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Phagocytic Cells as a Contributor to *In Vivo* Degradation of Alkyl Mercury

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A series of our studies clearly indicated that methyl mercury (MeHg) and ethyl Hg (EtHg) were dealkylated by reactive oxygen-producing systems (Suda et al. 1991,1992; Suda and Hirayama 1992; Suda and Takahashi 1992). Phagocytic cells are known to have reactive oxygen-producing systems. In vitro, several phagocytic cells, such as polymorphonuclear leukocytes (PMN), macrophages, mononuclear leukocytes (MNL) and eosinophils, could readily degrade EtHg (Suda et al. 1992). PMN having high EtHg degradation ability could also degrade MeHg to some extent (Suda et al. 1992). Therefore, it is considered that phagocytic degradation is one of alkyl Hg-degradation process in animal body. To elucidate the contribution of phagocytic cells to alkyl Hg degradation in vivo, incorporation of substrate (MeHg or EtHg) into phagocytic cells in vivo and intracellular degradation of them should be proved.

With regard to Hg distribution in blood after administration of MeHg, major attention has been paid to date to the distribution of Hg between erythrocytes and plasma (Norseth and Clarkson 1970; Clausing et al. 1984; Yasutake and Hirayama 1986). Yamada et al. (1978) studied incorporation of Hg into erythrocytes, plasma and leukocytes of rats poisoned with MeHg. However, there was no informations about organic and inorganic Hg distribution in these cells and fractionated leukocytes after alkyl Hg administration.

The present paper reports distribution of organic and inorganic Hg in erythrocytes, PMN, MNL and plasma of MeHg- or EtHg-treated rats. Furthermore, we studied the degradation ability of peritoneal PMN incorporated MeHg or EtHg in vitro.

MATERIALS AND METHODS

Male Wistar rats (200 ± 10 g) were used in this study. MeHgCl (Merck, Darmstadt, Federal Republic of Germany) or EtHgCl (Tokyo Kasei, Tokyo, Japan) was dissolved in 0.9% saline (pH 7.0) containing cysteine at a concentration of 1 mg Hg/ml (molar ratio of

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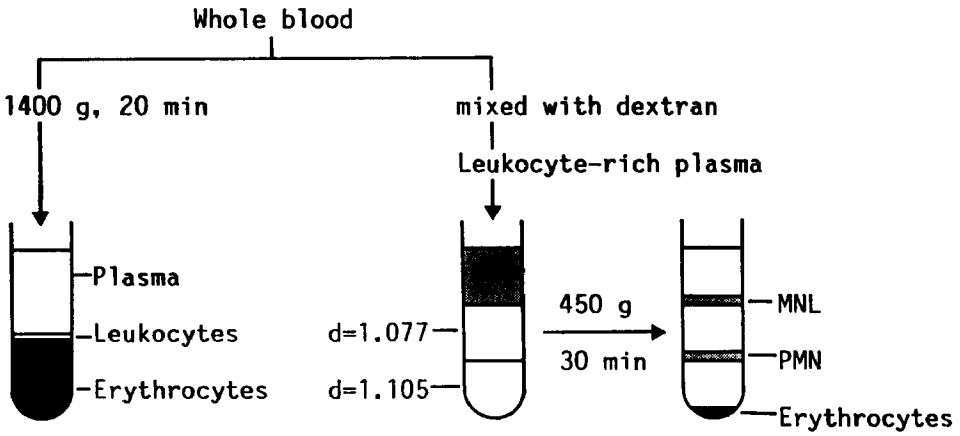

Figure 1. Separation of plasma, erythrocytes, PMN and MNL from rat whole blood. Details of procedure are given in the text.

cysteine/alkyl Hg=2.5), and administered orally at a dose of 10 mg Hg/kg, unless otherwise stated. Blood was collected in heparin-coated syringes from the heart of six rats 24 h after oral administration of alkyl Hg. The erythrocyte count [with 0.01 M phosphate-buffered 0.15 M saline, pH 7.4 (PBS)], total leukocyte count (with Türk solution), leukocyte differential (with Giemsa strain) and hematocrit (with a micromethod) were determined.

Plasma, erythrocytes, PMN and MNL were separated from whole blood as shown in Fig. 1. An aliquot of the whole blood was centrifuged at 1400 g for 20 min at 4°C. After the separation of plasma, leukocytes were removed by washing four times with PBS and residue was collected as erythrocyte fraction. The remainder of whole blood was mixed with an equal volume of 6% dextran T-500 (Pharmacia Fine Chemicals, Uppsala, Sweden) in PBS and allowed to keep at room temperature for 30 min (Dallegri et al. 1983). Then, separation of PMN and MNL from leukocyte-rich plasma was performed by a modification of the Ficoll discontinuous centrifugation method as described by English and Andersen (1974). Light (d=1.077 g/ml) and heavy separating fluid (d=1.105 g/ml) were prepared by mixing a commercial lymphocyte separation medium (Boehringer Manheim GmbH, Federal Republic of Germany), Ficoll (Pharmacia Fine Chemical, Uppsala, Sweden) and sodium metrizoate (Daiichi Pure Chemical, Tokyo, Japan). Slight contamination with erythrocytes were removed by hypotonic lysis in 1 ml of 1% bovine serum albumin (Sigma Chemical, St Louis, MO, U.S.A.) for 30 sec at 4°C. This albumin addition was done to avoid redistribution of Hg released from the hemolyzed erythrocytes to the leukocytes. After restore of isotonicity, each fraction was washed three times with PBS and resuspended in the same medium. These preparations of whole blood, plasma, erythrocytes, PMN and MNL were subjected to Hg analysis.

For preparation of peritoneal PMN, 24 h after po (or iv) administration of MeHg or EtHg rats were injected ip with 20 ml of 0.2%

glycogen (Tokyo Kasei, Tokyo, Japan) in 0.9% saline, and after 5 h PMN were collected by peritoneal lavage. The PMN were washed with PBS, and subjected to Hg analysis. Alkyl Hg-degrading ability of these PMN samples was also studied as described previously (Suda et al. 1992).

Total Hg was measured by flameless atomic absorption spectrometry in combination with gold amalgamation after acid hydrolysis (Suda and Takahashi 1986). Inorganic Hg was determined by a modification (Suda et al. 1990, 1991) of the method as originally described by Konishi and Takahashi (1983). The amount of organic Hg was calculated by subtracting the amount of inorganic Hg from the amount of total Hg.

RESULTS AND DISCUSSION

We have recently reported that several phagocytic cells, such as PMN, macrophages, MNL and eosinophils, can degrade alkyl Hg, but erythrocytes can not do so (Suda et al. 1992). To elucidate the contribution of phagocytic cells to in vivo degradation of alkyl Hg, the incorporation of the substrate (MeHg and EtHg) into the cells should be proved first. PMN and MNL in blood contained a considerable amount of MeHg or EtHg, though its concentration was about 1/4 to 1/10 of that in erythrocytes (Table 1). When PMN were collected in peritoneal cavity by glycogen ip injection, the peritoneal PMN contained also MeHg or EtHg (Fig. 2). These results indicated that MeHg and EtHg were taken up into PMN and MNL, which was necessary to prove intracellular degradation of them.

Interestingly, an appreciable amount of inorganic Hg was found in PMN and MNL (Table 1). The ratios of inorganic Hg/total Hg in PMN and MNL were higher than that in erythrocytes. The inorganic Hg concentration per cells was highest in PMN. Inorganic Hg in these cells may be the inorganic Hg which is biotransformed by other sites and then transported (Suda and Takahashi 1986, 1990; Suda and Hirayama 1992). Alternatively, there is a possibility that phagocytic cells themselves intracellularly degrade alkyl Hg. To obtain an evidence to support the latter possibility, we studied alkyl Hg-degrading ability of PMN. PMN were used as a representative phagocytic cells, because PMN have a high degradation ability of MeHg and EtHg as compared to MNL (Suda et al. 1992), and it is easy to collect them in peritoneal cavity from blood at a condensed condition. Phorbol myristate acetate (PMA) was used as a PMN-activating agent to produce inorganic Hg from alkyl Hg (Suda et al. 1992). When the peritoneal PMN incorporated MeHg or EtHg were incubated in Eagle-MEM medium with or without PMA, inorganic Hg production increased time-dependently, especially in the PMA-stimulated cells (Fig. 3). This finding indicates that PMN (probably also MNL) can intracellularly degrade MeHg and EtHg.

Furthermore, present data showed that peritoneal PMN degraded EtHg much faster than MeHg (Fig. 3). This observation is in a contrast to the findings in the studies using MeHg- and EtHg-

Table 1. Distribution of Hg in blood of rats 24 h after oral administration of MeHg or EtHg [a]

	MeHg-treated rats		EtHg-treated rats	
	Organic Hg	Inorganic Hg	Organic Hg	Inorganic Hg
Blood cells (μg Hg/ml of blood)[b]	67.60	0.64 (0.9%)	67.36	1.09 (1.6%)
Plasma (μg Hg/ml of blood)[b]	0.29	0.03 (9.4%)	0.29	0.08 (21.6%)
Erythrocytes (ng Hg/10^7 cells)	158.49	0.71 (0.4%)	185.42	2.58 (1.4%)
MNL (ng Hg/10^7 cells)	39.58	0.72 (1.8%)	20.38	1.01 (4.7%)
PMN (ng Hg/10^7 cells)	36.41	2.40 (6.2%)	26.54	3.31 (11.1%)

[a] Blood was collected from the heart of six rats 24 h after oral administration of MeHg or EtHg (10 mg Hg/kg), and fractionated as shown in Fig. 1. Results are expressed as means of three experiments. Values in parentheses indicate inorganic Hg/total Hg (%). In the experiments, erythrocytes/MNL/PMN ratio in cell numbers was 1680/1/0.10 in MeHg-treated rats and 1635/1/0.09 in EtHg-treated rats, respectively.

[b] Hg concentration in plasma and blood cells was calculated from the following equations:

Plasma Hg (μg Hg/ml of blood) = Plasma fraction Hg (μg Hg/ml) x (100-Ht)/100

Blood cells Hg (μg Hg/ml of blood) = Whole blood Hg (μg Hg/ml of blood) - Plasma Hg (μg Hg/ml of blood)

where, Ht is hematocrit value (%).

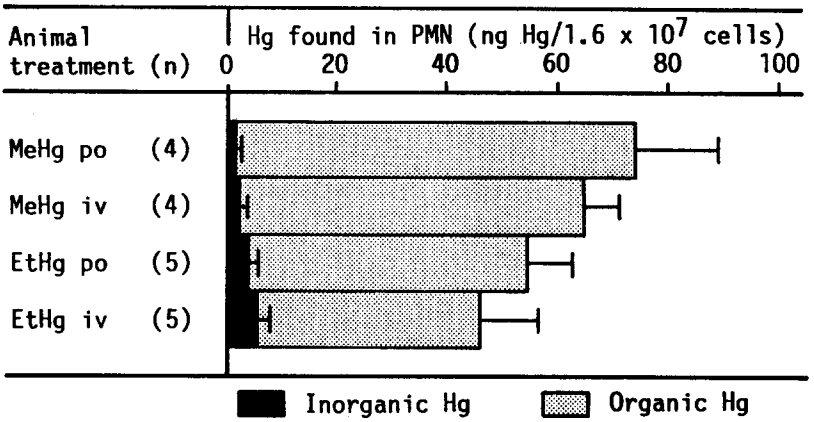

Figure 2. Organic and inorganic Hg found in peritoneal PMN collected from rats administered MeHg or EtHg. Glycogen-elicited rat peripheral PMN were collected 24 h after administration of MeHg or EtHg (10 mg Hg/kg, po or iv). The PMN were washed with PBS before Hg analyses. Results are expressed as means ± SD (standard deviation) of 4-5 experiments.

treated animals. Magos et al. (1985) reported that EtHg broke down faster than MeHg did in the rat body. We also confirmed the faster biotransformation of organic mercury in EtHg-treated rats as compared to MeHg-treated rats. Our data showed that the ratios of inorganic Hg/total Hg in kidneys (means ± SD) was 3.9 ± 1.2% (n=6) in MeHg-treated rats and 19.7 ± 4.2% (n=6) in EtHg-treated rats under the same conditions as shown in Table 1. Higher ratios of inorganic Hg/total Hg were also observed in erythrocytes, plasma, PMN and MNL collected from blood (Table 1), and peritoneal PMN (Fig. 2) in EtHg-treated rats. Such similar MeHg- and EtHg-degradation ability in PMN and rat body supported our hypothesis that PMN was one of contributors to biotransformation of alkyl Hg in animal body.

In conclusion, we found in the present study three evidences for the contribution of phagocytic cells to in vivo alkyl Hg degradation; firstly, PMN and MNL in blood incorporated a considerable amount of the substrate (MeHg or EtHg) essential for intracellular degradation; secondary, PMN could degrade their intracellular organic Hg to inorganic Hg; thirdly, PMN can readily degrade EtHg as compared to MeHg, and similar event was also found in the kidneys of MeHg- and EtHg-treated rats. We have already clearly demonstrated that rat liver microsomes can also degrade these mercurials in vitro (Suda and Hirayama, 1992). From these results, it is reasonable to conclude that phagocytic and microsomal degradation are alkyl Hg-degradation processes in the animal body.

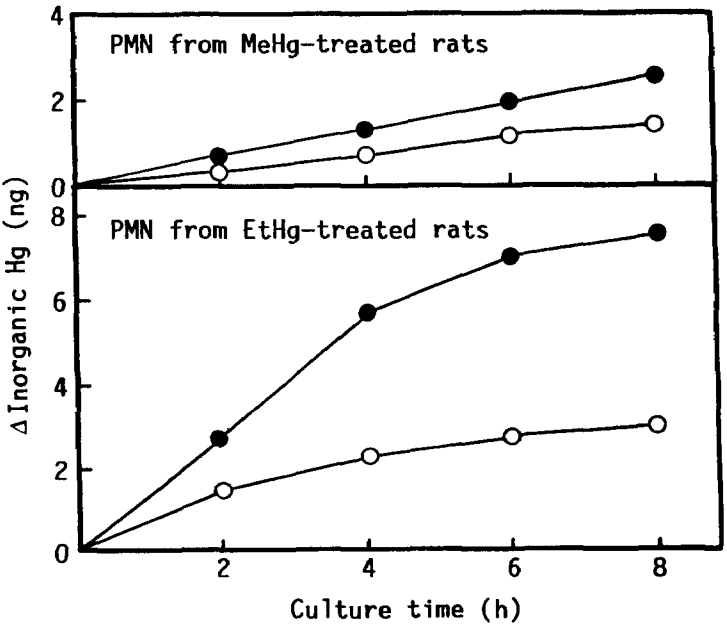

Figure 3. Inorganic Hg production from rat peripheral PMN incorporated MeHg and EtHg. Glycogen-elicited rat PMN were collected 24 h after po administration of MeHg or EtHg (10 mg Hg/kg). Washed cells (1.6 x 10^7 cells/dish) were cultured at 37°C under 95% air-5% CO_2 in 4 ml of Eagle-MEM medium with or without 10 ng/ml phorbol myristate acetate (PMA). The amount of inorganic Hg produced from PMN was calculated by subtracting the amounts of inorganic Hg found in the PMN before culture from that after culture. In this single representative experiment, Hg concentration found in PMN (ng Hg/1.6 x 10^7 cells) before culture was as follows; PMN collected from MeHg-treated rats (organic Hg=68.6, inorganic Hg=1.4), PMN collected from EtHg-treated PMN (organic Hg=40.7, inorganic Hg=3.0). Open circles, control cells; solid circles, PMA-stimulated cells.

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