Influence of Dietary Protein Levels on the Fate of Inorganic Mercury in Mice

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Influence of dietary protein levels on mercury (Hg) fate and on tissue metallothionein (MT) levels was investigated in mice. Twenty-four hr after single administration of mercuric chloride (2.5 mg Hg/kg, subcutaneous), the hepatic Hg concentration was enhanced by dietary protein deficiency, whereas the levels in other tissues and excrements were not affected. At that time, MT inductions by mercuric chloride in liver and kidney were suppressed by dietary protein deficiency, despite no observable differences in basal levels. Thus, Hg levels in the liver and kidney showed little correlation with MT levels. A further experiment demonstrated an enhancement of Hg concentration in the liver by dietary protein deficiency at 3 and 12 hr but not at 1 hr, and the Hg concentration in the kidney was transiently enhanced at 3 hr. Accordingly, the differences in Hg fate would arise considerably earlier, probably before MT induction. The present results suggest that dietary protein status modifies the fate of inorganic Hg, especially in the liver, probably independent of the differences in dietary protein level-dependent varying levels of MT.

Key words —— dietary protein, mercuric chloride, metallothionein

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INTRODUCTION

Mercury (Hg) compounds are major environmental pollutants as well as representative hazardous metals. In these compounds, methylmercury (MeHg) and mercuric ion (Hg^{2+}) have a high affinity for the thiol group.[1–4] Therefore, thiol compounds such as glutathione (GSH), an ubiquitous thiol-containing tripeptide, and metallothionein (MT), a cysteine-rich low molecular weight protein, play important roles in the fate and toxicity of these compounds.[2–11]

We have revealed that dietary levels of protein and sulfur amino acids are an important factor on the fate and toxicity of MeHg using rodents fed on a 24.8% protein diet (normal protein diet, NPD), a 7.5% protein diet (low protein diet, LPD) or either diet supplemented with sulfur amino acids.[12–18] In these experiments, the dietary modifications markedly altered the metabolism of low molecular weight thiol compounds including GSH,[12, 13, 19, 20] and the alterations have been suggested to be one reason for the marked differences in the fate of MeHg. In contrast, there are only a few reports revealing the influence of nutritional conditions such as dietary protein deficiency on the fate and toxicity of Hg^{2+}. For example, it is reported that feeding of protein-free diet could reduce the nephrotoxicity of Hg^{2+}, and demonstrated indirectly using $ZnSO_4$ that the reduction might result from suppressed MT induction caused by the diet.[21] However, in that study, whether dietary protein levels affect Hg accumulation as well as the metabolism of MT including basal and Hg^{2+}-induced levels remains unclear, although it is well known that MT plays important roles in retention of Hg^{2+} as well as protection against its acute nephrotoxicity.[10] Therefore, the relationship between Hg fate and MT metabolism must be further elucidated under several nutritional conditions such as dietary protein deficiency.

In the present study, mice fed on NPD or LPD were singly administered Hg^{2+}, and tissue distribution and excretion of Hg and tissue MT levels were examined. The results were then discussed as to whether or not differences in the fate of inorganic Hg were caused by variation of MT levels after the administration.

MATERIALS AND METHODS

Animals —— C57BL/6N male mice (aged 7 weeks) were obtained from CLEA Japan Co. (Osaka, Japan). The animals were maintained at 23 ± 2°C and 50–60% relative humidity and were exposed to a 12-hr light cycle from 7:00 a.m. They were housed individually, and fed on each of the two casein-based diets, NPD or LPD (CLEA Japan Co.), for 5 days before administration of Hg^{2+} or saline. The composition of the diets was reported previously.[12] All had free access to diet and tap water throughout the experiment. All experimental procedures were approved by the Ethics Committee on Animal Experiments of the National Institute for Minamata Disease (NIMD).

Treatment —— Mercuric chloride (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in saline and subcutaneously administered to mice at a dose of 2.5 mg Hg/kg. Control groups of mice were administered saline at that time. The animals were then housed in metabolism cages (1 mouse/cage). Twenty-four hr after the administration, urine and feces were collected and 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. After blood was removed from tissues by perfusion with ice-cold saline *via* the heart, the kidney and liver were excised. To examine time-dependent alterations in tissue Hg concentrations, tissues of another group of mice were obtained as described above 1, 3 and 12 hr after subcutaneous administration of mercuric chloride (2.5 mg Hg/kg). At least within 24 hr after the administration, the plasma creatinine concentration (an index of nephrotoxicity) and plasma alanine aminotransferase activity (an index of hepatotoxicity), determined as previously described,[14, 15] were not increased by the administration of Hg^{2+} compared with those in saline-administered control mice in either dietary group (data not shown), suggesting that little damage in the liver and kidney was caused by that dose of Hg^{2+}.

Analyses of MT and Hg —— MT levels in the liver and kidney were determined using the Hg-binding method[22] with modifications. Tissue homogenate in 1.15% KCl (approximately 5%; 1.0 ml) was mixed with diethylmaleate (5 μl) and incubated at room temperature for 15 min. After an addition of 10 mM cadmium chloride (25 μl), the samples were heated at 100°C for 5 min and centrifuged at 2000 rpm for 15 min. Mercuric chloride (5 mM; 25 μl) was added to the supernatant (0.5 ml), and MT-unbound Hg was removed by adding 1 mM ovalbumin (225 μl) followed by deproteinizing with 12.5% trichloroacetic acid (250 μl). After centrifugation at 8000 rpm for 5 min, the obtained supernatant was filtered using a 0.22 μm Millipore filter. The Hg amount in each filtrate (MT-bound Hg) was measured by the oxygen combustion-gold amalgamation method[23] using a Rigaku Mercury Analyzer SP-3 or MA2 (Nippon Instruments Co., Tokyo, Japan). MT levels were calculated based on a binding ratio of MT to metals of 1 : 7. Hg contents in the liver, kidney, blood, plasma and excrements were determined by the oxygen combustion-gold amalgamation method[23] as indicated above.

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

RESULTS AND DISCUSSION

We examined tissue distribution and excretion of Hg 24 hr after single administration of Hg^{2+} (2.5 mg Hg/kg, subcutaneous) in mice fed NPD or LPD. Hg concentration in liver was approximately 1.7 times higher in LPD-fed mice than in NPD-fed mice (Table 1). In contrast, Hg concentration in kidney, a major tissue to which inorganic Hg

Table 1. Influence of Dietary Protein Levels on Tissue and Excretory Hg Levels 24 hr after Administration of Hg^{2+}

		Diet	
		NPD	LPD
Liver	(μg/g)	1.27 ± 0.22	2.19 ± 0.40*
	(% of dosed Hg)	2.50 ± 0.42	3.76 ± 0.54*
Kidney	(μg/g)	35.59 ± 6.02	39.10 ± 0.53
	(% of dosed Hg)	23.05 ± 4.03	22.34 ± 0.07
Blood	(μg/ml)	0.30 ± 0.01	0.31 ± 0.01
Plasma	(μg/ml)	0.45 ± 0.03	0.50 ± 0.06
Urine	(% of dosed Hg)	38.53 ± 3.85	36.50 ± 0.85
Feces	(% of dosed Hg)	3.35 ± 0.55	4.97 ± 1.43

Mice were subcutaneously administered mercuric chloride (2.5 mg Hg/kg). Values represent the mean ± S.D. obtained from 3 to 4 mice. (*) indicates significant difference from NPD-fed mice at $p < 0.05$. The ratio of tissue weights to body weight in NPD- and LPD-fed mice were 0.0494 ± 0.0018 and 0.0438 ± 0.0021 in the liver, and 0.0162 ± 0.0005 and 0.0145 ± 0.0002 in the kidney, respectively.

distributed,[3] was similar in the two dietary groups (Table 1). The ratio of the weight of either the liver or kidney to body weight was significantly lower in LPD-fed mice than in NPD-fed mice (footnote to Table 1), since the weights in both tissues were lower in LPD-fed mice and there was no significant difference in the body weight (data not shown), as previously reported.[12, 13, 18] However, the difference in the percentage of accumulated Hg between the dietary groups showed the same trend as the Hg concentration in each tissue (Table 1). No significant differences were observed in Hg levels in blood, plasma, urine and feces (Table 1).

Influence of dietary protein levels and Hg^{2+} on tissue MT levels was determined, since it is well known that MT affects the retention of Hg^{2+}.[3, 10] In saline-administered control mice, MT concentrations in liver and kidney were similar in NPD- and LPD-fed mice (Fig. 1). Twenty-four hr after the Hg^{2+} administration, MT concentrations in NPD- and LPD-fed mice were approximately 8 and 3 times higher in the liver, and were approximately 19 and 15 times higher in the kidney compared to the respective controls (Fig. 1), suggesting that MT inductions by Hg^{2+} in these tissues are less when the dietary protein level is deficient than when it is adequate. As a result, the MT levels in both tissues in Hg^{2+}-administered mice were significantly lower in LPD-fed mice than in NPD-fed mice (Fig. 1). These results indicate that LPD-fed mice show a higher hepatic Hg level and a similar renal level compared to NPD-fed mice 24 hr after Hg^{2+} administration (Table 1), despite lower MT concentrations in both tissues at that time (Fig. 1). The mole ratio of Hg to MT calculated in both tissues was less than 1 regardless of the kind of diet (Table 2), suggesting that MT would be abundantly induced under the conditions in this study. The ratio in the liver was higher in LPD-fed mice than in NPD-fed mice, whereas there was no significant difference in the kidney (Table 2). These results would probably reflect the differences in accumulated Hg levels in the tissues rather than varying binding activity of MT to Hg^{2+}, given the below-mentioned fact that the differential in Hg fate between the dietary groups would arise earlier than the MT induction.

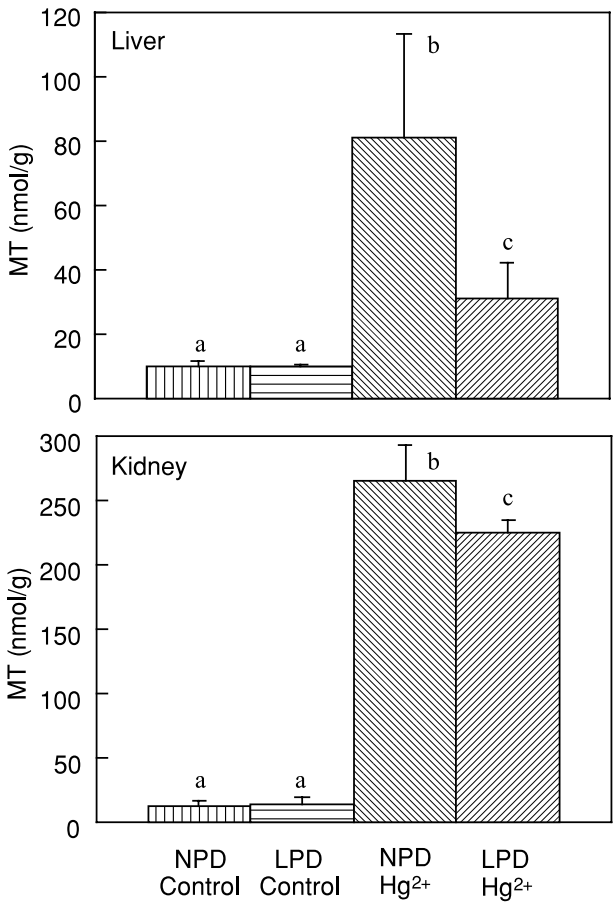

Fig. 1. Influence of Dietary Protein Levels on Tissue MT Levels in Mice 24 hr after Administration of Hg^{2+} or Saline

Mice were subcutaneously administered mercuric chloride (2.5 mg Hg/kg) or saline (controls). Values represent the mean ± S.D. obtained from 3 to 4 mice. Values with different superscripts (a–c) are significantly different ($p < 0.05$).

It has been reported that most Hg binds to high molecular weight substrates in cytosol a few hr after administration of inorganic Hg, whereas Hg binds to MT rather than them 24 hr after.[9] Therefore, it is important to clarify from when the differences in tissue Hg levels are observed between the dietary groups. As shown in Fig. 2, Hg concentrations in liver and kidney were significantly higher in LPD-fed mice than in NPD-fed mice at 3 hr, although no differences were observed at 1 hr. In addition, the difference in the liver remained at 12 hr but disappeared in the kidney (Fig. 2). Hg concentrations in blood and plasma were similar between the dietary groups at all times (data not shown). These results

Table 2. Influence of Dietary Protein Levels on Hg/MT Mole Ratio 24 hr after Administration of Hg^{2+}

	Diet	
	NPD	LPD
Liver	0.082 ± 0.016	0.384 ± 0.143*
Kidney	0.676 ± 0.127	0.868 ± 0.035

Mice were subcutaneously administered mercuric chloride (2.5 mg Hg/kg). Values represent the mean ± S.D. obtained from 3 to 4 mice. (*) indicates significant difference from NPD-fed mice at $p < 0.05$.

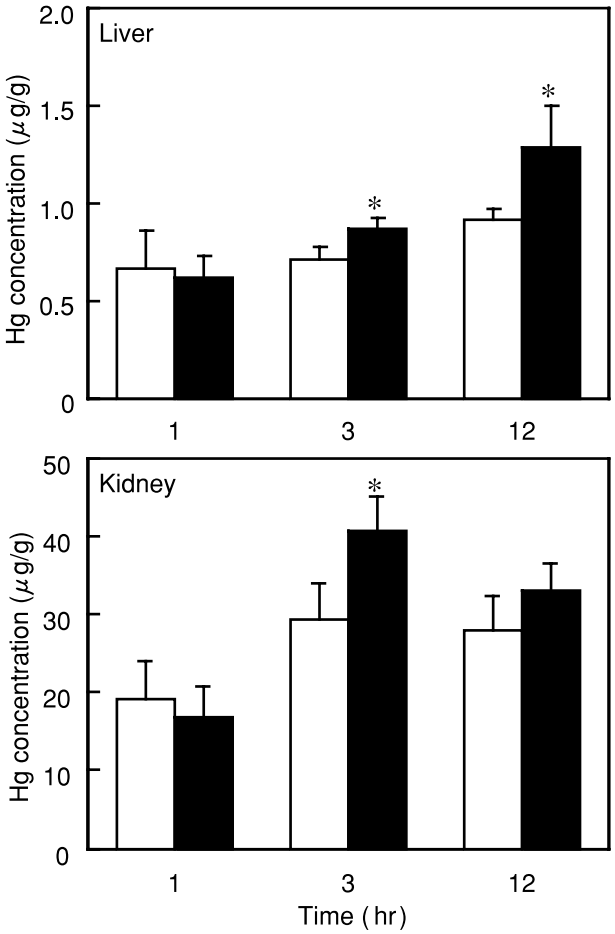

Fig. 2. Time-dependent Alterations in Hg Concentrations in Liver and Kidney in Mice fed NPD (□) or LPD (■) after Administration of Hg^{2+}

Mice were subcutaneously administered mercuric chloride (2.5 mg Hg/kg). Values represent the mean ± S.D. obtained from 3 to 5 mice. (*) indicates significant difference from NPD-fed mice at the specified times at $p < 0.05$.

suggest that the Hg level in the liver might continue to be higher in LPD-fed mice than in NPD-fed mice, probably at least from 3 to 24 hr, although the difference in the kidney is transient. In addition, the differences in Hg fate would arise considerably earlier, probably before MT induction. Our present results and the fact that most Hg binds to high molecular weight ligands rather than MT 2–4 hr after administration of inorganic Hg,[9, 10] suggest an involvement of factors other than the MT in the dietary protein level-dependent difference in Hg accumulation.

It has been suggested that GSH, particularly in the liver, plays an important role for the uptake and accumulation of Hg^{2+} in the kidney. That is, since inorganic Hg conjugated with GSH is secreted from the liver to plasma, and then transported to the kidney, the suppressed hepatic GSH level induces the lower renal Hg level.[7, 11] We previously found that the GSH concentration in the liver was lower in LPD-fed mice than in NPD-fed mice, but the levels in the kidney, blood and plasma were similar.[12, 19] Accordingly, LPD-fed mice that have the lower hepatic GSH level should show a lower renal Hg level than NPD-fed mice. However, that level is in reality similar (Table 1). We previously demonstrated that the efflux rate of GSH from liver was suppressed and the rate from kidney was not affected by dietary protein deficiency, and that this is one reason for the dietary protein level-dependent alterations in the fate of MeHg.[12] Since LPD-fed mice, which have a slower efflux rate of GSH from the liver,[12] show a higher hepatic Hg level (Table 1) compared to NPD-fed mice, alterations in GSH metabolism might partly account for the differences in the fate of Hg^{2+}. However, the Hg level in the kidney plus urine was not affected in this study (Table 1), although it was suppressed by dietary protein deficiency in the previous study regarding the fate of MeHg.[12, 18] Therefore, further detailed study regarding the influx, efflux and retention of Hg^{2+} in these tissues would be necessary to clarify the reason for the differences in its fate.

The present results suggest that dietary protein levels are an important factor in the fate of inorganic Hg and in Hg^{2+}-induced MT synthesis. It is also suggested that the alterations in Hg levels in various tissues caused by nutritional conditions such as dietary protein deficiency might not simply reflect the varying levels of MT induced after a single administration of inorganic Hg, at least in short-term experiments.

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