Sex Difference in the Influence of Dietary Protein Deficiency on the Fate of Methylmercury in Mice and Rats

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We investigated sex difference in the influence of dietary protein deficiency on the fate of methylmercury (MeHg) using both sexes of C57BL/6N mice and Wistar rats to determine the universality of the influence. One day after oral administration of MeHg (20 μmol/kg), regardless of sex and species, urinary Hg excretion was suppressed by dietary protein deficiency, whereas fecal excretion was not affected. At that time, tissue Hg concentrations in both sexes of the specified species were similarly influenced by dietary protein deficiency except for the gonads, although the influence on Hg concentration in each tissue was different between species. Regardless of sex, dietary protein deficiency resulted in the following alterations: in mice, the brain Hg concentration increased but the concentrations in the liver, kidney, blood and plasma were not affected, and in rats, Hg concentrations in the liver and blood increased but the renal concentration decreased with similar concentrations in the plasma and brain. Hg concentration in the testes was enhanced in mice but suppressed in rats by dietary protein deficiency, whereas that in the ovary was not affected in either species, suggesting that Hg accumulation in the gonads would be more changeable in males than in females by dietary protein deficiency. These results suggest that, regardless of sex, dietary protein deficiency similarly influences the fate of MeHg, except for the gonads. It is also suggested that a decrease in urinary excretion of MeHg by dietary protein deficiency might be universal.

Key words —— methylmercury, dietary protein, sex difference

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INTRODUCTION

It is well known that methylmercury (MeHg) has a high affinity for the thiol group.[1,2] Thus, the variations in the metabolism and structure of thiol compounds, including glutathione (GSH)[3,4] and hemoglobin,[5] have caused differences in the fate of MeHg between strains,[5] sexes[3,4,6] and ages[4,6] of mice. For example, C57BL male mice, in which GSH turnover is faster, show higher Hg levels in the kidney and urine and a lower level in the liver than females.[3,4,6] As a result, intoxication by repeated administration of MeHg occurs earlier in females than in males.[7] It also has been reported that sex differences in the fate and toxicity of MeHg are also observed in rats.[4,8–10] In contrast to C57BL mice, urinary Hg excretion in rats is higher in females than in males.[4,9]

We earlier demonstrated that dietary protein levels, which modulated GSH metabolism,[11–15] were a modifying factor in the fate and toxicity of MeHg 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).[14,16–19] Even within 24 hr after a single administration of MeHg at a dose of 20 μmol/kg, LPD-fed mice showed a higher brain Hg concentration and lower urinary Hg excretion compared with NPD-fed mice.[14,16,19] As described above, the fate of MeHg in C57BL male mice was, however, very different from that in female mice of this strain[3,4,6] and furthermore, was different from that in another strain of male mice.[4,5] Accordingly, it is possible that the influence of dietary protein deficiency on the fate of MeHg may be specially observed in C57BL male mice. On the other hand, we recently revealed that urinary Hg excretion was decreased by dietary protein deficiency in male Wistar rats as in C57BL male mice, but the influence on tissue Hg accumulation in the rats was markedly different from that in the mice.[20]

In the present study, to determine the universal influence of dietary protein deficiency on the fate of MeHg, tissue and excretory Hg after administration of MeHg (20 μmol/kg) were investigated in both

Table 1. Influence of Dietary Protein Levels on Body and Tissue Weights in Male and Female Mice and Rats

	Tissue	Diet	
		NPD	LPD
		Male mice	
Body weight (g)[a]		21.72 ± 0.40	21.75 ± 0.69
Tissue weight (g)[b]	Brain	0.410 ± 0.022	0.417 ± 0.037
	Liver	1.154 ± 0.017	0.944 ± 0.082**
	Kidney	0.286 ± 0.023	0.257 ± 0.005*
	Testes	0.184 ± 0.017	0.178 ± 0.020
		Female mice	
Body weight (g)[a]		19.28 ± 0.61	18.77 ± 0.27
Tissue weight (g)[b]	Brain	0.436 ± 0.003	0.439 ± 0.016
	Liver	0.931 ± 0.122	0.948 ± 0.119
	Kidney	0.252 ± 0.007	0.213 ± 0.012**
	Ovary	0.0053 ± 0.0010	0.0056 ± 0.0008
		Male rats	
Body weight (g)[a]		216.10 ± 4.71	182.04 ± 10.81**
Tissue weight (g)[b]	Brain	1.79 ± 0.03	1.75 ± 0.05
	Liver	11.25 ± 0.47	8.35 ± 0.93**
	Kidney	1.93 ± 0.08	1.42 ± 0.09**
	Testes	2.34 ± 0.09	2.20 ± 0.13
		Female rats	
Body weight (g)[a]		172.73 ± 1.57	165.79 ± 5.46
Tissue weight (g)[b]	Brain	1.70 ± 0.03	1.74 ± 0.04
	Liver	8.10 ± 0.73	7.01 ± 0.37*
	Kidney	1.54 ± 0.09	1.23 ± 0.04**
	Ovary	0.070 ± 0.004	0.068 ± 0.009

a) Five days after feeding of each diet (before MeHg administration). *b*) One day after MeHg administration. The values represent the mean ± S.D. obtained from 3 to 6 animals. Significantly different from NPD-fed animals in the specified species and sex, *$p < 0.05$, **$p < 0.01$.

sexes of C57BL mice and Wistar rats fed on NPD or LPD.

MATERIALS AND METHODS

Animals —— Both male and female C57BL/6N mice and Wistar rats (aged 7 weeks) were obtained from CLEA Japan Co. (Osaka, Japan). The animals were maintained at 23 ± 2°C in 50–60% relative humidity and were exposed to a 12-hr light cycle from 7:00 a.m. The animals were acclimated to either of two casein-based diets, NPD or LPD (CLEA Japan Co.), for 5 days before use in the experiment, and were given in each diet and tap water *ad libitum* throughout the experiment. The composition of the diets was reported previously.[14] All experimental procedures were approved by the Ethics Committee on Animal Experiment of the National Institute for Minamata Disease (NIMD).

MeHg Administration and Mercury Determination —— Methylmercuric chloride (Tokyo Chemical Industry Co., Tokyo, Japan) was dissolved in saline and administered orally to the animals at a dose of 20 μmol/kg on day 0. The animals were housed in metabolism cages (1 animal/cage), and urine and feces were collected for 1 day after the administration. On day 1, each animal was then anesthetized using pentobarbital. Blood was collected from the inferior caval vein in a heparinized syringe. After perfusion with ice-cold saline *via* the heart, kidney, liver, gonads and brain were excised. An aliquot of blood was centrifuged at 3000 rpm for 3 min to separate plasma. Hg content in each sample was determined by the oxygen combustion-gold amalgamation method[21] using a Rigaku Mercury Analyzer SP-3 or MA-2 (Nippon Instrument Co., Tokyo, Japan) and expressed as total Hg.

Statistical Analysis —— Significant differences between dietary groups in the specified species and

Table 2. Influence of Dietary Protein Levels on Tissue and Excretory Hg in Male and Female Mice and Rats 24 hr After MeHg Administration

Tissue	Diet			
	NPD	LPD	NPD	LPD
	Male mice		Male rats	
Brain (μg/g)	0.82 ± 0.09	1.23 ± 0.07**	0.57 ± 0.04	0.61 ± 0.04
(%)[a]	0.39 ± 0.03	0.59 ± 0.02**	0.12 ± 0.01	0.15 ± 0.01**
Liver (μg/g)	6.67 ± 0.30	7.31 ± 0.63	0.74 ± 0.03	1.69 ± 0.22**
(%)[a]	8.88 ± 0.37	7.89 ± 0.68	0.96 ± 0.07	1.92 ± 0.17**
Kidney (μg/g)	56.58 ± 2.54	61.23 ± 2.50	14.06 ± 0.80	10.46 ± 1.16**
(%)[a]	18.55 ± 0.66	18.19 ± 0.46	3.13 ± 0.28	2.04 ± 0.22**
Testes (μg/g)	2.87 ± 0.15	3.50 ± 0.43*	0.40 ± 0.04	0.30 ± 0.02**
(%)[a]	0.61 ± 0.04	0.70 ± 0.03**	0.108 ± 0.008	0.093 ± 0.015
Blood (μg/ml)	2.93 ± 0.17	3.08 ± 0.12	25.69 ± 1.82	31.19 ± 1.74**
Plasma (μg/ml)	0.83 ± 0.07	0.89 ± 0.07	0.170 ± 0.015	0.162 ± 0.026
Urine (%)[b]	4.03 ± 2.41	0.80 ± 0.20*	0.097 ± 0.029	0.036 ± 0.014**
Feces (%)[b]	1.27 ± 0.27	1.13 ± 0.43	2.47 ± 0.92	2.00 ± 0.39
	Female mice		Female rats	
Brain (μg/g)	1.00 ± 0.10	1.32 ± 0.09**	0.68 ± 0.06	0.69 ± 0.08
(%)[a]	0.57 ± 0.05	0.77 ± 0.07**	0.17 ± 0.02	0.18 ± 0.02
Liver (μg/g)	8.58 ± 0.67	7.58 ± 0.51	0.75 ± 0.04	1.51 ± 0.18**
(%)[a]	10.66 ± 1.09	9.53 ± 1.01	0.89 ± 0.05	1.59 ± 0.12**
Kidney (μg/g)	27.05 ± 2.57	29.82 ± 2.57	21.25 ± 0.81	16.76 ± 1.89**
(%)[a]	8.84 ± 0.51	8.42 ± 0.55	4.82 ± 0.26	3.11 ± 0.34**
Ovary (μg/g)	7.59 ± 1.50	7.63 ± 0.78	0.64 ± 0.06	0.63 ± 0.07
(%)[a]	0.051 ± 0.007	0.057 ± 0.004	0.0065 ± 0.0004	0.0064 ± 0.0006
Blood (μg/ml)	3.04 ± 0.34	3.46 ± 0.16	30.15 ± 0.65	34.18 ± 1.68**
Plasma (μg/ml)	1.18 ± 0.12	1.14 ± 0.09	0.163 ± 0.006	0.145 ± 0.013
Urine (%)[b]	0.51 ± 0.17	0.31 ± 0.13*	0.256 ± 0.173	0.035 ± 0.013*
Feces (%)[b]	1.43 ± 0.18	1.20 ± 0.17	1.40 ± 0.39	1.45 ± 0.36

a) Percentage of Hg accumulated in relation to Hg administered. *b*) Percentage of Hg excreted in relation to Hg administered. The animals were orally administered MeHg at a dose of 20 μmol/kg. One day after the administration, urine and feces were collected, and tissues were excised under pentobarbital anesthesia. The values represent the mean ± S.D. obtained from 3 to 6 animals. Significantly different from NPD-fed animals in the specified species and sex, *$p < 0.05$, **$p < 0.01$.

sex were determined by Student's *t*-test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Sex Difference in MeHg Fate Alteration in Mice

After feeding either NPD or LPD for 5 days, body weight was similar in NPD- and LPD-fed mice regardless of sex (Table 1, above). In males, the weights of liver and kidney in LPD-fed mice were lower than in NPD-fed mice, whereas the brain weight was identical (Table 1, above). A similar tendency was observed in females except for the liver, the weight of which was similar in the two dietary groups (Table 1, above). The weights of gonads, testes in males and ovary in females, were identical in each sex (Table 1, above).

The influence of dietary protein deficiency on tissue and excretory Hg was investigated in male and female mice orally administered MeHg at a dose of 20 μmol/kg (Table 2, left). Regardless of sex, the Hg concentration and percentage of accumulated Hg in relation to administered Hg in the brain were higher in LPD-fed mice than in NPD-fed mice, although there were no significant differences in levels in the liver, kidney, blood and plasma between the dietary groups 1 day after MeHg administration. Hg concentration and percentage of accumulated Hg in the testes were higher in LPD-fed mice, but those in the ovary were similar. In both sexes, urinary Hg excretion was markedly lower in LPD-fed mice than in NPD-fed mice, although fecal excretion was identical. Thus, there was little sex difference in the in-

fluence of dietary protein deficiency on the fate of MeHg in mice except for the gonads.

Sex Difference in MeHg Fate Alteration in Rats

After feeding each diet for 5 days, body weight in male rats was markedly higher in NPD-fed rats than in LPD-fed rats, but no weight difference was observed in female rats (Table 1, below). In each sex, the weights of liver and kidney in LPD-fed rats were lower than in NPD-fed rats, whereas the weights of brain and gonads were similar between the dietary groups (Table 1, below).

Tissue and excretory Hg were investigated in both sexes of rats fed NPD or LPD after oral administration of MeHg at a dose of 20 μmol/kg (Table 2, right). One day after MeHg administration, Hg concentration in the brain was similar in the two dietary groups in both sexes. The percentage of Hg accumulation in the brain was higher in LPD-fed rats than in NPD-fed rats only in males, probably due to the difference in the body weight in males alone (Table 1, below). Regardless of sex, both Hg concentration and percentage of accumulated Hg in the liver were much higher and those in the kidney were much lower in LPD-fed rats. In the gonads, a significant difference was observed only in Hg concentration in the testes: the concentration was lower in LPD-fed rats than in NPD-fed rats. Regardless of sex, Hg concentration in the blood was higher in LPD-fed rats, whereas no marked difference was observed in the plasma Hg concentration. Urinary Hg excretion in LPD-fed male and female rats was approximately one-third and one-seventh of that in NPD-fed rats, respectively, although the fecal excretion was identical in both sexes. Thus, the influence of dietary protein deficiency on the fate of MeHg in rats was similar in male and female rats with the exception of the percentage of Hg accumulated in the brain and Hg concentration in the gonads.

Universality of MeHg Fate Alteration

In the present study, the influence of dietary protein deficiency on the fate of MeHg showed virtually the same tendency at 24 hr after administration of MeHg between both sexes in mice and rats (Table 2) regardless of the marked sex difference in its fate in intact animals.[3,4,6] A most remarkable finding is that urinary Hg excretion is always suppressed by dietary protein deficiency regardless of sexes and species, whereas fecal excretion is not affected (Table 2). Although tissue Hg accumulation except for gonads was similarly altered by dietary protein deficiency between sexes in the specified species, the influence was different in mice and rats, *i.e.*, the concentrations in the liver, kidney and blood were affected by dietary protein deficiency in rats, but not in mice (Table 2). We have demonstrated that dietary protein deficiency affects the metabolism of thiol compounds and/or the transport activity of the neutral amino acids, and these changes would be the reasons for the differences in the fate of MeHg.[14,16,19,20] In addition, we previously reported that sex hormones controlled the turnover rates of GSH in the liver and kidney, and this would cause the marked sex differences in the MeHg levels in these tissues and urine.[3] Thus, dietary protein deficiency might similarly influence the metabolism and the transport activity at least in both sexes of the specified species even if those are very different in intact animals. A support for this speculation might be that in both male and female rats, a lowered dietary protein level decreases the hepatic GSH concentration within a few hr, and the lower GSH levels are maintained for 6 weeks.[12] In contrast, Hg concentration in the testes was enhanced by dietary protein deficiency in mice but suppressed in rats, whereas that in the ovary was not affected in either species (Table 2). Therefore, Hg accumulation in the gonads would be more changeable in males than in females by dietary protein deficiency, although the influence is opposite in mice and rats. The present results suggest that, regardless of sex, dietary protein deficiency similarly influences the fate of MeHg except for the gonads, and that the decrease in urinary excretion of MeHg by dietary protein deficiency might be universal.

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