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Effect of Reticuloendothelial System Blockade on the Biotransformation of Methyl Mercury in the Rat

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It has been reported that methyl mercury (MeHg) administered to animals is biotransformed to inorganic mercury (Norseth and Clarkson 1970; Suda and Takahashi 1986). Several studies have presented that there are two degradation process, by intestinal microflora (Rowland 1980; 1984) and by animal tissues themselves (Norseth 1971; Takahashi and Suda 1986). We became interested in the sites and mechanism of the latter process. In a previous paper (Suda and Takahashi 1986), we reported the biotransformation of MeHg in the rat was enhanced by phenylhydrazine administration, and inhibited by splenectomy or treatment with carrageenan (CAR). On the basis of these informations, we suggested that spleen and liver might be the important sites for formation of inorganic mercury, and that reticuloendothelial system (RES) cells in these organs might play a major role in this biotransformation.

The major function of the RES is the clearance from the circulation of a wide variety of materials such as effete red blood cells, denatured proteins and other intruded foreign substances (Brouwer and Knook 1983). The clearance activity of RES cells, mainly located in the liver and spleen, can be depressed by saturating those cells with CAR, colloidal carbon (CC), trypan blue (TB), colloidal iron (CFe), dextran sulfate, silica, etc. (Fisher 1966; Souhami and Bradfield 1974; Yoshikai et al. 1979; Brouwer and Knook 1983). Purpose of this study is to confirm the relationship between RES function and biotransformation of MeHg by using four representative blockers, CC, TB, CFe and CAR. The inhibited biotransformation of MeHg in RES-blocker-treated rats was evaluated by measuring the amount of total and inorganic mercury in tissues. On the other hand, RES cell activity was measured by carbon clearance tests.

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Abbreviations:
MeHg, methyl mercury; CAR, carrageenan;
CC, colloidal carbon; TB, trypan blue;
CFe, colloidal iron; RES, reticuloendothelial system.

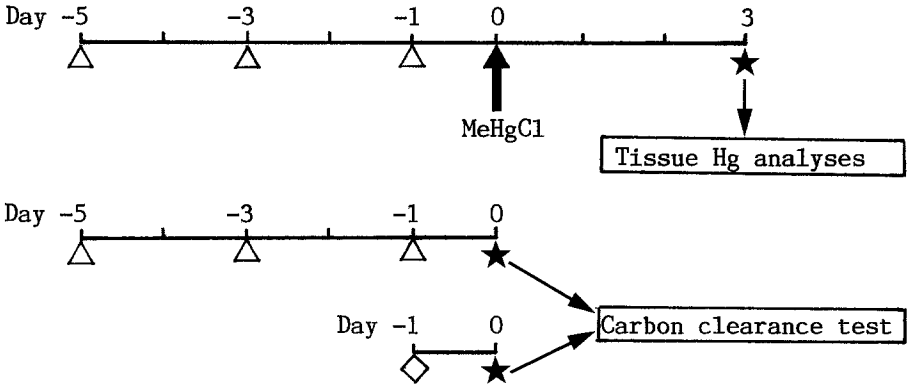

Figure 1. Flow diagram of experimental design. Details of treatment are given in the text. Symbols: (△), administration of colloidal carbon (CC), trypan blue (TB) or colloidal iron (CFe); (◇), administration of carrageenan (CAR); (★), sacrifice.

MATERIALS AND METHODS

Three groups of male Wistar rats were used in the studies on MeHg biotransformation. Figure 1 shows the flow diagram of experimental design illustrating the schedule of the drug administration and the day of sacrifice. The range of body weights at the time of MeHgCl administration was 205-210 g for CC-treated rats, 190-195 g for TB-treated rats and 195-200 g for CFe-treated rats. All rats were injected intravenously (iv) via the lateral tail vein with 1 mg Hg/rat as MeHgCl (Merck, Darmstadt, West Germany) on Day 0. To produce RES depression, each RES-blocker was given iv on Day -5, Day -3 and Day -1. As CC, Pelican Fount India Ink 518 (Günther Wagner, Hanover, West Germany) was used in this study. After the ink was dialyzed overnight against saline in visking cellophane tube, the carbon concentration was determined spectrophotometrically at 660 nm by comparing its absorbance with that of a Pelican C11/1431a (Günther Wagner, Hanover, West Germany) solution kindly provided by Dr. K. Matsuno (Kumamoto University Medical School, Japan). CC was suspended in 0.9% saline solution at a concentration of 32 mg/ml and administered at a dose of 80 mg/kg or 160 mg/kg. TB (Chroma Gesellschaft Schmid & Co., Stuttgart-Untertürkheim, West Germany) was dissolved in saline (2 mg/ml) and administered at a dose of 5 mg/kg or 10 mg/kg. CFe (Chondroitin ferrous sulfate, Blutal, 4 mg iron/ml, Daiichiseiyaku Co., Tokyo, Japan) was injected at a dose of 10 mg/kg or 20 mg/kg. On Day 3, all rats were sacrificed under deep ether anesthesia. After taking blood sample from the heart, a sufficient volume of saline solution was perfused through the heart to wash out the blood remaining in the body. The excised organs were stored at -20°C until they were individually homogenized in deionized water, and subjected to the mercury analyses.

Total mercury was measured by flameless atomic-absorption spectrometry in combination with gold amalgamation after acid

hydrolysis as previously reported (Suda and Takahashi 1986). Determination of inorganic mercury was done according to the method of Konishi and Takahashi (1983), except that the potassium cyanide concentration was increased to 1%. Excess potassium cyanide is needed to convert sugar aldehyde, which leads to organic mercury decomposition during alkali digestion, to cyanohydrin. This direct determination method used for inorganic mercury can detect >1 ng of inorganic mercury in the sample without decomposing organic mercury in the tissue.

To know the effects of above treatments on RES activity, colloidal carbon clearance was studied in the rats treated at the highest doses of CC, TB, CFe and CAR (Fig. 1). Male Wistar rats weighing 180-200 g were divided into five groups; saline control group, CC-treated group, TB-treated group, CFe-treated group and CAR-treated group, respectively. Each group consisted of six rats. CC, TB or CFe was given iv on Day -5, Day -3 and Day -1 as described above. CAR (type IV, Sigma Chemical Co., St. Louis, Mo.) was dissolved in saline (10 mg/ml) and given intraperitoneally (ip) at a dose of 200 mg/kg as a single injection on Day -1. Phagocytic capacities of RES were determined on Day 0 with a slight modification of the carbon clearance test of Biozzi et al. (1953). Under light ether anesthesia, all rats were injected with 80 mg/kg of CC through the tail vein. Blood samples of 0.03 ml were obtained at 3 min intervals for 15 min using heparinized capillary tubes by cutting the tip of the tail. These blood samples were hemolyzed in 3 ml of 0.1% Na_2CO_3, and the carbon concentration was determined by a spectrophotometer from the absorbance at 660 nm. The global phagocytic index K and the corrected phagocytic index α were calculated from the following equations:

$$K=(\log C_1-\log C_2)/(t_2-t_1) \qquad \alpha=K \times W/wls$$

C_1: carbon concentration at time t_1
C_2: carbon concentration at time t_2
W: animal body weight
wls: fresh weight of liver and spleen

All data are reported as mean ± SD (standard deviation). The statistical significance was evaluated using Student's t test, and $p<0.05$ was taken as significant.

RESULTS AND DISCUSSION

The inhibited biotransformation of MeHg in RES-blocker-treated rats was evaluated by using two important parameters. The first was the amount of inorganic mercury trapped in kidneys, probably because in this organ metallothionein was correlatively induced along with the inorganic mercury generated in the whole body. The amount of MeHg accumulating within the brain appeared to be another valuable parameter, because this value reflected the MeHg remaining in the body. As shown in Table 1, decreases in both the inorganic portion of total mercury and the absolute amount of inorganic mercury were observed in the kidneys of 160 mg/kg x 3 CC group, 5 or 10 mg/kg x 3 TB group, and 10 or 20 mg/kg x 3 CFe group. The ratios of renal inorganic mercury concentration

Table 1. Effect of RES-blockers on the biotransformation of MeHg in rats.

Treatment			Kidney			Brain
RES-blocker	Doses x times	n	Total Hg (μg/g)	Inorganic Hg (μg/g)	Inorganic Hg (%)	Total Hg (μg/g)
Colloidal carbon	0 mg/kg x 3	8	12.28 ± 0.99	1.85 ± 0.13	15.1 ± 1.0	1.39 ± 0.06
	80 x 3	8	10.74 ± 0.74**	1.53 ± 0.17**	14.3 ± 1.6	1.46 ± 0.07*
	160 x 3	8	13.20 ± 0.62	1.61 ± 0.11**	12.3 ± 1.0***	1.49 ± 0.07*
Trypan blue	0 mg/kg x 3	8	16.51 ± 1.23	3.01 ± 0.33	17.7 ± 1.7	1.43 ± 0.08
	5 x 3	8	16.78 ± 1.61	1.91 ± 0.32***	11.6 ± 2.2***	1.50 ± 0.08
	10 x 3	8	16.43 ± 1.04	2.17 ± 0.16***	13.3 ± 1.6***	1.53 ± 0.09*
Colloidal iron	0 mg/kg x 3	8	15.28 ± 0.85	2.76 ± 0.19	18.1 ± 1.4	1.35 ± 0.13
	10 x 3	8	16.87 ± 1.14**	2.14 ± 0.28***	12.8 ± 2.0***	1.61 ± 0.10***
	20 x 3	8	18.55 ± 1.08***	1.83 ± 0.51***	9.9 ± 2.7***	1.58 ± 0.12**

Colloidal carbon, trypan blue or colloidal iron was given iv on Day -5, Day -3 and Day -1. After MeHgCl (1 mg Hg/rat) iv injection on Day 0, all the rats were killed on Day 3. Each value represents the mean ± SD. Asterisks indicate significant differences from control rats. (*), $p<0.05$; (**), $p<0.01$; (***), $p<0.001$.

(μg/g) of RES-blocker-treated rat groups to those of the corresponding control groups were 0.87 ± 0.06 in 160 mg/kg x 3 CC group, 0.72 ± 0.05 in 10 mg/kg x 3 TB group, 0.66 ± 0.19 in 20 mg/kg x 3 CFe group, and 0.60 ± 0.09 in 200 mg/kg x 1 CAR group. Here, the result obtained from the rats treated with CAR, one of the best RES-blockers, was calculated from our previous report (Suda and Takahashi 1986). The total amount of mercury in brain, most of which was found as MeHg (>98%), was greater in three RES-blocker-treated rat groups than in the corresponding control groups (Table 1). When MeHg concentrations were calculated by subtracting the absolute amount of inorganic mercury from the total amount of mercury, the ratios of MeHg contents (μg/g) in brain of RES-blocker-treated rat groups to those of the corresponding control groups were 1.07 ± 0.05 in 160 mg/kg x 3 CC group, 1.07 ± 0.06 in 10 mg/kg x 3 TB group, 1.17 ± 0.09 in 20 mg/kg x 3 CFe group, and 1.72 ± 0.15 in 200 mg/kg x 1 CAR group. Thus, judging from the decreased amount of inorganic mercury trapped in kidneys and the increased amount of MeHg which accumulated within the brain, pretreatment with three agents (CC, TB and CFe) resulted in the decreased metabolism of MeHg, though the inhibitory effects of three were weaker than that of CAR.

The K indices were decreased significantly in 10 mg/kg x 3 TB group, 20 mg/kg x 3 CFe group, and 200 mg/kg x 1 CAR group (Table 2). These results indicate the suppression of phagocytic activity in these rats. In addition, index α, which measures phagocytic activity per unit weight of liver and spleen, also decreased significantly in 160 mg/kg x 3 CC group, 10 mg/kg x 3 TB group, 20 mg/kg x 3 CFe group, or 200 mg/kg x 1 CAR group. Judging from these two phagocytic indices, the RES function appeared to be blocked more severely in the following order; 160 mg/kg x 3 CC group < 10 mg/kg x 3 TB group, 20 mg/kg x 3 CFe group < 200 mg/kg x 1 CAR group. This order correlated with the inhibiting effects of agents on the biotransformation of MeHg. This good correlation between clearance and biotransformation indicated that the phagocytic activity of RES cells might be

Table 2. The carbon clearance rates in rats treated with colloidal carbon, trypan blue, colloidal iron and carrageenan

Treatment		Phagocytic indices	
RES-blocker	Doses x times	K index	α index
Control	-	0.0340 ± 0.0031	6.52 ± 0.39
Colloidal carbon	160 mg/kg x 3	0.0308 ± 0.0034	6.22 ± 0.39*
Trypan blue	10 mg/kg x 3	0.0229 ± 0.0065**	6.14 ± 0.52*
Colloidal iron	20 mg/kg x 3	0.0266 ± 0.0045**	6.17 ± 0.49*
Carrageenan	200 mg/kg x 1	0.0106 ± 0.0018***	4.33 ± 0.31***

Colloidal carbon, trypan blue, or colloidal iron was given iv on Day -5, Day -3, and Day -1. Carrageenan was given ip as a single injection on Day -1. Phagocytic indices were measured on Day 0 by carbon clearance in different groups consisting of six rats. Each value represents the mean ± SD. Asterisks indicate significant differences from control rats. (*), $p<0.05$; (**), $p<0.01$; (***), $p<0.001$.

involved in in vivo metabolism of MeHg.

RES cells are found throughout the body, and the liver, spleen and lymph nodes are known to be three major sites (Brouwer and Knook 1983). Hirokawa and Hayashi (1980) reported that mercury granules were detected histochemically in macrophages of the thymus, spleen, lymph nodes, lung, liver, kidney and bone marrow after MeHg administration. Mercury granules detected by their technique might be inorganic mercury, but not MeHg, since recent reports (Rodier and Kates 1988; Suda et al. 1989) have indicated that similar histochemical methods revealed only inorganic mercury. These facts may support a speculation that RES cells might have the ability to metabolize MeHg to inorganic mercury.

Until now, intestinal microflora has been considered as a major potential site of demethylation of MeHg in animal body rather than animal tissues themselves (Rowland et al. 1984). However, our study demonstrated that CAR, CFe, TB or CC treatment could cause an inhibited biotransformation of MeHg in rats, with a concomitant RES blockade. These considerable evidences indicated a meaningful role of RES cells in the biotransformation of MeHg by animal tissues themselves, and suggested that the degree of relevance of the RES pathway in mammalian MeHg metabolism was not so small. Studies of the ability of phagocytic cells to metabolize alkyl mercury to inorganic mercury in vitro (Takahashi et al. 1988; and unpublished paper) will further elucidate the role of RES in the biotransformation of MeHg.

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