Effect of Selenium Compounds on the Permeability of Rat Small Intestine to Mercury Compounds

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The effect of selenium compounds on the transport of methylmercury (MMC) and HgCl₂ was studied by circulating perfusion experiments in an improved in vitro apparatus.

When the initial mercury content in the inner solution was 0.5 mM, promotion of mercury transport was observed upon the addition of 0.01 mM selenite.

The transport of MMC was greatly increased with addition of increasing amounts of selenite, while in the case of HgCl₂, mercury transport rather decreased with increasing amounts of selenite added. When selenite was added to the inner solution containing MMC, its increasing effect on mercury transport was considerably weaker than that of selenite. Transport of HgCl₂ was slightly increased by the addition of selenate.

Accumulation of mercury in the intestine was hardly changed with increasing addition of selenite or selenate in the case of MMC, but increased in the case of HgCl₂. Accumulation of selenium in the intestine was higher with selenite than selenate.

The above observations may be closely related to the mechanisms of absorption, distribution, and toxicity of mercury compounds in vivo in the presence of selenium compounds.

Keywords—mercury; selenium; permeability; small intestine; in vitro circulation; accumulation

Absorbed mercury compounds must permeate through various biologic membranes to reach the target organs. One of the authors showed by in vitro perfusion experiments that mercury compounds penetrate poorly through the small intestinal wall, but easily permeate through it in the presence of low concentrations of SH compounds which might release the mercury from protein.

Recently, Mitani et al. showed that cysteine accelerates the transport of methylmercury through the placenta. On the other hand, Yamane et al. reported that the mercury concentration in rat brain increased when methylmercury was given simultaneously with selenium compounds, but the mechanism was not clarified at all. In this work, we attempted to examine the effect of selenium compounds upon mercury permeation through the rat small intestine.

Experimental

In Vitro Circulating Perfusion Apparatus

—The circulating perfusion apparatus described in the previous report was slightly modified.

Figure 1 shows the structure of this apparatus. A roller pump (RP-V₂, Furuse Science Co.) was used for circulation of the inner solution. Rat small intestine (unverted) was attached between the glass projections of the upper chamber. The inner
solution circulates on the mucosal side of the small intestine and the outer solution comes into contact with its serosal side.

**Preparation of Solutions for Circulating Perfusion Experiments**—Inner Solution: Isotonic buffer solution at pH 7.4 as reported by Koizumi, but at 4-fold higher concentration (12.5 ml), 100 mM d-glucose (1.25 ml), 1 mM mercury solution (5.0 ml), and 10 mM selenium solution (0.05–5.0 ml) were diluted with water to give a total volume of 50 ml.

Outer Solution: Isotonic buffer solution (pH 7.4), with the same contents as the inner solution, containing 19.1 g of Na₂HPO₄·12H₂O, 1.8 g of KH₂PO₄, and 4.0 g of NaCl in a total volume of 1000 ml.

**In Vitro Circulating Perfusion of Rat Small Intestine**—Male rats of the Wistar strain weighing 250–400 g were fasted for a whole night prior to the experiments and allowed water freely. The animals were anesthetized with ether and the abdominal cavity was opened by a midline incision. The ileum part of the small intestine was removed by cutting the mesentery. The small intestine was connected to a cannula and the contents were washed out with 0.9% NaCl.

Then, the small intestine was connected between glass projections of the upper chamber unit with moderate tension and tied with cotton threads. As shown in Fig. 1, the length of the intestine utilized was 15 cm.

The upper chamber with the small intestine attached was inserted into the lower chamber containing 95 ml of isotonic buffer solution, and 30 ml of the inner solution was poured into the upper chamber. The inner solution was recirculated with a roller pump at a speed of 20 ml/min. This apparatus was immersed in the warm water bath at 37°C.

Aliquots of the outer solution (2 ml) were withdrawn at 0, 10, 30, 60, 90, 120 and 150 min after the starting of perfusion for determinations of Hg and Se. The total volume of the outer solution used for the determination was only 16 ml and the effect on the permeation was considered to be negligible.

Contents of the inner solutions in these series of experiments (Exp. 1–6) are shown in Table I.

<table>
<thead>
<tr>
<th>Table I. Contents of Inner Solution in Each Experiment</th>
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<tbody>
<tr>
<td>Exp. number</td>
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<tr>
<td>-------------</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>
Analytical Procedure — Reagents used in these analyses were of super special grade or were special reagents for trace metal analysis.

Microdetermination of Mercury* : An intestine sample (ca. 2 g) or perfusion sample solution (2 ml) was placed in a sample tube, and HNO₃ (1.5 ml) was added. After one day, (1 : 1) H₂SO₄ (1 ml) and 4 % KMnO₄ (10 ml) were added and the whole was heated at a temperature sufficiently low to prevent loss of mercury by evaporation. After digestion of the sample and decolorization of the permanganate with hydroxylamine, distilled water was added up to 100 ml. An aliquot of the solution was used for mercury determination by reductive vaporization—atomic absorption spectrophotometry.

Fluorometric Determination of Selenium* : Sample solutions prepared by wet digestion were extracted into cyclohexane after the addition of 2,3-diaminonaphthalene and the fluorescence of the extracts was measured at 520 nm.

Results and Discussion

Effect of Selenium on the Transport of Mercury through Rat Small Intestine

The effect of selenite added to the inner solution on the transmural movement of MMC contained in the inner solution is shown in Fig. 2 (a). Mercury transport, expressed as ppm in the outer solution, was increased at the lowest selenite concentration, 0.01 mm, and greatly increased at 0.04 mm, and 0.5 mm. At 1.0 mm, however, no further increase was observed. The enhancing effect of selenite on mercury transport at a lower concentration than that of MMC (0.1 mm) is quite distinct from the effect of SH compounds reported in the previous paper.23

The amounts of mercury and selenium that appeared in the outer solution after 150 min perfusion and the extent of transport (percentage with respect to the initial amount) are shown in Table II.

<table>
<thead>
<tr>
<th>Na₂SeO₃ (mm)</th>
<th>Hg transfer</th>
<th>Se transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.99 µg</td>
<td>0.82 %</td>
</tr>
<tr>
<td>0.01</td>
<td>21.5 µg</td>
<td>3.57 %</td>
</tr>
<tr>
<td>0.04</td>
<td>37.6 µg</td>
<td>6.25 %</td>
</tr>
<tr>
<td>0.5</td>
<td>77.0 µg</td>
<td>12.8 %</td>
</tr>
<tr>
<td>1.0</td>
<td>67.9 µg</td>
<td>11.3 %</td>
</tr>
</tbody>
</table>

The amount of transported mercury was 4.95 µg (0.82 %) in the absence of selenite, and increased to 21.5 µg (3.57 %) with 0.01 mm selenite. Maximum transport of mercury, 77.0 µg (12.8 %), was observed in the presence of 0.5 mm selenite.

Transport of selenium increased with increasing addition of selenite to the inner solution, as shown in Fig. 2 (b). The extent of transport was 10–16 % except when 0.04 mm selenite was added.

The effect of selenate addition on the permeation of MMC is shown in Fig. 3 (a). Promotion of MMC transport by selenate was not observed at 0.01 mm selenite, but the transport increased a little at 0.04 mm and increased appreciably at 0.5 mm. At 1.0 mm selenate addition, transport of MMC was rather decreased, as in the case of selenite addition. As a whole, selenite showed a stronger accelerating effect on MMC transport than selenate. This is also apparent in Table III, which shows the amount of MMC transport after 150 min perfusion.

The amount of selenium transport increased with increasing addition of selenate into the inner solution, but this was not the case with selenite (Fig. 3 (b)).

The effects of selenite addition on the transport of mercuric chloride are shown in
Fig. 2. Transport of Solutes through Rat Small Intestine in \textit{in Vitro} Circulation Experiments (Exp. 1)

Effect of Na$_2$SeO$_3$ added to the inner solution.

- ○: Na$_2$SeO$_3$ not added.
- ●: Na$_2$SeO$_3$ 0.01 mM.
- △: Na$_2$SeO$_3$ 0.04 mM.
- ▲: Na$_2$SeO$_3$ 0.5 mM.
- □: Na$_2$SeO$_3$ 1.0 mM.

(a) methylmercury (b) selenium

Fig. 3. Transport of Solutes through Rat Small Intestine in \textit{in Vitro} Circulation Experiments (Exp. 2)

Effect of Na$_2$SeO$_3$ added to the inner solution.

- ○: Na$_2$SeO$_3$ not added.
- ●: Na$_2$SeO$_3$ 0.01 mM.
- △: Na$_2$SeO$_3$ 0.04 mM.
- ▲: Na$_2$SeO$_3$ 0.5 mM.
- □: Na$_2$SeO$_3$ 1.0 mM.

(a) methylmercury (b) selenium
The effect of sodium selenate addition on the permeation of mercuric chloride is shown in Fig. 5 (a). In this case, a small promotion of mercury transport was recognized on the addition of 0.01 mM selenate and increasing transport was observed with the addition of increasing concentrations of selenate. The effect of selenate on the permeation of HgCl₂ is considered to be nearly the same as that on methylmercury permeation, as presented in Fig. 5 (a) and Table V.

The amount of selenate transported was larger than that of selenite regardless of the sort of mercuric used.

**Transport of Selenium in the Absence of Mercury Compounds**

As indicated in Fig. 6 (a), (b), and Table VI and Table VII, the presence of HgCl₂ or MMC had no influence on selenium transport irrespective of the chemical form of selenium.
Fig. 4. Transport of Solutes through Rat Small Intestine in *in Vitro* Circulation Experiments (Exp. 5)

Effect of Na$_2$SeO$_3$ added to the inner solution.
- ○: Na$_2$SeO$_3$ not added.
- ●: Na$_2$SeO$_3$ 0.01 mM.
- △: Na$_2$SeO$_3$ 0.04 mM.
- ▲: Na$_2$SeO$_3$ 0.5 mM.
- □: Na$_2$SeO$_3$ 1.0 mM.

(a) inorganic mercury  
(b) selenium
Accumulation of Mercury and Selenium in the Small Intestine after 150 min Perfusion

In experiment No. 1, no increase of mercury accumulation in the intestine was observed with increasing selenite addition, as shown in Table VIII. Accumulation of selenium in the intestine increased with increasing selenite addition.

As described in the previous report of Matsumoto, low molecular SH compounds, e.g. cysteine, promoted mercury transport through the intestine, and this was considered to be due to the interfering effect of the SH compound on the binding of mercury to protein.

In the case of selenium compounds, a different mechanism should be considered. Sumino et al. reported that MMC bound to protein was converted in vitro to a free benzene-soluble substance by the addition of selenite. The benzene-extractable substance formed in rabbit blood from MMC and Na₂SeO₃ was then identified by Naganuma and Imura as bis (methylmercuric) selenide.

Therefore, when MMC and selenite permeate into the intestinal wall in vivo, production of a lipid-soluble substance must be considered. Lipophilic substances are known to pass easily through biologic membranes, and might be involved in the accelerating effect of selenite on the mercury transport. Further experiments are necessary.

Accumulation of mercury in the intestine increased a little upon the addition of selenate. Accumulation of selenium from selenate in the intestine was far less than in the case of selenite, as shown in Tables VIII and IX.

In experiment No. 3, mercury accumulation in the intestine tended to increase when selenite was added to HgCl₂ solutions. Selenium accumulation was greatly increased, as in the case of MMC-selenite, as shown in Table X. Komiya et al. reported that colloidal HgSe appeared in rat serum when HgCl₂ and sodium selenite were dosed simul-
Table VIII. Accumulation of Hg and Se in the Small Intestine after 150 min Perfusion (Exp. 1)

<table>
<thead>
<tr>
<th>Se added to inner soln. (Na_2SeO_3 (mm))</th>
<th>Accumulation in the intestine Hg ppm</th>
<th>µg</th>
<th>Se ppm</th>
<th>µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86.9</td>
<td>147.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.01</td>
<td>115.4</td>
<td>196.1</td>
<td>0.81</td>
<td>1.38</td>
</tr>
<tr>
<td>0.04</td>
<td>91.1</td>
<td>154.9</td>
<td>6.47</td>
<td>11.0</td>
</tr>
<tr>
<td>0.5</td>
<td>80.5</td>
<td>136.8</td>
<td>68.8</td>
<td>117.0</td>
</tr>
<tr>
<td>1.0</td>
<td>67.1</td>
<td>114.0</td>
<td>202.3</td>
<td>344.0</td>
</tr>
</tbody>
</table>

Table IX. Accumulation of Hg and Se in the Small Intestine after 150 min Perfusion (Exp. 2)

<table>
<thead>
<tr>
<th>Se added to inner soln. (Na_2SeO_3 (mm))</th>
<th>Accumulation in the intestine Hg ppm</th>
<th>µg</th>
<th>Se ppm</th>
<th>µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86.9</td>
<td>147.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.01</td>
<td>113.7</td>
<td>193.4</td>
<td>0.39</td>
<td>0.66</td>
</tr>
<tr>
<td>0.04</td>
<td>116.5</td>
<td>198.0</td>
<td>3.26</td>
<td>5.54</td>
</tr>
<tr>
<td>0.5</td>
<td>92.0</td>
<td>156.4</td>
<td>11.4</td>
<td>19.4</td>
</tr>
<tr>
<td>1.0</td>
<td>89.2</td>
<td>151.7</td>
<td>36.0</td>
<td>61.1</td>
</tr>
</tbody>
</table>

Table X. Accumulation of Hg and Se in the Small Intestine after 150 min Perfusion (Exp. 3)

<table>
<thead>
<tr>
<th>Se added to inner soln. (Na_2SeO_3 (mm))</th>
<th>Accumulation in the intestine Hg ppm</th>
<th>µg</th>
<th>Se ppm</th>
<th>µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25.1</td>
<td>42.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.01</td>
<td>55.3</td>
<td>94.1</td>
<td>2.61</td>
<td>4.44</td>
</tr>
<tr>
<td>0.04</td>
<td>51.9</td>
<td>88.3</td>
<td>11.6</td>
<td>19.7</td>
</tr>
<tr>
<td>0.5</td>
<td>71.3</td>
<td>121.2</td>
<td>109.6</td>
<td>186.3</td>
</tr>
<tr>
<td>1.0</td>
<td>70.5</td>
<td>119.9</td>
<td>132.6</td>
<td>225.7</td>
</tr>
</tbody>
</table>

Table XI. Accumulation of Hg and Se in the Small Intestine after 150 min Perfusion (Exp. 4)

<table>
<thead>
<tr>
<th>Se added to inner soln. (Na_2SeO_3 (mm))</th>
<th>Accumulation in the intestine Hg ppm</th>
<th>µg</th>
<th>Se ppm</th>
<th>µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25.1</td>
<td>42.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.01</td>
<td>30.2</td>
<td>51.3</td>
<td>0.47</td>
<td>0.81</td>
</tr>
<tr>
<td>0.04</td>
<td>50.7</td>
<td>86.2</td>
<td>4.25</td>
<td>7.23</td>
</tr>
<tr>
<td>0.5</td>
<td>96.5</td>
<td>164.0</td>
<td>34.9</td>
<td>59.4</td>
</tr>
<tr>
<td>1.0</td>
<td>72.0</td>
<td>122.3</td>
<td>23.7</td>
<td>40.2</td>
</tr>
</tbody>
</table>

Simultaneously. In the intestine, a similar reaction might occur and the colloidal products might scarcely permeated into the serosal solution.

In perfusion experiment No. 4, mercury accumulation in the intestine tended to increase with increasing addition of selenate to the inner mucosal solution as in the case of selenite, but selenium accumulation was not increased as much as in the case of selenite (Table XI). The mechanism of the weak promoting effect of selenate on inorganic mercury have not been clarified.

Reaction mechanism between mercury and selenium compound in the intestine is not yet clarified, so more investigation is necessary from now on.

References and Notes