Effects of Tiopronin on Excretion and Distribution of $^{203}$Hg-Labeled Mercury Compounds in Mice

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Abstract—Effects of repeated doses of tiopronin [N-(2-mercaptopropionyl)glycine] on elimination of $^{203}$Hg were determined by means of whole body counting after intravenous injection of $^{203}$Hg-methylmercuric chloride (Me $^{203}$HgCl), phenylmercuric acetate (Ph $^{203}$HgAc) or mercuric chloride ($^{203}$HgCl$_2$) into mice. The tissue distribution of $^{203}$Hg-mercury compounds was also observed, using a autoradiographic technique, and the effects of tiopronin were compared with those of 2,3-dimercaptopropanol (BAL). Subcutaneous administrations of tiopronin stimulated excretion of radioelements from the whole body of mice given Me $^{203}$HgCl, the biological half-life of Me $^{203}$HgCl being shortened from 6.5 to 2.9 days. Autoradiographic studies showed that the radioactivities after Me $^{203}$HgCl injection were less in all organs in the tiopronin-treated mice than in the controls after a temporary rise of radioactivities in the blood and secretory glands. The excretion of $^{203}$Hg from the mice given Ph $^{203}$HgAc or $^{203}$HgCl$_2$ was also stimulated by tiopronin treatment, but the stimulation was much less than when the mice had been given Me $^{203}$HgCl. Repeated administrations of BAL remarkably increased the excretion of radioelement in the mice given Ph $^{203}$HgAc or $^{203}$HgCl$_2$ but not Me $^{203}$HgCl. However, autoradiograms demonstrated that BAL enhanced accumulation of these compounds in the nervous system and muscle compared with the control after injection of three different types of mercury compounds.

A number of reports have been published on Minamata Disease, a chronic methylmercury poisoning resulting from the ingestion of fish contaminated with mercurial wastes (1–3). Chelating agents, 2,3-dimercaptopropanol (BAL) and calcium disodium edetate, were clinically prescribed for treatment of these patients. However, the results obtained were not always satisfactory (4).

Certain chelating agents such as BAL and cysteine enhance the accumulation of mercury compounds in different tissues, especially in the brain of experimental animals (5–8). Tiopronin has been recently reported to promote excretion of mercury compounds in man (9) and animals (10–11). There is, however, no actual proof that tiopronin is clinically effective for Minamata Disease.

In the present investigation, the effects of tiopronin on the elimination and distribution of $^{203}$Hg-mercury compounds were compared with those of BAL by means of whole body counting and whole body autoradiography, respectively.

MATERIALS AND METHODS

Animals and Compounds

Animals used (ddY-SLC strain) were adult male mice, weighing from 20 to 24 g, fed...
203Hg-labeled methylmercuric chloride (Me 203HgCl) and phenylmercuric acetate (Ph 203HgAc) were purchased from the New England Nuclear Corp. (U.S.A.). 203Hg-labeled mercuric chloride (203HgCl2) was obtained from Daiichi Pure Chemicals Co., Ltd. Tiopronin was a product of Santen Pharmaceutical Co., Ltd., and the BAL used was a reagent grade of Wako Pure Chemical Industry, Ltd.

Testing the effect of drugs on excretion of 203Hg-mercury compounds

Me 203HgCl, Ph 203HgAc and 203HgCl2 having specific activities of 36, 56 and 65 μCi/mgHg, respectively, were used. In each experiment, 25 mice were separated into 5 groups and treated as follows: (1) Control group; No drug treatment after 203Hg-mercury compound injection, (2) Tiopronin-treated group (A); Tiopronin was given daily for 6 days, commencing with the day of injection of 203Hg-mercury compound, (3) Tiopronin-treated group (B); Tiopronin was given daily for 6 days from day 7 after injection of 203Hg-mercury compound, (4) Pre-mixed group; No drug treatment after injection of 203Hg-mercury compound. The mercury compound was mixed in a test tube with twice the equimolar amount of tiopronin, and this mixture was injected, (5) BAL-treated group; BAL was given daily for 6 days, commencing with the day of injection of 203Hg-mercury compound.

203Hg-mercury compounds and their mixtures with tiopronin were given into the tail vein at a dose of 0.5 mgHg/kg. Tiopronin (dissolved in saline, adjusted to pH 7.0 with NaOH) and BAL (dissolved in olive oil) were given s.c. at a dose of 5.0 mg/kg. The volume of the infusate was 0.1 ml/20 g of body weight.

During the experiment period (14 days), animals were placed individually in metabolism cages in order to prevent contamination with their excrement.

Whole body radioactivity of animals was counted daily for 13 days between 9:00 a.m. and 10:00 a.m. and BAL or tiopronin was administered immediately after the counting. Animals were sacrificed on day 14. Radioactivities of whole animal and organs, such as the kidney, brain and remaining carcass, were measured using a Armac Scintillation Spectrometer (Packard model 446). The correction of the observed counts for radioactive decay was made by the use of standard samples.

Preparation of autoradiograms

Twenty mice were separated into groups of 4 (control group, tiopronin-treated group (A), tiopronin-treated group (B) and BAL-treated group), and treated with 203Hg-mercury compounds and drugs in the same manner to those mentioned above, except that Me 203HgCl, Ph 203HgAc and 203HgCl2 used had specific activities of 0.80, 0.88 and 0.96 μCi/mgHg, respectively. One mouse from each group was sacrificed with an inhalation of chloroform on day 1, 3, 5 and 12 after injection of 203Hg-mercury compounds. The drugs were not given to the animals on the day of sacrifice. Mice were also given drugs immediately after injection of 203Hg-mercury compounds and sacrificed 1 hr later. According to the methods of Ullberg modified by Matsuoka (12), the mice were immersed in the mixture of dry-ice and acetone, and whole body autoradiography was performed. Sagittal sections of 40 μ
thickness were exposed to industrial X-ray film (Sakura type N) for 7 to 30 days.

RESULTS

Effect of drugs on elimination of $^{203}\text{Hg}$-mercury compounds

Figure 1 shows the time-course of the effect of drugs on whole body retention of $^{203}\text{Hg}$-mercury compounds expressed as mean percentage of injection dose in the semilogarithmic graph.

$\text{Me}^{203}\text{HgCl}_2$: Whole body retention of $^{203}\text{Hg}$ of the control group decreased linearly, and the biological half-life was 6.5 days. In the tiopronin-treated groups (A) and (B), the elimination rates were remarkably enhanced by tiopronin administration, and biological half-life of both groups was about 2.9 days during the treatment period. On the other hand, a significant increase in the elimination rates was not observed in the pre-mixed and BAL-treated groups.

$\text{Ph}^{203}\text{HgAc}$: Whole body retention of $^{203}\text{Hg}$ of the control group decreased in a curve for a few days after injection, and thereafter, decreased in a straight line. In the tiopronin-treated groups (A), (B) and pre-mixed group, the elimination rates increased slightly. In the BAL-treated group, the increase in the elimination rate was comparable to that of the tiopronin-treated group (A) in a few days, and thereafter was enhanced to a considerable extent. This may be due to the potent activity of BAL in initiating excretion of inorganic mercury to which $\text{Ph}^{203}\text{HgAc}$ was converted in the animal tissues (13–14).

![Fig. 1. Effects of tiopronin and BAL on the time course of $^{203}\text{Hg}$ elimination after injection of three different types of $^{203}\text{Hg}$-labeled mercury compounds into mice. $^{203}\text{Hg}$-mercury compounds or their mixtures with tiopronin were given i.v. and then tiopronin and BAL were given s.c., as described in the "MATERIALS AND METHODS". Whole body radioactivity was counted. Each point represents mean percentage of injected dose from five animals. The arrows indicate the administration of tiopronin (†) or BAL (△). ○ Control group; ● ● Tiopronin-treated group (A), given tiopronin for the first 6 days; △ △ Tiopronin-treated group (B), given tiopronin for 6 days from day 7; ■ ■ Pre-mixed group, given the mixture of $^{203}\text{Hg}$-mercury compound and tiopronin: ○——○ BAL-treated group, given BAL for the first 6 days.](image-url)
Effect of tiopronin on $^{203}$Hg content in the kidney, brain and remaining carcass of mice sacrificed on day 14 after injection of $^{203}$Hg-mercury compounds

<table>
<thead>
<tr>
<th>Mercury compounds</th>
<th>Group</th>
<th>Kidney</th>
<th>Brain</th>
<th>Remaining carcass</th>
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<tr>
<td>Me $^{203}$HgCl</td>
<td>Control</td>
<td>4.01±0.34</td>
<td>0.15±0.02</td>
<td>15.23±1.85</td>
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<td>Tiopronin-treated (A)</td>
<td>1.55±0.42**</td>
<td>0.08±0.02**</td>
<td>5.56±0.45**</td>
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<td>Tiopronin-treated (B)</td>
<td>1.45±0.27**</td>
<td>0.07±0.02**</td>
<td>6.47±0.44**</td>
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<td>Pre-mixed</td>
<td>3.25±0.67</td>
<td>0.12±0.05</td>
<td>12.80±2.41</td>
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<tr>
<td>Ph $^{203}$HgAc</td>
<td>Control</td>
<td>3.13±0.35</td>
<td>0.04±0.01</td>
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<td>Tiopronin-treated (A)</td>
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<td>Tiopronin-treated (B)</td>
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<td>0.04±0.01</td>
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<td>$^{203}$HgCl$_2$</td>
<td>Control</td>
<td>5.10±1.10</td>
<td>0.05±0.01</td>
<td>3.41±0.38</td>
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<td>Tiopronin-treated (A)</td>
<td>3.31±1.65*</td>
<td>0.05±0.01</td>
<td>3.21±0.40</td>
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<tr>
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<td>Tiopronin-treated (B)</td>
<td>3.34±0.41*</td>
<td>0.05±0.01</td>
<td>3.21±0.32</td>
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<tr>
<td></td>
<td>Pre-mixed</td>
<td>4.31±0.23</td>
<td>0.03±0.01</td>
<td>3.86±0.83</td>
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</table>

Each value represents mean percentage± standard deviation of injected dose from five mice. Tiopronin-treated group (A) was given tiopronin for 6 days commencing with the day of injection of $^{203}$Hg-mercury compound and tiopronin-treated group (B) was given tiopronin for 6 days from day 7 after the injection of $^{203}$Hg-mercury compound. Pre-mixed group was given the mixture of $^{203}$Hg-mercury compound and tiopronin instead of $^{203}$Hg-mercury. Significance of difference in respect to control group: *P<0.05, **P<0.01.

$^{203}$HgCl$_2$: A slight increase in the elimination rates was observed and such was similar to the case of Ph $^{203}$HgAc, in the tiopronin-treated groups (A), (B) and pre-mixed group. On the contrary, the elimination rate remarkably increased in the BAL-treated group. The biological half-life of the control group was 4.1 days, while that of the BAL-treated group was 0.8 days.

**Effect of tiopronin on $^{203}$Hg content in organs on day 14 after injection of $^{203}$Hg-mercury compounds:** Table 1 shows the content of $^{203}$Hg, expressed as the percentage of injection dose, in the kidney, brain and remaining carcass at the end of 13 days treatment period. In this experiment, the BAL-treated group was not included.

The $^{203}$Hg content in the kidney, brain and remaining carcass of the mice injected with Me $^{203}$HgCl in the tiopronin-treated groups (A) and (B) was reduced by half to one third of that in the control group. In the mice injected with Ph $^{203}$HgAc and $^{203}$HgCl$_2$, only the kidney content of $^{203}$Hg decreased in the tiopronin-treated groups (A), (B) and pre-mixed group.

**Autoradiogram observation**

**Effect of drugs on distribution of $^{203}$Hg-mercury compounds:** Autoradiograms of sagittal sections of the drug-treated and control mice sacrificed at one hour after injection of $^{203}$Hg-mercury compounds are shown, as a negative picture, in Fig. 2. In the mice injected with Me $^{203}$HgCl, the radioactivities in the blood, lacrimal gland, lung and salivary gland of the
tiopronin-treated animals were higher than those of the control animals (A1:A3). However, tiopronin showed no effect on distribution of Ph $^{203}$HgAc or $^{203}$HgCl$_2$ (B1:B3, C1:C3). The autoradiograms of the BAL-treated mice injected with the three different $^{203}$Hg-mercury compounds revealed that BAL enhanced the accumulation of $^{203}$Hg in the central nervous system and muscle (A1:A2, B1:B2, C1:C2).

Whole body autoradiograms prepared from the tiopronin-treated and control mice
sacrificed on day 3 and 12 after injection of Me $^{203}\text{HgCl}$ (Fig. 3) depict that organs of the animals given tiopronin clearly contain less $^{203}\text{Hg}$ than the corresponding organs of the control animals. It was particularly evident that except for the kidney and liver there was almost no radioactivity detected in other organs of mice in the tiopronin-treated groups (A) and (B) sacrificed on day 12.

**Accumulation of $^{203}\text{Hg}$-mercury compounds in brain:** Fig. 4 shows the autoradiograms, as a positive picture, of the brain of the drug-treated and control mice sacrificed on day 5 after injection of $^{203}\text{Hg}$-mercury compound.

In the BAL-treated mice, the radioactivities of the brain were apparently higher than those of the control animals injected with any of the $^{203}\text{Hg}$-mercury compounds, particularly Ph $^{203}\text{HgAc}$ (A1:A2, B1:B2, C1:C2). Whereas, in the tiopronin-treated mice, the radio-

![Fig. 4](image)

**Fig. 4.** Autoradiograms showing effects of tiopronin and BAL on distribution of $^{203}\text{Hg}$ in brain on day 5 after injection of $^{203}\text{Hg}$-mercury compounds. Mice were given $^{203}\text{Hg}$-mercury compound in a single dose of 0.5 mg Hg/kg i.v. and then 50 mg/kg of tiopronin or BAL s.c. daily for 5 days. A, Me $^{203}\text{HgCl}$; B, Ph $^{203}\text{HgAc}$; C, $^{203}\text{HgCl}$. 1, Control group; 2, BAL-treated group; 3, Tiopronin-treated group (A).

![Fig. 5](image)

**Fig. 5.** Autoradiograms showing the accumulation of $^{203}\text{Hg}$ in peripheral nerves after injection of Ph $^{203}\text{HgAc}$. Mice were given Ph $^{203}\text{HgAc}$ in a single dose of 0.5 mg Hg/kg i.v. and then 50 mg/kg s.c. of BAL daily for 5 days. A, after 3 days (Control group); B, after 5 days (Control group); C, after 5 days (BAL-treated group).
activity of brain after Me $^{203}$HgCl decreased remarkably as compared with that of the control mice (A1:A3). No difference was observed in the radioactivities of the brain between the tiopronin-treated and control animals after Ph $^{203}$HgAc and $^{203}$HgCl$_2$ (B1:B3, C1:C3).

Accumulation of $^{203}$Hg-mercury compounds in peripheral nerves: A considerable amount of radioactivity was observed in spinal or sciatic nerves a few days after injection of $^{203}$Hg-mercury compounds. Fig. 5 shows typical autoradiographic data of the peripheral nerves of the control and BAL-treated mice sacrificed on day 3 or 5 after Ph $^{203}$HgAc injection. BAL enhanced the accumulation of $^{203}$Hg in the peripheral nervous system. Tiopronin had no effect on the accumulation of $^{203}$Hg in the peripheral nervous system (data not shown).

DISCUSSION

The present studies showed that tiopronin enhanced the excretion of $^{203}$Hg-mercury compounds in the animal body. This effect was more pronounced after injection of Me $^{203}$HgCl than after Ph $^{203}$HgAc or $^{203}$HgCl$_2$. Furthermore, tiopronin exerted its stimulative effect not only when the treatment started on the same day of administration of $^{203}$Hg-mercury compounds but also one week after $^{203}$Hg-mercury compounds. Autoradiographic studies showed that the radioactivities after Me $^{203}$HgCl were less accumulated in all organs of tiopronin-treated mice than those of control mice, after a temporary rise of radioactivities in the blood and secretory glands.

On the other hand, BAL was effective in decreasing the body burden after injection of Ph $^{203}$HgAc and $^{203}$HgCl$_2$ but not after Me $^{203}$HgCl. However, the autoradiographic studies showed that BAL induced a redistribution demonstrated by increased radioelements in the peripheral and central nervous systems and muscle after injection of three types of mercury compounds.

Sulfhydryl compounds are well known to make stable complexes with mercury compounds in vitro. Therefore, it seems that the complex formation of tiopronin with mercury compounds plays an important role in enhancement of the excretion of mercury compounds after tiopronin administration. However, it was also demonstrated that when the mixture of tiopronin and mercury compounds (mole ratio, 2:1) was injected into mice, the stimulatory effect on the excretion of mercury compounds was much less than when a large excess amount of tiopronin was given repeatedly after $^{203}$Hg-mercury compound injection. These results indicate that the complex of tiopronin and mercury compounds formed by mixing may be easily decomposed in the animal body, and that the separated mercury compounds may be taken up by endogenous cysteine, glutathione, or protein sulfhydryl groups when tiopronin is not present in excess of the mercury compounds. This consideration is supported by the data of Funae et al. (15) who showed that the stability constant of the mercury complex with tiopronin was less than that with glutathione or cysteine.

In mice treated with Me $^{203}$HgCl, the tiopronin-induced temporary rise in the concentration of $^{203}$Hg in the blood and secretory glands may be associated with acceleration of $^{203}$Hg elimination from the whole body. In mice given Ph $^{203}$HgAc or $^{203}$HgCl$_2$, the ac-
celerated reduction of tiopronin was due to the removal of $^{203}$Hg from the kidney. The mechanism of accelerated excretion by tiopronin after Ph $^{203}$HgAc or $^{203}$HgCl$_2$ seems to be considerably different from that after Me $^{203}$HgCl.

Among various drugs (9-11, 16-21) used experimentally for the release of mercury compounds from the organism, tiopronin appears to be one of the most effective. Moreover, this compound has the distinct advantage of being much less toxic (22) than the compounds mentioned above. From the above considerations, tiopronin is considered to have a therapeutic value in cases of mercury poisoning, particularly methylmercury poisoning.

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