Inhibitory Effect of Selenium on Biliary Secretion of Methyl Mercury in Rats

Tsutomu Urano,*† Nobumasa Imura,* and Akira Naganuma*‡

*Department of Public Health and Molecular Toxicology, School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, J apan; †Department of Hygiene, Yokohama City University, School of Medicine, Yokohama 236, J apan; and ‡Department of Molecular and Biochemical Toxicology, Faculty of Pharmaceutical Sciences, Tohoku University, Sendai 980-77, J apan

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The inhibitory effect of sodium selenite on biliary secretion of methyl mercury was examined in rats. The biliary secretion of methyl mercury in rat treated with 1 μmol/kg of methyl mercury was significantly decreased by administration of selenite at doses of 0.05 μmol/kg or higher. In rats given 10 μmol/kg of methyl mercury, marked depression of biliary secretion of mercury was observed when selenite was injected at a dose of 0.2 μmol/kg. On the other hand, secretion of substantial amounts of selenium was observed when biliary secretion of mercury was depressed. When the concentration of selenium in the bile was higher than 5 nmol/ml, biliary secretion of mercury was markedly depressed independently of the dose of methyl mercury administered (1 μmol/kg or 10 μmol/kg). These results suggest that the degree of inhibitory effect of selenite may be determined by the selenium concentration in the liver or the bile after treatment with selenite rather than the molar ratio of the dose of methyl mercury and selenite. We concluded that the decrease in biliary secretion of methyl mercury induced by selenite may result from inhibition of pathway for secretion of methyl mercury from liver to bile rather than the direct formation of a complex between methyl mercury and selenium. Methyl mercury has been considered to be secreted from liver to bile as a complex with glutathione (GSH). However, administration of selenite did not affect biliary secretion of GSH or hepatic glutathione S-transferase activity. Moreover, gel filtration of liver cytosol demonstrated that the distribution pattern of hepatic methyl mercury between macromolecules and GSH was not significantly changed by administration of selenite. These results suggest that selenite does not affect complex formation of methyl mercury with GSH at least in the liver. Selenite might specifically inhibit the activity of the canalicular transporter(s) which transport complexes of methyl mercury and GSH from the liver to bile.

Methylmercury is as an environmental pollutant which produces several neurological disorders in man (1, 2). Although many studies of the fate of methylmercury in animals have been performed, the mechanisms regulation of tissue distribution and excretion of methylmercury have not been examined in detail. Entero-hepatic circulation of methylmercury is considered to play an important role in metabolism and toxicity of methylmercury, because a considerable amount of methylmercury is secreted into the bile most of which is reabsorbed through the intestine (3). The main form of methylmercury observed in the bile of animals is present as a complex with glutathione (GSH) (4-7). Depletion of hepatic GSH significantly decreased the biliary secretion of both GSH and methylmercury (8, 9). The biliary secretion of methylmercury may be determined by the rate of secretion of GSH into the bile (8). On the other hand, the biliary secretion of methylmercury is strongly inhibited by selenium-containing compounds (10). However, the mechanism of this effect has not been elucidated. Selenium compounds have been shown to reduce the toxicity of methylmercury (11, 12) and to modify its tissue distribution (13-15). The effects of selenium compounds on the toxicity and tissue distribution of methylmercury might be due to direct interaction of both compounds in animals to form a complex of methylmercury and selenium (15, 16). The present study was performed to examine the involvement of the direct interaction of methylmercury and selenium in the inhibitory effect of selenium on the biliary secretion of methylmercury.
MATERIALS AND METHODS

Chemicals. [\(^{203}\text{Hg}\)]-Methylmercuric chloride (4.3 Ci/g) was synthesized from [\(^{203}\text{Hg}\)]-mercuric chloride (New England Nuclear, Boston MA) and methylcobalamin by the method of Naganuma et al. (17). [\(^{75}\text{Se}\)]-Sodium selenite (5.6 Ci/g) was purchased from Amersham Japan Co. Ltd. All other chemicals used for the experiments were commercially obtained.

Bile collection. Male Wistar rats weighing 180-220 g were purchased from Charles River Japan, Ind. The animals were anesthetized with sodium pentobarbital (100 mg/kg, with additional administration if necessary) and body temperature was maintained constant by means of infrared lamps throughout the experiments. Bile ducts were cannulated with polyethylene tubing (id, 0.5 mm; od, 0.8 mm). Then, various doses of [\(^{75}\text{Se}\)]-sodium selenite (0.025-2.0 \(\mu\)mol/1.5 \(\mu\)Ci/kg) were injected intravenously 2 hr after i.v. administration of [\(^{203}\text{Hg}\)]-methylmercuric chloride (1 \(\mu\)mol or 10 \(\mu\)mol/20 \(\mu\)Ci/kg). Bile was collected every 15 min for 1 hr after administration of selenite and then every 30 min for an additional 3 hr.

The radioactivities of \(^{203}\text{Hg}\) and \(^{75}\text{Se}\) in the bile samples were calculated from the values measured at 0.28 MeV and 0.4 MeV with an Aloka Auto Well Gamma System.

Determination of GSH. Bile samples were collected in ice-chilled tubes containing EDTA (final concentration; about 100 mM) to prevent oxidation of GSH in bile. Concentrations of GSH, cysteinylglycine (CysGly) and cysteine (Cys) in bile were determined by high performance liquid chromatography (HPLC) using ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F) as a fluorogenic reagent according to our modification (6) of the method of Toyo'oka et al. (18).

Enzyme assay. The liver was homogenized in 4 volumes of ice-cold 0.25 M sucrose and centrifuged at 105,000 \(\times\) g for 60 min. Activity of glutathione S-transferase (GST) in the supernatant was measured by the spectrophotometric method of Habig et al. using 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) as substrates (19). Protein concentration was determined by the method of Lowry et al. (20).

Gel filtration. The rats were injected with sodium selenite (0.2 mmol/kg) 2 hr after injection of [\(^{203}\text{Hg}\)]-methylmercuric chloride (1 \(\mu\)mol/kg), and were killed 30 min after injection of selenite. The liver was homogenized in 3 volumes of ice-cold phosphate buffered saline and then the homogenate was centrifuged at 105,000 \(\times\) g at 4 °C for 60 min. The supernatants (4 ml) were applied to an Sephadex G-15 column (25 mm \(\times\) 450 mm) and eluted with 50 mM Tris-HCl buffer (pH 7.6) containing 1 mM EDTA and 0.5 M NaCl. The eluate was fractionated into 2 ml portions and the concentration of \(^{203}\text{Hg}\) in each fraction was determined.

RESULTS

Figures 1 and 2 show the effects of selenite on biliary secretion of mercury in rats. Various doses of sodium selenite were administered i.v. 2 hr after injection of methylmercury at a dose of 1 \(\mu\)mol/kg (Fig. 1) or 10 \(\mu\)mol/kg (Fig. 2). When methylmercury was administered rats at a dose of 1 \(\mu\)mol/kg, the biliary secretion of mercury was significantly decreased by administration of selenite at a dose of 0.05 \(\mu\)mol/kg (one-twentieth of the dose of methylmercury) and at doses more than 0.05 \(\mu\)mol/kg (Fig. 1). In rats given 10 \(\mu\)mol/kg of methylmercury, marked depression of biliary secretion of mercury was observed even when selenite was injected at a dose of 0.2 \(\mu\)mol/kg (one-fiftieth of the dose of methylmercury) (Fig. 2). These results indicated that a specific molar ratio of methylmercury dose to that of selenite is not necessary to obtain the same extent of inhibition of biliary secretion of methylmercury by selenite.

![Graphs showing the effects of selenite on biliary secretion of mercury in rats.](image-url)
Moreover, as shown in Figs. 1 and 2, secretion of substantial amounts of selenium was observed when biliary secretion of mercury was depressed. Figure 3 illustrates the relationship between the biliary concentration of mercury and selenium until 4 hr after injection of selenite. When the concentration of selenium in the bile was higher than 5 nmol/ml, biliary secretion of mercury was markedly depressed regardless of the dose of methylmercury and concentration of mercury in the bile. These results suggest that the decrease in biliary secretion of methylmercury induced by selenite may result from inhibition of the pathway for secretion of methylmercury from liver to bile rather than the formation of a complex between methylmercury and selenium.

Methylmercury has been considered to be secreted from liver to bile as a complex with GSH (8, 21). Treatment of animals with GSH-depleting agents decreases hepatic GSH concentration and biliary secretion of both methylmercury and GSH (9, 22). Figure 4 shows the effects of selenite on secretion of GSH and other thiol compound into the bile of rats. Concentrations of GSH, CysGly and cysteine were not significantly influenced by administration of selenite.

Inhibition of biliary secretion of methylmercury by administration of an inhibitor for GST has also been

**FIG. 2.** Effect of selenite on secretion of methylmercury into rat bile. Various doses of selenite were injected i.v. 2 hr before injection of 10 μmol/kg of methylmercury. The mean pretreatment value was 18.96 ± 3.40 nmol Hg/ml bile.

**FIG. 3.** Relation between mercury and selenium concentrations in bile of rat treated with methylmercury and selenite. Various doses of selenite were administered 2 hr after administration of methylmercury (see Figs. 1 and 2). Rats received methylmercury at doses of 1 μmol/kg (A) or 10 μmol/kg (B).
Administration of sodium selenite at non-toxic doses strongly inhibited the secretion of methylmercury into bile (Figs. 1 and 2). Selenium compounds interact with methylmercury and modify its tissue distribution in animals (13, 15, 24). Selenium and methylmercury can directly react in animal tissue and form bis(methylmercuric) selenide (15, 16). This complex formation of methylmercury and selenium after administration of selenium compounds may result in modification of the fate of methylmercury in animals. Therefore, we examined the involvement of the interaction of methylmercury and selenium in the inhibitory effect of selenite on the biliary secretion of methylmercury. As bis(methylmercuric) selenide is unstable and determination of tissue concentration of this compound is difficult, a kinetic study was performed. The results indicated that the extent of the inhibitory effect of selenite on biliary secretion of methylmercury is dependent on the dose of selenite and the concentration of selenium in bile, but not the molar ratio of methylmercury and selenite administered (Figs. 1 and 2). The inhibition of biliary secretion of methylmercury was observed when selenite was administered at the dose of one-fifties of the dose of methylmercury (Fig. 2). Moreover, selenite administration did not significantly affect the hepatic accumulation of methylmercury under the experimental conditions used in the present study (Table 2). These results suggested that the inhibition of biliary secretion of methylmercury induced by selenite may result from the effects of selenium on the pathway of hepatobiliary transport of methylmercury rather than interaction with methylmercury.

Methylmercury has been considered to be secreted from liver to bile as a complex with GSH (8, 21). Some reagents which decrease methylmercury secretion into bile have been reported. GSH-depleting agents such as diethylmaleate (9) and inhibitors of hepatobiliary transport of GSH such as ICG (21) decreased biliary secretion of not only methylmercury but also GSH. On the other hand, GSH-depletion induced by selenite did not significantly affect the hepatic accumulation of methylmercury (Table 2). These results suggested that the inhibition of biliary secretion of methylmercury induced by selenite may result from the effects of selenium on the pathway of hepatobiliary transport of methylmercury rather than interaction with methylmercury.

Finally, we examined the possibility that administration of selenite decreases accumulation of methylmercury in the liver. However, methylmercury concentration in the liver was not significantly decreased by administration of selenite as shown in Table 2.

### TABLE 1

<table>
<thead>
<tr>
<th>Time after Se treatment (min)</th>
<th>GST activity (nmol/min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>DCNB</td>
</tr>
<tr>
<td></td>
<td>Se (-)</td>
</tr>
<tr>
<td>15</td>
<td>5.41 ± 1.23</td>
</tr>
<tr>
<td>30</td>
<td>6.94 ± 2.12</td>
</tr>
<tr>
<td>60</td>
<td>7.73 ± 4.13</td>
</tr>
<tr>
<td>120</td>
<td>8.16 ± 2.05</td>
</tr>
</tbody>
</table>

Rats were treated with selenite (0.2 μmol/kg) 2hr after methylmercury (1 μmol/kg) administration. Each value represents the mean ± S.D. of 3 ~ 6 animals.
the other hand, S-methylcysteine, an inhibitor of GST, decreased biliary secretion of methylmercury without affecting the secretion of GSH into bile (23). In the present study, selenite significantly decreased the biliary secretion of methylmercury (Figs. 1 and 2), but did not affect that of GSH (Fig. 4) or hepatic GST activity (Table 1). These results indicated that selenite is a novel inhibitor of the secretion of methylmercury into bile.

Hepatobiliary transport of methylmercury may be regulated by complexation of methylmercury with GSH and efflux of the complex from liver cells to bile through the canalicular membrane. Complexes of methylmercury and GSH have been detected in the liver of rats (25). Refsvik (23) reported that administration of inhibitors of GST decreased biliary secretion of methylmercury as mentioned above. The complex formation of methylmercury and GSH seems to be catalyzed by GST in the liver. However, selenite did not inhibit hepatic GST activity (Table 1) or the formation of methylmercury-GSH complex in the liver cytosol (Fig. 5) in the present study. These results suggested that the inhibitory effect of selenite on methylmercury secretion into bile did not result from the reduction of rate of formation of methylmercury-GSH complex in the liver. In the canalicular membrane of liver cells, transporter proteins have been found to deliver GSH or its S-conjugates into the bile (26, 27). Dutczak and Ballatori (28) reported that methylmercury-GSH complex was not a substrate for the canalicular glutathione S-conjugate carriers, but appears to be a substrate for canalicular carriers that transport GSH. Selenium may specifically inhibit the activity of the canalicular transporter(s) involved in active transport of GSH from liver to bile.

TABLE 2

<table>
<thead>
<tr>
<th>Time after Se treatment (min)</th>
<th>203Hg Concentration (nmol/g liver)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Se (−)</td>
</tr>
<tr>
<td>15</td>
<td>1.19 ± 0.35</td>
</tr>
<tr>
<td>30</td>
<td>1.31 ± 0.11</td>
</tr>
<tr>
<td>60</td>
<td>0.96 ± 0.11</td>
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<tr>
<td>120</td>
<td>1.06 ± 0.29</td>
</tr>
</tbody>
</table>

* Significantly different from Se (−) (p<0.005).

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