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Selective Quantification of Inorganic Mercury in Tissues of Methylmercury–Treated Rats

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It is well documented that methylmercury (MeHg) is gradually converted to inorganic Hg (Hg-i) in various animal tissues (Norseth and Clarkson 1970a; Mehra and Choi 1981). Hg-i, thus accumulated in the tissues, sometimes causes adverse effects that are not observed with MeHg itself. For example, renal damage observed in MeHg-treated animals is believed to be due to Hg-i biotransformed from MeHg (Fowler 1972). It is necessary to separate the effects of these two mercurial species (organic and inorganic) in order to understand the toxic effects in MeHg-treated animals. Since most tissue Hg usually exists in organic form after MeHg administration, especially in the case of short-term animal experiments, an accurate and selective determination of the Hg-i levels in tissue is necessary to know the exact level of this mercurial species.

Although there are several methods reported for selective determination of Hg-i in tissue samples in the presence of MeHg (Norseth and Clarkson 1970b; Magos 1971; Magos and Clarkson 1972; Yamamoto et al. 1980; Konishi and Takahashi 1983), their use is often inconvenient because of time-consuming measurements, considerable blank values, or requirement for radioisotopes or specialized apparatus. This paper describes a convenient method for selective Hg-i determination based on a simple principle using a conventional Hg analyzer.

MATERIALS AND METHODS

MeHg chloride and $HgCl_2$ were obtained from Tokyo Chemical Co. (Tokyo) and Wako Chemical Co. (Osaka), respectively, and used without further purification. Hydrochloric acid, NaOH and benzene were analytical grade.

Hg standard samples containing Hg-i at 0, 2.5, 5, 10, 20 and 100 % of total Hg were prepared by adding $HgCl_2$ and MeHg chloride to 10 % (wt/wt) homogenate of liver (background Hg content was less than 4 ng/mL in the homogenate) of a female Wistar rat (aged 9 wk) to a final total Hg concentration of 5 μg Hg/mL and used for Hg analysis. In the separate experiment, tissue samples were obtained from female Wistar rats (aged 9

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wk) which had been sacrificed 1 and 7 d after oral administration of MeHg chloride (4 mg Hg/kg body weight). After perfusion with saline, brain, liver and kidney samples were excised and homogenated in water (1:9 tissue:water; 10 %, wt/wt) for Hg analysis.

Preparation of samples for Hg-i analysis was performed as follows. We used 0.2 mL of 6 N HCl to acidify 0.5 mL of tissue homogenate in a micro tube (1.5 mL). The acidified homogenate was shaken vigorously with about 0.6 mL of benzene in a micro tube mixer for 3 min. The mixture was centrifuged at 12,000 rpm for 3 min. The benzene was removed using an aspirator; an insoluble layer at the interface was left undisturbed. The benzene extraction was repeated a total of 6 times to remove the MeHg completely. A wash with petroleum ether was done once to remove the benzene. A stream of air was used to remove the residual petroleum ether. MeHg in the 5th benzene wash was undetectable by ECD gas chromatography (less than 2 ng Hg/mL). A portion (0.5 mL) of the aqueous phase-suspension was neutralized with the same volume of 1.71 N NaOH and used for Hg analysis.

Total Hg and Hg-i levels were determined by the oxygen combustion-gold amalgamation method (Jacobs et al. 1960) using a Rigaku Mercury Analyzer SP-3. MeHg levels were determined as described previously (Hirayama et al. 1987). Hg-i was also determined by the method of Konishi and Takahashi (1983).

RESULTS AND DISCUSSION

The present method is based on quite a simple principle. Addition of excess HCl changed coexisting MeHg into its chloride which could be extracted easily from water into benzene. Under the described experimental conditions, about 80 % of MeHg was extracted by a single benzene partitioning (data not shown). Accordingly, the amount of MeHg remaining after 6 extractions was calculated to be as low as 0.0064 % of the initial amount. When chloroform, which is also effective in extracting MeHg, was used in the place of benzene, the recovery of Hg-i was as low as about 90 % of the initial amount (data not shown). In preliminary experiments, various concentrations of HCl (1 to 3 N) and homogenate ratios (0.1 to 10 %) were examined. The extractive removal of MeHg with benzene and retention of Hg-i in the samples were found to be satisfactory within these ranges. 1.71 N HCl, which was obtained by the addition of 6 N HCl to the samples, and 10 % homogenate were employed here.

Hg-i levels obtained from the standard samples by using the present MeHg-removal method are summarized in Table 1; the values obtained using the method of Konishi and Takahashi (1983) are also reported. The values obtained here corresponded well to the calculated ones throughout the range of Hg-i/MeHg ratios. Recovery of Hg-i and removal of added MeHg were almost complete in the present procedure; it was concluded that demethylation of MeHg and loss of Hg-i during preparation of the samples were negligible. Although the values obtained by both methods were consistent with each other when Hg-i contents were higher than 10 % of total Hg, some overestimation of the values was observed by the Konishi-Takahashi' method in the samples containing lower Hg-i probably due to

Table 1. Inorganic mercury levels (μg/mL) obtained from the standard samples [a]

Analytical method	Hg-i content (%)						
	0	2.5	5	10	20	40	100
Experimental value							
Present method (SD)	0.0022 (0.0008)	0.127 (0.030)	0.254 (0.030)	0.503 (0.008)	1.03 (0.02)	1.98 (0.03)	4.94 (0.18)
Konishi-Takahashi' method (SD)	0.058 (0.003)	0.158 (0.030)	0.263 (0.040)	0.489 (0.015)	1.00 (0.02)	2.03 (0.04)	4.98 (0.12)
Calculated value	0	0.125	0.25	0.5	1	2	5

[a] Each sample was prepared by adding MeHg chloride and/or $HgCl_2$ to a 10 % homogenate of rat liver; total Hg concentration was 5 μg/mL. Mean values and SD were obtained from four experiments.

Table 2. Inorganic, methyl and total mercury levels in brain, liver, and kidney of methylmercury-treated rats sacrificed 1 and 7 d after the administration [a]

Tissue	Hg-i (A)	MeHg (B)	Total Hg (C)	% of Hg-i	(A + B)/C
1 d					
Brain	1.5 ± 0.3	158 ± 9	163 ± 7	0.94 ± 0.16	0.98
Liver	0.22 ± 0.02	4.37 ± 0.31	4.55 ± 0.37	4.86 ± 0.59	1.01
Kidney	2.10 ± 0.21	16.9 ± 1.4	19.1 ± 1.4	11.0 ± 1.2	0.99
7 d					
Brain	5.6 ± 1.1	238 ± 9	245 ± 10	2.29 ± 0.52	0.99
Liver	0.42 ± 0.04	2.51 ± 0.16	2.93 ± 0.17	14.5 ± 1.4	1.00
Kidney	13.1 ± 0.8	13.8 ± 1.1	26.9 ± 1.6	48.8 ± 2.3	1.00

[a] One and 7 days after oral administration of MeHg chloride (4 mg Hg/kg body weight), rats were sacrificed and the tissues were excised. Hg values were expressed as ng Hg for the brain and μg Hg for liver and kidney per g wet tissue. Values represent mean ± SD obtained from 5 animals.

demethylation of MeHg during the operation. The overestimation of Hg-i in the presence of MeHg in large excess was also reported by the method of Magos (1971). Furthermore, since the blank value of the reagents in the Konishi-Takahashi' method reached nearly 7 ng of Hg (reported value of about 3 ng), evaluation of lower values would be liable to be accompanied by the experimental error. The blank value for the reagents was also reported to be 2.5 to 3.0 ng by the method of Magos and Clarkson (1972). The blank value in the present method was much lower (less than 0.2 ng Hg/mL of the final mixture); thus the data for lower levels should be more reliable.

Inorganic, organic and total Hg levels in brain, liver and kidney of rats sacrificed 1 and 7 d after MeHg administration are summarized in Table 2. The sum of Hg-i and MeHg values fitted well with the total Hg values in every tissue sample at both experimental times. The highest ratio of Hg-i was shown in the kidney of 7 d; 48.8% of the renal Hg was accounted for by inorganic species. On the other hand, the brain of 1 d showed the lowest Hg-i ratio of 0.94%. Although the present method does not contain any novel reagent or procedure, it yielded satisfactory Hg-i values in tissue samples even if its ratio was lower than 1% of total Hg using a conventional Hg analyzer.

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