- Research Letter -

Influence of Dietary Protein Levels on the Acute Toxicity of Inorganic Mercury in Mice

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The influence of dietary protein levels on the acute toxicity of inorganic mercury (Hg) was investigated using mice fed a 24.8% protein diet (normal protein diet, NPD) or a 7.5% protein diet (low protein diet, LPD), and relationships between tissue susceptibility and both levels of Hg and metallothionein (MT) were examined. Twenty-four hr after administration of inorganic Hg, the plasma creatinine concentrations, an index of nephrotoxicity, increased in LPD-fed mice but not in NPD-fed mice at 5 mg Hg/kg, and in both dietary groups at 7.5 mg Hg/kg compared to the respective controls. However, plasma alanine aminotransferase (ALT) activities, an index of hepatotoxicity, increased in both groups only at 7.5 mg Hg/kg. Hg concentrations in the liver was higher in LPD-fed mice than in NPD-fed mice only at 5 mg Hg/kg, although dietary protein levels did not affect concentrations in the liver at 7.5 mg Hg/kg or in the kidney at both doses. MT concentrations were similar in the two dietary groups except for the liver, in which the lowered MT level was observed in LPDfed mice only at 7.5 mg/kg. The present results suggest that dietary protein levels can modify the acute toxicity of singly administered inorganic Hg, at least in the kidney. It is also suggested that MT induction by toxic doses of inorganic Hg is suppressed by dietary protein deficiency, especially in the liver, but this difference would not lead to the variations in the toxicity or in the Hg retention at least within 24 hr.

Key words —— inorganic mercury, dietary protein, metallothionein, nephrotoxicity, hepatotoxicity

INTRODUCTION

Mercury (Hg) is a major environmental pollutant and a highly toxic heavy metal. It is well known that the mercuric ion (Hg^{2+}) induces acute renal failure in experimental animals and humans, and that the fate and toxicity of Hg^{2+} are modified by metallothionein (MT), a cysteine-rich low molecular-weight protein.¹⁻³⁾ In addition, Hg^{2+} also reportedly shows hepatotoxicity in experimental animals.^{4, 5)}

We recently demonstrated that dietary protein levels are a modifying factor on the Hg fate after Hg²⁺ administration at 2.5 mg Hg/kg, which dose of Hg²⁺ did not show both hepatotoxicity and nephrotoxicity, using C57BL male mice fed on a 24.8% protein diet (normal protein diet, NPD) or a 7.5% protein diet (low protein diet, LPD).⁶⁾In that study, dietary protein levels varied the fate of Hg^{2+} , especially in the liver; that is LPD-fed mice showed a higher Hg concentration in the liver than NPD-fed mice 24 hr after administration. In addition, we found that a dietary protein deficiency suppressed MT induction by Hg^{2+} at that dose in the liver and kidney.⁶⁾ Although varying MT levels could not simply explain the dietary protein leveldependent alterations in Hg levels in these tissues, these facts lead to the possibility that dietary protein levels might modify the toxicity of Hg^{2+} . We previously revealed that the dietary protein leveldependent difference in susceptibility to the toxic doses of methylmercury (MeHg) did not simply reflect the Hg fate after its single administration at almost a non-toxic dose, as mentioned below. NPDfed mice showed similar Hg concentrations in the liver and kidney despite higher urinary Hg excretion 24 hr after the administration of MeHg at 20 or 40 µmol/kg,⁷⁻¹⁰) whereas renal toxicity was observed in both dietary groups but hepatotoxicity was observed only in NPD-fed mice at 120 µmol/kg.¹⁰⁾ Therefore, alterations in the susceptibility to Hg²⁺ caused by dietary protein deficiency should be studied separately from alterations in its fate at almost a non-toxic dose. However, there is not so much information about the modifications in susceptibility to toxic doses of Hg²⁺ by dietary protein deficiency and the reasons for their differences. A previous report using rats revealed that a protein-free diet compared to a control diet reduced renal damage induced by Hg²⁺, and suggested that this differ-

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ence was involved in the suppressed MT induction caused by feeding the protein-free diet;¹¹⁾ however, the levels of both Hg and MT were not determined in that study. In addition, almost no reports demonstrated the modification in Hg^{2+} -induced hepatotoxicity caused by dietary protein deficiency.

In the present study, C57BL male mice fed on NPD or LPD were administered Hg^{2+} at toxic doses (5 or 7.5 mg Hg/kg), and the dietary protein level-dependent difference in susceptibility to the acute toxicity of Hg^{2+} in the liver and kidney was determined. The influence of dietary protein levels on the tissue accumulation and excretion of Hg and on tissue MT levels after administration was also investigated, and the relationships between the toxicity and these levels were discussed.

MATERIALS AND METHODS

Animals — C57BL/6N male mice (aged 7 weeks) were obtained from CLEA Japan Co. (Osaka, Japan). The animals were maintained at $23 \pm 2^{\circ}$ C and 50–60% relative humidity and were exposed to a 12-hr light cycle daily from 7:00 *a.m.* The animals were housed individually, and acclimated for 5 d before Hg²⁺ administration on either NPD or LPD (CLEA Japan Co.), the composition of which was reported previously.⁷) They had free access to both diet (NPD or LPD) and tap water throughout the experiments. All experimental procedures were approved by the Ethics Committee on Animal Experiments of the National Institute for Minamata Disease (NIMD).

Treatment — Mice were subcutaneously administered mercuric chloride (Wako Pure Chemical Industries, Osaka, Japan) dissolved in saline at a dose of 5 or 7.5 mg Hg/kg or saline, and then housed in metabolism cages (1 mouse/cage). Twenty-four hr after 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.

Analyses of MT, Hg, Creatinine and Alanine Aminotransferase (ALT) Activity — MT levels in the liver and kidney were determined using the Hg-binding method¹²⁾ with modifications as previously described.⁶⁾ Hg contents in the liver, kidney, blood, plasma and excrement were determined by the oxygen combustion-gold amalgamation method¹³⁾ using a Rigaku Mercury Analyzer SP-3 or MA2 (Nippon Instruments Co., Tokyo, Japan). The creatinine concentration and ALT activity in each plasma were determined using a CRE-EN Kainos assay kit (Kainos Co., Tokyo, Japan) and by the method of Reitman and Frankel¹⁴⁾ using a S.T-A-Test Wako assay kit (Wako Pure Chemical Industries), respectively.

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

Figure 1 shows the creatinine concentration (an index of nephrotoxicity) and ALT activity (an index of hepatotoxicity) in plasma of mice fed NPD or LPD 24 hr after a single administration of Hg^{2+} (5 or 7.5 mg Hg/kg) or saline. Plasma creatinine



Fig. 1. Creatinine Concentration and ALT Activity in Plasma in Mice Fed NPD or LPD 24 hr after Administration of Hg²⁺ or Saline

■, NPD; \equiv , LPD. Mice were subcutaneously administered mercuric chloride (5 or 7.5 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) at the specified indices.



Fig. 2. Influence of Dietary Protein Levels on Hg Fate in Mice 24 hr after Administration of Hg²⁺
□, NPD; □, LPD. Mice were subcutaneously administered mercuric chloride (5 or 7.5 mg Hg/kg). Values represent the mean ± S.D. obtained from 3 to 4 mice. (*) indicates significant difference from NPD-fed mice at the specified doses at p < 0.01.

concentrations increased in LPD-fed mice but not in NPD-fed mice at 5 mg Hg/kg, and in both dietary groups at 7.5 mg Hg/kg compared to the respective controls administered saline (Fig. 1A). It was previously reported that renal damage induced by Hg²⁺ was reduced in rats fed a protein-free diet compared to those fed a control diet in a relatively short-tem experiment (within 2 d).¹¹⁾ Although the reasons for that difference have not been resolved. the opposite result might be due to the differences between species and/or from variations in the protein contents (low protein or protein-free). In contrast to plasma creatinine concentrations, plasma ALT activities increased in both groups only at 7.5 mg Hg/kg (Fig. 1B). These results suggest that the kidney is damaged by a lower dose of Hg^{2+} than the liver, at least under limited nutritional conditions, and that a dietary protein deficiency enhances the acute toxicity of Hg^{2+} to the kidney but not to the liver. In addition, acute toxicity by toxic doses of Hg^{2+} in the liver and kidney would not simply reflect their Hg concentrations after a single administration of Hg^{2+} at the lower dose, since Hg concentrations in LPD-fed mice were lower in the liver and similar in the kidney compared to those in NPD-fed mice 24 hr after administration at 2.5 mg Hg/kg.⁶

We then investigated the tissue distribution and excretion of Hg 24 hr after administration of Hg²⁺ at toxic doses to clarify the relationship between the toxicity and the fate of Hg²⁺. At 5 mg Hg/kg, Hg concentrations in liver were significantly higher in LPD-fed mice than in NPD-fed mice as described in our earlier report⁶⁾ that used half the dosage, whereas this difference was not found at 7.5 mg Hg/kg (Fig. 2A). Hg concentrations in kidney, a major tissue to which inorganic Hg is distributed,¹⁾ were similar in the two dietary groups at both doses (Fig. 2B). Hg concentrations in blood

and plasma were significantly higher in LPD-fed mice than in NPD-fed mice at 5 mg Hg/kg, although no significant difference was observed at 7.5 mg Hg/kg (Fig. 2C and 2D). Hg excretion levels in urine and feces were similar in the two dietary groups at both doses (Fig. 2E and 2F), as same as at almost a non-toxic dose (2.5 mg Hg/kg).⁶⁾ Therefore, dietary protein levels might not affect the Hg excretion levels at least within 24 hr after the administration of Hg²⁺ even at toxic doses. However, after renal dysfunction such as a proximal tubular necrosis induced by Hg²⁺ or other agents, urinary Hg excretion generally increased.²⁾ Accordingly, it might thereafter differ between the dietary groups at least at 5 mg/kg, due to the renal toxicity observed only in LPD-fed mice (Fig. 1A). At 5 mg Hg/kg, the level of creatinine increased only in LPD-fed mice (Fig. 1A), despite similar renal Hg concentrations in the two dietary groups (Fig. 2B), suggesting that a dietary protein deficiency could enhance the susceptibility to Hg²⁺, at least in the kidney. In contrast, although a dietary protein deficiency caused a higher Hg concentration in the liver (Fig. 2A), the hepatic toxicity of Hg²⁺ was not observed at that dose (Fig. 1B). In addition, higher plasma and blood Hg concentrations were caused by dietary protein deficiency at the same dosage (Fig. 2C and 2D). Accordingly, these might provide a key to explain the dietary protein level-dependent difference in renal susceptibility, although it is uncertain that those phenomena lead to or are induced by the enhanced susceptibility caused by dietary protein deficiency. As different from the case at 5 mg Hg/kg, there were no significant differences in either the fate or acute toxicity of Hg²⁺ between the dietary groups at 7.5 mg Hg/kg (Figs. 1 and 2).

It is well known that Hg²⁺ can induce MT, and that MT suppresses its toxic action, especially in the kidney.¹⁾ We earlier found that although basal levels of MT in the liver and kidney were similar in the two dietary groups (as shown in Fig. 3), its induction by Hg^{2+} at a dose of 2.5 mg Hg/kg was less than when the dietary protein level was lowered.⁶⁾ Twenty-four hr after the administration of higher doses of Hg²⁺, MT concentrations in the liver dosedependently increased in NPD-fed mice, but not in LPD-fed mice (Fig. 3A). In contrast, no difference was observed in renal MT concentrations regardless of the dosages or dietary protein levels (Fig. 3B). These results suggest that MT induction by Hg²⁺ would reach a plateau at a lower dose in the kidney than in the liver regardless of dietary protein levels,



Fig. 3. Influence of Dietary Protein Levels on Tissue MT Concentrations in Mice 24 hr after Administration of Hg²⁺ or Saline

 \square , NPD; \blacksquare , LPD. Mice were subcutaneously administered mercuric chloride (5 or 7.5 mg Hg/kg) or saline (controls). Values represent the mean \pm S.D. obtained from 3 to 4 mice. Values with different superscripts (a-d) are significantly different (p < 0.05) at the specified tissues.

and that hepatic MT induction by Hg^{2+} would reach its ceiling at the lower dose by dietary protein deficiency.

As described above, MT induction was affected by dietary protein deficiency, especially in the liver. In that tissue, although MT concentrations were lower in LPD-fed than in NPD-fed mice at 7.5 mg Hg/kg (Fig. 3A), the Hg concentrations were similar and the hepatotoxicity was observed in both dietary groups 24 hr after the administration of Hg²⁺ (Figs. 1B and 2A). In addition, although the renal concentrations of MT and Hg were similar regardless of doses or diets (Figs. 2B and 3B), nephrotoxicity was observed only in LPD-fed mice at 5 mg Hg/kg and in both groups at 7.5 mg Hg/kg (Fig. 1A). There is a complex relationship between the metabolism of intracellular thiol compounds, including MT, and the fate and toxicity of Hg^{2+} .²⁾ In the kidney, MT induction appears to lead to increased Hg accumulation and decreased toxicity.²⁾ In addition, the renal toxicity of Hg²⁺ was markedly enhanced in MT-null mice compared to wild-type mice, although the Hg accumulation was lower in the former than in the latter 24 hr after Hg²⁺ administration.³⁾ Therefore, it might be difficult to determine the relationships between the tissue dysfunctions and MT levels in those tissues. We recently suggested that the alterations in Hg levels in the liver and kidney caused by dietary protein deficiency might not simply reflect the varying levels of MT induced by singly-administered Hg²⁺ at 2.5 mg Hg/kg within at least 24 hr. Our present results support that hypothesis even after the administration of toxic doses of Hg²⁺, especially in the liver.

The present results suggest that dietary protein levels can modify the acute toxicity of singlyadministered inorganic Hg, at least in the kidney, probably due to some factor other than the MT level. It is also suggested that MT induction by toxic doses of inorganic Hg is suppressed by dietary protein deficiency, especially in the liver, but this difference would not lead to the variations in the toxicity and the Hg retention within at least 24 hr.

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