

Effects of Reactive Oxygen Modulators on *in vivo* Demethylation of Methylmercury

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(Received November 30, 1998; Accepted December 25, 1998)

To elucidate the involvement of reactive oxygen in *in vivo* demethylation of methylmercury (MeHg), the effects of paraquat (PQ) and other reactive oxygen modulators on inorganic-Hg (I-Hg) production in MeHg-administered rats were examined. Rats were intravenously (i.v.) injected with MeHgCl (2 mg/kg). After MeHg administration, I-Hg levels time-dependently increased in the liver up to 9 h, whereas the renal levels did not change during the first 3 h, and then increased up to 24 h in a time-dependent manner. PQ stimulated I-Hg production in the liver but not in the kidney, whereas it increased 2-thiobarbituric acid-reactive substance (TBA-RS) levels in both tissues. PQ-induced stimulation of I-Hg production was not further accelerated by an OH· enhancer, such as FeSO₄ or Fe(III)EDTA. Hepatic I-Hg production in MeHg-administered rats (without PQ) was suppressed by NaCN (a potent inhibitor of mitochondrial cytochrome oxidase) but not by desferal or Fe(III)EDTA (an OH· modulator). These results suggest that hepatic mitochondria may play an important role in *in vivo* demethylation of MeHg, and that reactive oxygen species other than OH· may participate in it.

Key words—methylmercury, demethylation, reactive oxygen species, mitochondria, liver, rat

INTRODUCTION

Methylmercury (MeHg) is gradually transformed to inorganic mercury (I-Hg) *in vivo*. This

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biotransformation is an important factor for the evaluation of MeHg toxicity in organisms, particularly in a chronic phase. The cleavage of the C-Hg bond was suggested to occur *via* a reactive oxygen-mediated process *in vitro*.^{1,2)} Our previous study indicated that the hydroxyl radical (OH·) produced in rat liver microsome preparations was responsible for the degradation of MeHg.³⁾ Thus, it seems likely that reactive oxygens (RO), probably OH·, are also involved in the *in vivo* degradation of MeHg.

To elucidate the involvement of RO in the *in vivo* demethylation of MeHg, the effects of paraquat (PQ) and other RO modulators on I-Hg production in MeHg-administered rats were examined.

MATERIALS AND METHODS

MeHgCl was purchased from Tokyo Kasei Co. (Tokyo, Japan) at a purity of more than 99%. PQ was purchased from Nakarai Tesque, Inc. (Kyoto, Japan). Desferoximine mesylate (desferal) was purchased from Sigma Chemical Co. (St. Louis, MO). The other reagents were purchased from Wako Pure Chemical Ind. (Osaka, Japan). All chemicals used were of the purest grade available. Each reagent was dissolved in phosphate buffered saline (PBS, pH 7.4) for injection.

Male Wistar rats (9 weeks of age) were intravenously (i.v.) injected with MeHgCl (2 mg/kg). At the appropriate times (1, 3, 6, 9 and 24 h), rats (5 for each group) were perfused with ice cold saline under ethylether anesthesia to excise liver and kidneys for Hg analysis.

In another experiment, groups of 5 to 6 rats were i.v. administered MeHgCl (2 mg/kg). Some groups were intraperitoneally (i.p.) or subcutaneously (s.c.) administered the following reagents 3 h after MeHgCl administration: 1) PQ (50 or 75 mg/kg, i.p.), 2) Fe(III)EDTA (75 mg/kg, s.c.), 3) PQ (75 mg/kg, i.p.) + Fe(III)EDTA (75 mg/kg, s.c.), 4) PQ (75 mg/kg, i.p.) + FeSO₄ (75 mg/kg, s.c.) or 5) PBS alone. Other groups were administered the following reagents 1 h before MeHgCl administration: 1) NaCN (2.5 mg/kg, i.p.), 2) Fe(III)EDTA (75 mg/kg, s.c.), 3) desferal (400 mg/kg, i.p.) or 4) PBS alone.

Total-Hg (T-Hg) levels were determined according to the oxygen-combustion gold amalgamation method using a Rigaku mercury analyzer SP-3, which was also used to determine I-Hg levels as described elsewhere.⁴⁾

2-Thiobarbituric acid reactive substance (TBA-RS) levels in the tissues were determined according to Ohkawa *et al.*⁵⁾

All data were indicated by mean \pm S.D. Significant differences were calculated by Student's *t* test, and $p < 0.05$ was taken as significant.

RESULTS AND DISCUSSION

After i.v. administration of MeHg to the rats, I-Hg levels time-dependently increased in the liver up to 9 h, whereas the renal levels were unchanged from control value during the first 3 h, then increased up to 24 h in a time-dependent manner (Fig. 1). This result suggests that demethylation of MeHg takes place primarily in the liver, after which I-Hg is gradually transported to the kidney *via* the circulation.

PQ treatment increased hepatic I-Hg levels in a dose-dependent manner without significant change in T-Hg levels, whereas it did not alter T-Hg or I-Hg levels in the kidney (Fig. 2). In the PQ (75 mg/kg)-treated group, TBA-RS levels, an indicator of RO generation, increased in both

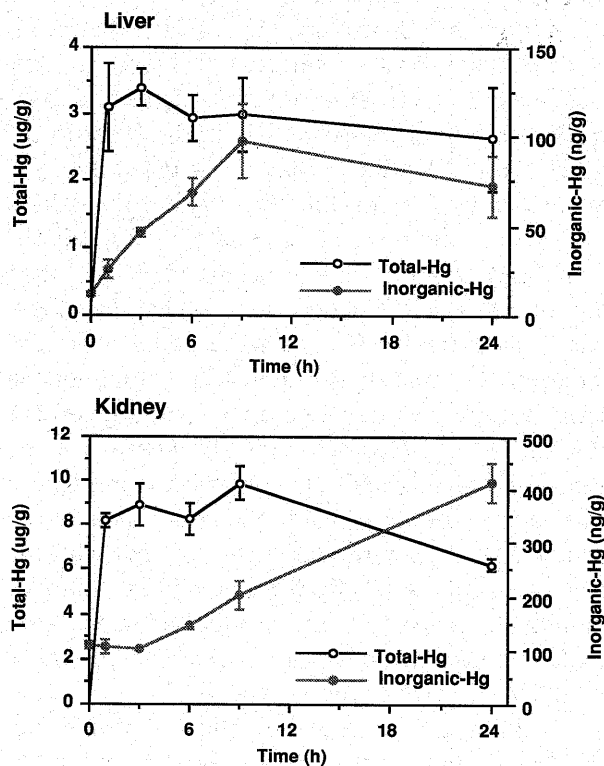


Fig. 1. Time Course of Total- and Inorganic-Hg Levels in Liver and Kidney of MeHg-administered Rats

Rats were i.v. injected with MeHgCl (2mg/kg). The tissues were excised at 1, 3, 6, 9 and 24 h after administration of MeHgCl. Each point represents mean \pm S.D.

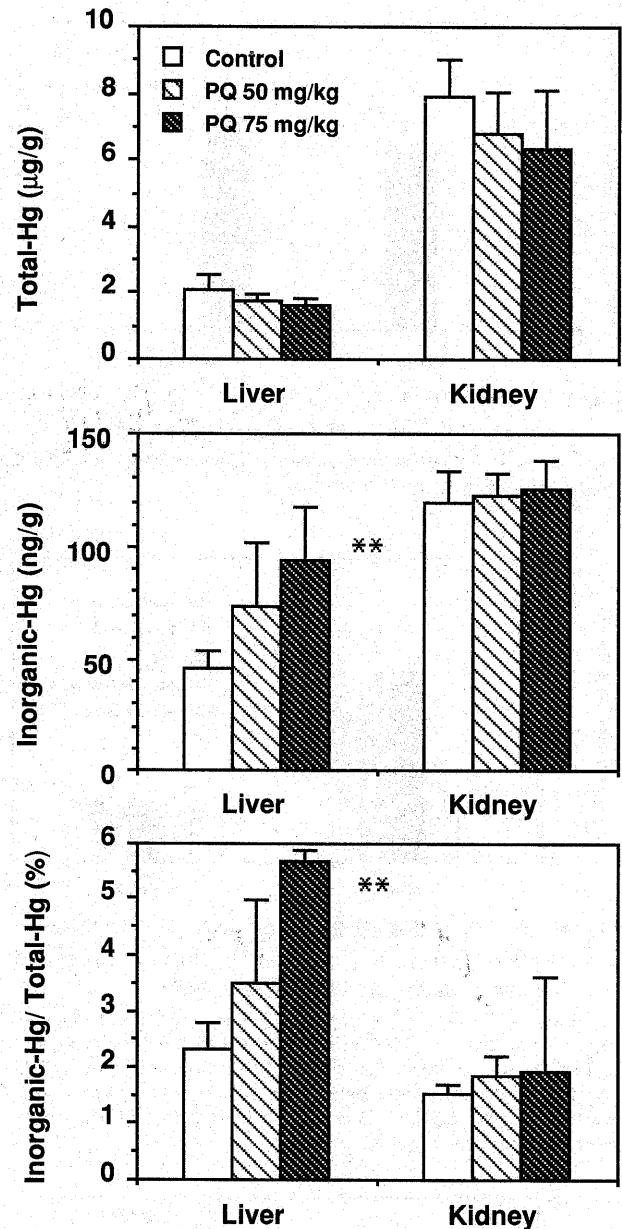


Fig. 2. Effects of Paraquat (PQ) on Total- and Inorganic-Hg Levels and Its Ratio in Liver and Kidney of MeHg-administered Rats

Rats were i.v. injected with MeHgCl (2mg/kg), and after 3 h they were i.p. injected with PQ (50 or 75 mg/kg) or PBS. The tissues were excised 6 h after MeHgCl injection. **Significantly different from control (PBS-injected group), $p < 0.05$.

liver and kidney (Fig. 3). Thus, PQ increased demethylation in the liver with a concomitant increase in TBA-RS levels but not in the kidney. PQ primarily produces superoxide anion (O_2^-) in aerobic conditions, but $OH\cdot$ in anaerobic conditions.⁶⁾ Since the liver is an aerobic organ and accounts for 20% of total oxygen consumption in the body, O_2^- production by PQ might be more active in the liver than in the kidney.

It is still unclear whether O_2^- itself or its

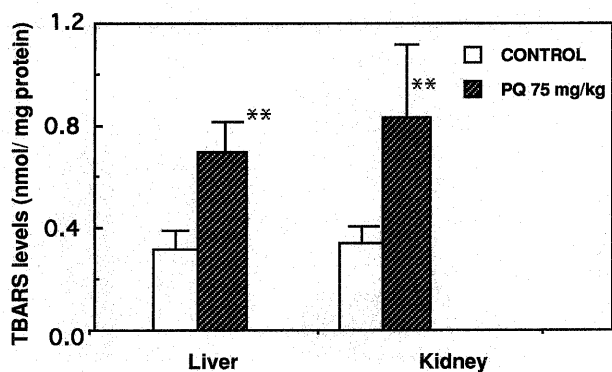


Fig. 3. Effects of PQ (75 mg/kg) on TBA-RS Levels in Liver and Kidney of MeHg-administered Rats

Rats were treated as described in the legends of Fig. 2. **Significantly different from control, $p < 0.05$.

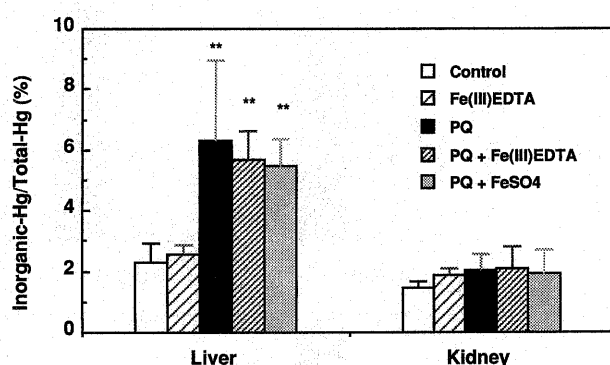


Fig. 4. Effects of Fe(III)EDTA or FeSO₄ in Addition to PQ on Inorganic-Hg/Total-Hg Ratio in Liver and Kidney of MeHg-administered Rats

Rats were i.v. injected with MeHgCl (2mg/kg), and after 3 h they were i.p. injected with PQ (75 mg/kg), Fe(III)EDTA (75 mg/kg, s.c.) or PBS. FeSO₄ (75 mg/kg, s.c.) or Fe(III)EDTA (75 mg/kg, s.c.) in addition to PQ were administered to the rats. The tissues were excised 6 h after MeHgCl injection. **Significantly different from control (PBS injected group), $p < 0.05$.

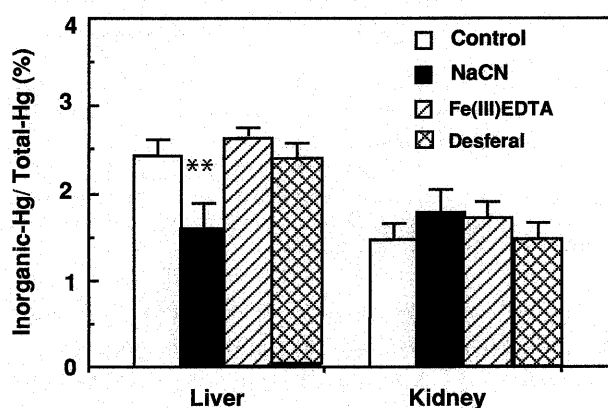


Fig. 5. Effects of NaCN, Fe(III)EDTA or Desferal on Inorganic-Hg/Total-Hg Ratio in Liver and Kidneys of MeHg-administered Rats

Rats were i.p. injected with NaCN (2.5 mg/kg), Fe(III)EDTA (75 mg/kg, s.c.) or desferal (400mg/kg) or PBS, and after 1 h they were i.v. injected with MeHgCl (2mg/kg). The tissues were excised 6 h after MeHgCl administration. **Significantly different from control (PBS group), $p < 0.05$.

metabolites participate in *in vivo* demethylation. OH \cdot is a highly reactive oxygen species, and it enhanced demethylation in hepatic microsome preparations.³⁾ Accordingly, OH \cdot seems to also participate in the *in vivo* demethylation observed here. It is reported that mortality from PQ was enhanced by FeSO₄, an enhancer of OH \cdot generation, and depressed by desferal, a depressor of OH \cdot generation.⁷⁾ However, coadministration of FeSO₄ or Fe(III)EDTA with PQ did not affect I-Hg production in either tissue (Fig. 4), though TBA-RS levels showed a marked increase (data not shown). In order to examine the involvement of OH \cdot in *in vivo* demethylation in physiological conditions (without PQ), MeHg-administered rats were treated with an OH \cdot modulator, desferal or Fe(III)EDTA. I-Hg production in the liver was not affected by these reagents at all (Fig. 5). These results suggest that the involvement of OH \cdot is inconsequential both in PQ-stimulated and physiological conditions. O₂⁻ is known to be a weak reactant in hydrophilic solvents but a powerful reactant in a hydrophobic solvents.⁸⁾ O₂⁻ produced in hydrophobic environment, such as mitochondrial membrane, might be responsible for *in vivo* demethylation.

Although PQ produces O₂⁻ by the NADPH-cytochrome P450 reductase system in microsomes, a recent study demonstrated that O₂⁻ produced in liver mitochondria in the presence of NADH and PQ participates in PQ toxicity.⁹⁾ Accordingly, it is possible that the mitochondrial mechanism also plays a role in PQ-induced MeHg-degradation. Since the main intracellular site of native RO generation is the mitochondria,¹⁰⁾ an intrinsic *in vivo* demethylation might occur in the mitochondria just like PQ-induced demethylation. In this study, CN⁻ suppressed the hepatic I-Hg/T-Hg ratio (Fig. 5), whereas it enhanced both I-Hg and OH \cdot production in hepatic microsome preparations.³⁾ Since it is reported that CN⁻, a potent inhibitor of cytochrome oxidase, suppressed TBA-RS levels in sub-mitochondrial particles,¹¹⁾ I-Hg production suppressed by CN⁻ also suggests the involvement of mitochondria in *in vivo* demethylation.

Taking all this together, these findings suggest that hepatic mitochondria play an important role in *in vivo* demethylation of MeHg, and that reactive oxygen species other than OH \cdot participates in it. O₂⁻ is a possible candidate. The role of the hepatic mitochondria in demethylation is

currently under study by our group.

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