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Comparison of Mercury Accumulation Among the Brain, Liver, Kidney, and the Brain Regions of Rats Administered Methylmercury in Various Phases of Postnatal Development

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Several animal studies have indicated that a developing organism in its prenatal and early postnatal stage may be at higher risk in toxic metal exposure than in adult stage (Kostial et al. 1978; Jugo 1979; Kostial 1983). Many infants were congenitally affected by methylmercury in the epidemics in Japan and Iraq (WHO, 1990). The infants reported from Minamata, Japan, had severe cerebral palsy, whereas their mothers had mild or no manifestations of poisoning (Harada 1978). Some of the high susceptibility in infants may resulted from the specific features of the methylmercury metabolism in the developing organisms. Prenatal or postnatal development is characterized by functional immaturity of organs, which may affect the mercury (Hg) accumulation among organs. It seems possible that the Hg distribution might, in fact, reflect the toxic effects of methylmercury during a given developing phase. Thus, its distribution deserves closer examination. In our previous study, when a toxic level of methylmercury was administered, the Hg distribution and its effects on body weight gain and neurological disorders were found to be different among the rat postnatal developing phases (Sakamoto et al. 1993). In the present study, the Hg distribution among organs and brain regions was investigated during the several development phases with a nontoxic level of methylmercury treatment.

MATERIALS AND METHODS

Adult male and female Wistar rats supplied by CLEA Japan were mated, and females were maintained on a 12-hr light/12-hr dark cycle at 23° C with free access to rat chow and tap water. Within 24 hours of birth, a litter was randomly reduced to five male neonates, which were then maintained by a dam until weaning on postnatal day 30. After postnatal day 30, the rats were maintained with free access to rat chow and tap water. Three groups of

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rats (five rats for each group) on postnatal days 1 (PD-1), 14 (PD-14) and 35 (PD-35) were orally administered 1 mg/kg/day of methylmercury chloride (98% of purity, Merck) for 10 consecutive days, and Hg contents in the tissues were determined on the day after the final treatment. This administration level was employed as a nontoxic level, since the rats did not show the toxic signs of either methylmercury poisoning or weight loss even at 2.5 mg/kg/day dosage of methylmercury chloride for 10 consecutive days (Sakamoto et al. 1993). Methylmercury chloride (36.4 mg) and cysteine in a molecular ratio 1:1 were dissolved in 8.5 ml of distilled water and 1.5 ml of condensed milk, and then orally administered, using a microman-pipette (Gilson) for suckling (PD-1 and PD-14) rats and a stainless catheter for weaning (PD-35) rats.

Blood samples were taken from the heart under pentobarbital anesthesia prior to whole-body perfusion with physiological saline to remove remaining blood from tissues. The brain, liver ,and kidney were removed. Brain was separated from spinal cord at the decussation of pyramids, and separated into 4 regions (cerebral cortex + hippocampus, striatum + diencephalon, midbrain - oblongata, and cerebellum) according to Schubert and Sedvall (1972).

Each tissue was homogenized in 9 volumes (w/v) of ice-cold deionized water for 3 min. Total Hg concentrations (μg/g) in the brain regions, spinal cord, liver, kidney, and blood were determined by the oxygen combustion-gold amalgamation method of Jacobs et al. (1960) using a Mercury Analyzer MV 250R (Sugiyama-gen Environmental Science Co., Tokyo). The absolute limit of detection was 0.1 ng. The coefficient of variation was 1.1% for replicated samples containing 20 ng of Hg in 20 μl of homogenate. The Hg levels in tissue were expressed in μg/g (concentration) and in percentage (%) of total administered dose of Hg (amount of Hg in tissue × 100/amount of total Hg administered (mg)).

Statistical significance was tested by Student's t test and one-way analysis of variance (ANOVA). The level of significance was put at $p<0.01$.

RESULTS AND DISCUSSION

In order to evaluate the effects of methylmercury during the several postnatal development phases of rats, Hg distribution among the organs and brain regions during the phases was examined. Takeuchi (1982) suggested that methylmercury affected the nerve cells after considerable differentiation had occurred during the intermediate or later period of human fetal life. Rapid brain growth occurs primarily during the third

trimester in humans, whereas in rats it occurs after parturition (Dobbing and Sands 1979; West et al. 1984). Accordingly, we used early postnatal developing rats to evaluate the effects of methylmercury on the developing central nervous system in the human fetus (Sakamoto 1993). The distributions of Hg among organs and brain regions during the several development phases of rats with a nontoxic level of methylmercury treatment were examined in the present study.

Special care was exercised for the adequate whole-body perfusion in order to remove blood from organs, since the Hg concentration in blood is considerably higher than in other organs in the rats during the several days after the final dose of continuous methylmercury treatment (Magos et al. 1978, 1981). The Hg concentration in the brain of decapitated rats was about 40% higher than that of the perfused ones at 3, 6 or 9 days after the final dose (Magos et al. 1978).

The differences in the body weights, organ weights and organ-body weight ratios of rats administered methylmercury for 10 consecutive days (1 mg/kg/day) from postnatal days (PD) 1, 14, and 35 are shown in Table 1. Although the weights of body, kidney and liver in PD-1 rats ranged about 7 to 12% of those in PD-35 rats, the brain weight in PD-1 rats were about 50% of that in PD-35 rats. The brain/body weight ratios differed remarkably according to the postnatal phases. The brain/body weight ratios in PD-1 rats were about 2 and 5 times as high as those in PD-14 and PD-35 rats, respectively. On the other hand, kidney/body and liver/body weight ratios

Table 1. Body weights, organ weights and organ/body weight ratios of rats administered 1 mg/kg/day of methylmercury chloride for 10 consecutive days from postnatal days 1, 14, and 35. Rats sacrificed and tissues taken on day 11.

Tissue	Group	PD-1	PD-14	PD-35
Body weight (B.W.)	(g)	20.0 ± 1.4*	66.2 ± 3.8*	181.1 ± 9.2
Brain	Weight (g)	1.06 ± 0.03*	1.58 ± 0.04*	1.91 ± 0.04
	% of B.W.	5.31 ± 0.14*	2.40 ± 0.12*	1.05 ± 0.04
Kidney	Weight (g)	0.23 ± 0.01*	0.77 ± 0.05*	1.92 ± 0.07
	% of B.W.	1.18 ± 0.03*	1.16 ± 0.03*	1.06 ± 0.03
Liver	Weight (g)	0.65 ± 0.06*	2.79 ± 0.16*	8.73 ± 0.73
	% of B.W.	3.23 ± 0.13*	4.21 ± 0.01*	4.81 ± 0.16

Values are mean ± SD, n=5.
* Significantly different ($p<0.01$) from PD-35 rats.
PD=postnatal days

Table 2. Mercury concentrations (μg/g) and percentage of total mercury in the tissues of rats administered 1 mg/kg/day of methylmercury chloride for 10 consecutive days from postnatal days 1, 14, and 35. Rats sacrificed and tissues taken on day 11.

Tissue	Group	PD-1	PD-14	PD-35
Brain	Hg (μg/g)	1.58 ± 0.04*	2.34 ± 0.08*	1.42 ± 0.04
	Hg % dose	1.17 ± 0.04*	0.80 ± 0.05*	0.18 ± 0.01
Kidney	Hg (μg/g)	8.80 ± 0.29*	22.88 ± 1.77*	30.60 ± 2.60
	Hg % dose	1.39 ± 0.05*	3.79 ± 0.34	4.03 ± 0.36
Liver	Hg (μg/g)	3.49 ± 0.25*	7.67 ± 0.30*	4.04 ± 0.27
	Hg % dose	1.59 ± 0.09*	4.62 ± 0.17*	0.82 ± 0.04
Blood	Hg (μg/g)	16.3 ± 1.66*	25.4 ± 1.83	25.5 ± 1.52

Values are mean ± SD, n=5.
* Significantly different ($p<0.01$) from PD-35 rats.
PD=postnatal days

were comparatively constant among the phases. Table 2 gives the mercury concentrations (μg/g) and % of total mercury administered (total dose, 1 mg/kg/day of methylmercury chloride for 10 consecutive days from postnatal days 1, 14, and 35) in tissues taken on day 11. The Hg distribution was comparatively uniform among the organs in PD-1 rats, and the difference in the Hg distribution became very evident with development of the postnatal phase. The Hg concentration in the brain was highest in PD-14 rats, followed by PD-1 and PD-35 rats. The % of total Hg administered in the brain was highest in PD-1 rats and decreased with the postnatal phase. The Hg concentration and % of total Hg administered in the kidney increased markedly with the postnatal phase. The Hg concentration and % of total Hg administered in the liver were highest in PD-14 rats. The blood Hg concentrations in PD-14 or PD-35 rats were higher than in PD-1 rats.

The % of total Hg administered in the organs should reflect the differences in the organ/whole body weight ratios among the development phases. The % of the total Hg administered in the brain was highest in PD-1 rats and decreased with development, reflecting the decrease in the brain/whole body weight ratio with postnatal development. This indicates that the earlier the postnatal phase, the higher the % of total Hg administered in the brain. The change in the % of total Hg administered in the organs during the development period is worth noting in the experiment using the developing animals. However, Hg concentration should be more important than that of the % of total Hg administered to evaluate the susceptibility of the target tissue to the toxic effects of methylmercury.

The Hg distribution among the organs was comparatively uniform in PD-1 rats, and the difference in Hg concentrations among the organs became very evident with the development of the postnatal phase. The early postnatal period is characterized by functional immaturity of organs, which could be the main reason for the uniform Hg distribution among the organs in PD-1 rats. From the practical point of view, the earlier the postnatal phase was, the higher the brain/kidney or brain/liver ratios of Hg concentration. The brain, therefore must be the predominant organ receiving the toxic effect of methylmercury in the postnatal development phases.

The kidney Hg concentration increased markedly with the postnatal phases, a phenomenon possibly resulting from the functional maturation of the kidney during infancy. The glomerular filtration rate is known to be very low in immature kidneys (Spitzer 1985). The slow blood flow into the renal tissue may lead to less accumulation of methylmercury in the kidney of neonatal rats. Renal γ-glutamyl transpeptidase plays an important role in renal uptake of methylmercury (Berndt and Baggett 1985). Therefore, the low activity of this enzyme in the neonatal period (Tanaka et al. 1992) may be another reason for the low Hg accumulation in the kidney during that period. Inouye et al. (1986) reported that Hg accumulation in the kidney of the mouse fetus was considerably lower than with the dam.

The other characteristic of Hg accumulation during development was that its concentration in the brain and liver was highest in PD-14 rats. Shi et al. (1990) reported that newborn mice had a unique hair growth cycle, and the pelt during the active hair growth stage constituted the large tissue compartment of methylmercury distribution. They also mentioned that at 20 days of age, when most hair had ceased growing, methylmercury was not taken up by the hair, and the amount of methylmercury in the pelt was at the lowest level. Chase et al. (1951) documented that the hair growth activity of mice was high during postnatal days 5 to 14 and days 27 to 37 and ceased around postnatal days 15 to 25. PD-14 rats were administered methylmercury from postnatal days 14 to 23, and this period corresponded to the cessation of hair growth. Shi et al. (1990) have mentioned that methylmercury has a very high affinity for reduced sulfhydryl (SH) groups and proliferating hair follicle cells produce high-sulfur proteins which may lead to the high Hg concentration in the hair. Therefore, the growth cycle during the postnatal developing phases may affect the amount of mercury available to the rest of the body and raise the mercury concentrations in organs such as liver and brain in PD-14 rats. In our previous study (Sakamoto et al. 1993), with a toxic level of methylmercury treatment (10 mg/kg/day of methylmercury chloride for 10 consecutive days) the neurological disorders and deficit of motor coordination were far more

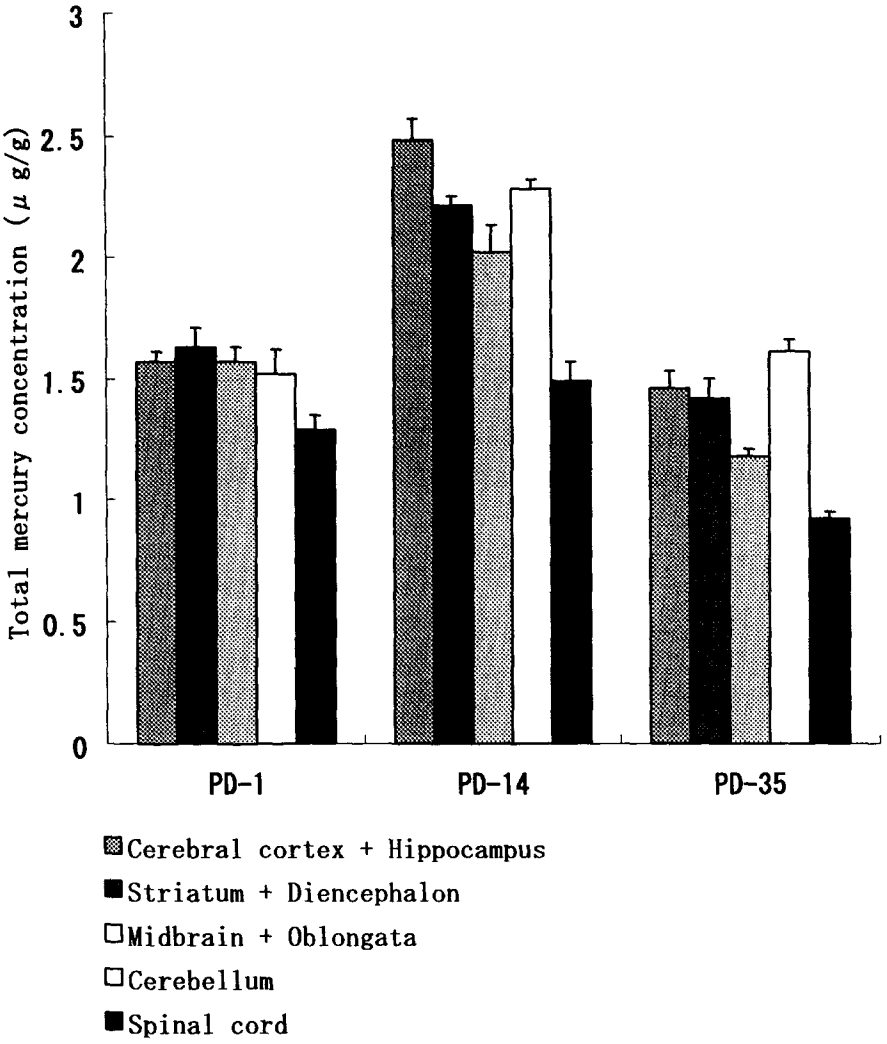

Figure 1. Mercury distribution in the different brain regions and spinal cord of rats treated with 1 mg/kg/day of methylmercury chloride for 10 consecutive days from postnatal days 1, 14, and 35. Animals were sacrificed on day 11. Values are mean + SD, n=5, PD=postnatal days.

severe in PD-14 rats than in PD-1 and 35 rats. The brain Hg concentration was highest in PD-14 rats, which might partly explain their high sensitivity. Therefore, the uptake of methylmercury by the growing hair should be also considered as a factor which affect the toxicity of methylmercury among the developing phases in rats or mice.

Figure 1 shows the Hg concentration in the different brain regions and spinal cord of PD-1, PD-14 and PD-35 rats on the day after the final

treatment with 1 mg/kg/day of methylmercury chloride for 10 consecutive days. The Hg accumulation in the early postnatal phase (PD-1) was comparatively uniform not only among the organs, but also among the brain regions. No significant difference in Hg concentration was found among the brain regions (cerebral cortex + hippocampus, striatum + diencephalon, midbrain - oblongata, and cerebellum) in PD-1 rats (F value=1.12; p=0.395). There were significant differences in Hg levels among the brain regions in PD-14 and -35 rats (F value=18.21; $p<0.01$ and F value=87.05; $p<0.01$, respectively). The highest Hg concentrations were observed in the cerebral cortex + hippocampus for PD-14 rats and in the cerebellum for PD-35 rats. On the other hand, the Hg concentration in the spinal cord was lower than that in each brain region in every development stage of rats ($p<0.01$). Also, the difference in Hg concentrations between the spinal cord and brain regions in PD-1 rats was less than in PD-14 and -35 rats.

However, little is known about the mechanism of the methylmercury distribution in the brain regions. A possible explanation of the difference may be in the varying amounts of proteins and other molecules containing SH group in the different brain regions. Because, methylmercury has a high affinity for the SH compounds (Carty and Malone, 1979). The striking difference in Hg concentration between the spinal cord and each brain regions may help our understanding about the mechanism of the methylmercury distribution. Nevertheless, additional studies are needed to reveal the mechanism(s) of the difference in methylmercury distribution among the brain regions.

From comparison of adult versus fetal cases of methylmercury poisoning, Takeuchi (1968) indicated that the distribution of damage was generalized in the brain of fetal cases, in contrast to that of adult cases whose focal lesions were predominant in the cerebellum and occipital cortex. The generalized damage in the brain in the case of congenital Minamata disease may be partly explained by the uniform Hg distribution among the brain regions in the early developing period. The regional difference in Hg accumulation became evident with development, and the highest Hg concentration was obtained in cerebellum in PD-35 rats. Several investigators also indicated that the Hg concentration in the cerebellum was highest among brain regions in adult rats (Magos et al., 1982; Arito 1982; Miyakawa 1973). The difference in Hg concentration among the brain regions appear to play a part in determining the selectivity of its effect in the case of adult Minamata disease, but can not be the only cause of variation in the sensitivity of the regions.

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