender, D. A. (1975) *Amino Acid Metabolism*, John Wiley and Sons, London, pp. 112–142.
22) Jacobs, M. B., Yamaguchi, S., Goldwater, L. J. and Gilbert, H. (1960) Determination of mercury in blood. *Am. Ind. Hyg. Ass. J*., **21**, 475–480.
23) Simmons-Willis, T. A., Koh, A. S., Clarkson, T. W. and Ballatori, N. (2002) Transport of a neurotoxicant by molecular mimicry: the methylmercury-L-cysteine complex is a substrate for human L-type large neutral amino acid transporter (LAT) 1 and LAT2. *Biochem. J*., **367**, 239–246.
24) Fukasawa, Y., Segawa, H., Kim, J. Y., Chairoungdua, A., Kim, D. K., Matsuo, H., Cha, S. H., Endou, H. and Kanai, Y. (2000) Identification and characterization of a Na^+-independent transporter that associated with the 4F2 heavy chain and exhibits substrate selectivity for small neutral D- and L-amino acids. *J. Biol. Chem*., **275**, 9690–9698.
25) Ennis, S. R., Ren, X. and Betz, A. L. (1994) Transport of α-aminoisobutyric acid across the blood-brain barrier studied with *in situ* brain perfusion of rat brain. *Brain Res*., **643**, 100–107.
26) Aw, T. Y., Ookhtens, M. and Kaplowitz, N. (1984) Inhibition of glutathione efflux from isolated rat hepatocytes by methionine. *J. Biol. Chem*., **259**, 9355–9358.
27) Fernandez-Checa, J. C., Maddatu, T., Ookhtens, M. and Kaplowitz, N. (1990) Inhibition of GSH efflux from rat liver by methionine: effects of GSH synthesis in cells and perfused organ. *Am. J. Physiol*., **258**, G967–G973.
28) Adachi, T., Yasutake, A. and Hirayama, K. (1992) Influence of dietary protein levels on the fate of methylmercury and glutathione metabolism in mice. *Toxicology*, **72**, 17–26.
29) Adachi, T., Yasutake, A. and Hirayama, K. (1994) Influence of dietary levels of protein and sulfur amino acids on the fate of methylmercury in mice. *Toxicology*, **93**, 225–234.
30) Adachi, T., Yasutake, A. and Hirayama, K. (1995) Influence of dietary levels of protein and sulfur amino acids on the subacute toxicity of methylmercury in mice. *Jpn. J. Toxicol. Environ. Health*, **41**, 411–418.
31) Adachi, T. and Hirayama, K. (1998) Dietary protein levels cause different effects of methionine supplement on the fate of methylmercury in mice. *Jpn. J. Toxicol. Environ. Health*, **44**, 226–232.
32) Adachi, T. and Hirayama, K. (2005) Influence of dietary protein levels on the fate of methylmercury

and on amino acid transport at the renal brush border membrane in rats. *J. Health Sci*., **51**, 138–146.
33) Adachi, T., Kuwana, T., Pan, H. S. and Hirayama, K. (2005) Sex difference in the influence of dietary protein deficiency on the fate of methylmercury in mice and rats. *J. Health Sci*., **51**, 207–211.
34) Adachi, T., Yasutake, A. and Hirayama, K. (2002) Influence of dietary levels of protein and sulfur amino acids on metabolism of glutathione and related amino acids in mice. *J. Health Sci*., **48**, 446–450.
35) Adachi, T. (2006) Dietary protein level-dependent alterations in urinary excretion of thiol compounds caused by L-methionine supplement in mice. *J. Health Sci*., **52**, 63–66.