Protective Effect of Sodium Molybdate against the Acute Toxicity of Mercuric Chloride in Rat. VI. The Mechanism of Stimulative Action of Sodium Molybdate on Urinary Excretion of Mercury

Toshiaki Koizumi,* Masamitsu Honma and Yasuhiro Yamane

Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoi-cho, Chiba 260, Japan. Received August 11, 1989

In order to gain further insight into the protective action of Na₂MoO₄ pretreatment (1.24 mmol/kg, once a day, i.p.) against the acute toxicity of HgCl₂ (30 μmol/ml/kg, once, s.c.), changes of renal function, tissue accumulation of mercury, and urinary excretions of mercury and phenolsulfonphthalein after exposure to HgCl₂ were investigated.

Lactate content in the kidney and serum calcium were also measured.

Na₂MoO₄ pretreatment enhanced urinary excretion of mercury. Renal function of Na₂MoO₄-pretreated rats was better maintained as compared to that of the rats given HgCl₂ alone at either dose (30 or 15 μmol/kg) although the metal content in the kidney of this group was almost the same as that of the latter HgCl₂-alone rats. This pretreatment prevented the rise in lactate content in the kidney and the reduction of urinary excretion of phenolsulfonphthalein caused by HgCl₂. Na₂MoO₄ reduced serum calcium. These results suggest that Na₂MoO₄ prevented mercury-induced acute renal failure by decreasing tissue accumulation of the metal through urinary excretion of mercury. Better renal hemodynamics attributable to hypocalcemia may be a causative factor in the enhancement of urinary excretion of mercury.

Keywords: mercuric chloride; sodium molybdate; acute renal failure; glomerular filtration; tubular secretion; renal blood flow; phenolsulfonphthalein

Previous papers from this laboratory reported that pretreatment of rats with Na₂MoO₄ protected them against the acute toxicity of HgCl₂, and the protective action of Na₂MoO₄ was exerted by enhancing mercury-induced renal metallothionein induction and urinary excretion of mercury. It has been assumed that the latter action of Na₂MoO₄ would be effective to prevent tissue injury by mercury because it is generally thought that excretion through main avenues such as urine and feces is one detoxication mechanism for harmful heavy metals. However, no quantitative evaluation to support such an assumption has been made so far. In the present study, therefore, renal function and tissue accumulation of mercury after exposure to HgCl₂ were evaluated in relation to urinary excretion of mercury. The mechanism of stimulative action of Na₂MoO₄ on urinary excretion of mercury is discussed.

Materials and Methods
The reagents used in this study were of analytical grade, obtained from Wako Pure Chemical Industries (Osaka, Japan).

Male Wistar rats (Matsumoto Labo-animals Laboratory, Kimitsu, Japan) weighing 160–180 g were given solid diet (CE-2, Clea Japan Inc., Tokyo, Japan) and water ad libitum.

Urinary Excretion and Tissue Accumulation of Mercury Rats were randomly divided into 2 groups of 15 rats each and were individually placed in metabolic cages. One group received i.p. injection of Na₂MoO₄ dissolved in saline once a day for 3 days at a dose of 1.24 mmol/kg and was given one s.c. injection of HgCl₂ dissolved in saline at a dose of 30 μmol/kg 24 h after the final i.p. injection of Na₂MoO₄. The other was treated with saline instead of Na₂MoO₄ for 3 d and received the same dose of HgCl₂ as Na₂MoO₄-treated rats but 24 h after the final i.p. injection of saline. Urine was collected in an ice bath every 4 h from 0 to 12 h after treatment with HgCl₂. Five rats from each group were killed by cervical dislocation 4, 8 and 12 h after exposure to HgCl₂ and the livers and kidneys were immediately perfused with iced 1.15% KCl. The tissue were rapidly removed, rinsed with the same solution, blotted and used for measurement of mercury. Mercury in the urine and tissue was analyzed as described previously.

Dose–Response Study on Mercury Accumulation in Renal Nuclear Fraction A total of 25 rats were randomly divided into 5 groups of 5 rats each. The rats in groups 1 to 4 were treated with saline for 3 d as in the above experiment and received one s.c. injection of HgCl₂ at a dose of 11, 15, 19 or 30 μmol/kg 24 h after the final i.p. injection of saline. Group 5 rats were pretreated with Na₂MoO₄ as in the above experiment and were given one s.c. injection of HgCl₂ at a dose of 30 μmol/kg 24 h after the final i.p. injection of Na₂MoO₄. All the rats in the experimental groups were killed by cervical dislocation 12 h after exposure to HgCl₂ and the kidneys were processed and removed in the above experiment. Renal nuclear fraction was prepared according to the method of Hogeboom. Mercury in the crude nuclei was analyzed as described above.

Renal Function after Exposure to HgCl₂ Rats were randomly divided into 4 groups of 5 rats each. The rats in group 1 were treated with saline alone. Group 2 and 3 rats were injected with saline for 3 d as in the above experiment and were given one s.c. injection of HgCl₂ either 15 or 30 μmol/kg 24 h after the final i.p. injection of saline. Group 4 rats were pretreated with Na₂MoO₄, as in the above experiment and received one s.c. injection of HgCl₂ at a dose of 30 μmol/kg 24 h after the final i.p. injection of Na₂MoO₄. The rats were killed by exsanguination by cardiac puncture 12 h after exposure to HgCl₂. The kidneys were perfused with 1.15% KCl and were analyzed for mercury. Blood urea nitrogen and serum creatinine were determined by the methods of Haré and Kimura and Nishina, respectively.

Lactate Content in the Kidney A total of 40 rats were divided into two groups (groups 1 and 2) of 5 rats each and two groups (groups 3 and 4) of 15 rats each. Groups 1 and 2 rats were treated with either saline or Na₂MoO₄ for 3 d as in the above experiment. Group 3 rats received saline for 3 d, then were given 30 μmol/kg of HgCl₂ (s.c.). Group 4 rats were pretreated with Na₂MoO₄, then one s.c. injection of HgCl₂ as in the above experiment. Group 1 and 2 rats were killed by cervical dislocation 0 h after exposure to HgCl₂ and the kidneys were analyzed for lactate. Five rats from groups 3 and 4 were killed 4, 8 and 12 h after exposure to HgCl₂ and the kidneys were removed. Lactate was determined by the method of Guttmann and Wahlefeld.

Urinary Excretion of Phenolsulfonphthalein in Rats Given HgCl₂ with or without Na₂MoO₄ Pretreatment Rats were randomly divided into 4 groups of 5 rats each and were individually placed in metabolic cages. The rats were treated with either saline or Na₂MoO₄ or HgCl₂ or both Na₂MoO₄ and HgCl₂ as in the above experiment. All the rats in all the groups were given one s.c. injection of phenolsulfonphthalein dissolved in saline at a dose of 0.6 mg/kg 0 h after exposure to HgCl₂. Urine was collected in an ice bath from 0 to 4 h after mercury treatment. Phenolsulfonphthalein in the urine was measured by the method of Ishii.

Calcium Concentration in Serum and Urine of Rats after Pretreatment with Na₂MoO₄ Rats were divided into 2 groups of 5 rats each and were individually placed in metabolic cages. The rats were treated with either saline or Na₂MoO₄ as in the above experiment. Urine was collected in an ice bath from 20 to 24 h after the final i.p. injection of Na₂MoO₄. Blood was collected by cardiac puncture 24 h after the final i.p. injection of Na₂MoO₄. Calcium in the serum and urine was determined by
atomic absorption spectrophotometry.

Results and Discussion

It is generally believed that mercury is excreted in urine through glomerular filtration and tubular secretion. However, it is expected that such excretory functions of the kidney for mercury will be increasingly impaired after exposure to HgCl₂ because of the development of mercury toxicity. Actually, many renal cells are found in the urine of mercury-treated rats. Considering that in our previous study, urine samples were collected every 24 h after exposure to HgCl₂, these results suggest that Na₂MoO₄ enhances the urinary excretion of mercury by stimulating normal renal functions as described above or rather by aggravating mercury toxicity. To examine this question, in the present study, urinary mercury and enzyme were measured at earlier time points than in the previous study.

Figure 1 shows urine excretion of mercury up to 12 h after exposure to HgCl₂. HgCl₂-alone rats excreted only a total of 20 μg of mercury up to 12 h whereas Na₂MoO₄ pretreated rats excreted more than 60 μg of Hg. On the other hand, enzyme activity (alkaline phosphatase) in the urine collected between 8 and 12 h after exposure to HgCl₂ was 12-fold higher in the former than the latter (not shown in this paper). These results suggest that the stimulative effect of Na₂MoO₄ on urinary excretion of mercury is independent of renal tubular damage.

Mercury contents in the liver and kidney of these animals are shown in Fig. 2. Na₂MoO₄ did not cause any significant difference in mercury content in these tissues up to 4 h but thereafter resulted in a lower concentration of the metal in both tissues. These findings suggest that Na₂MoO₄ reduced tissue accumulation of mercury through enhancement of urinary excretion of the metal.

A dose response study on the accumulation of mercury in renal nuclear fraction was carried out to assess how Na₂MoO₄ reduced tissue accumulation of mercury. The kidney is a target organ of the toxicity after administration of an acute dose of mercury. Mercury taken up by the kidney is mainly found in nuclei and cytosol. Nuclei should be a good marker to evaluate accumulation of mercury in the tissue from the point of view of toxicology because of their sensitivity to mercury toxicity. Figure 3 shows mercury content in renal nuclear fraction prepared from rats (open circle) given HgCl₂ at either dose as indicated in the figure and Na₂MoO₄-pretreated rats (closed triangle). Mercury accumulated sigmoidally in the renal nuclear fraction with increase of the administration dose of the metal. The metal content in the renal nuclear fraction from Na₂MoO₄-pretreated rats was almost identical to that from the rats treated with 15 μmol/kg of HgCl₂ alone, even though they had received 30 μmol/kg.

Renal function of Na₂MoO₄-pretreated rats was compared to that of the rats given 15 μmol/kg of HgCl₂ to see whether Na₂MoO₄ could alleviate renal toxicity of mercury through enhancement of its urinary excretion. The results shown in Table 1 suggest that the renal function of Na₂MoO₄-pretreated rats was better maintained as compared to that in rats given 15 μmol/kg of HgCl₂, although there was no difference in mercury content of the kidney between the two groups. The above data clearly suggest that Na₂MoO₄ could alleviate renal toxicity of HgCl₂ by decreasing tissue accumulation of mercury through enhancement of urinary excretion of the metal.

![Fig. 1. Urinary Excretion of Mercury in Rats Given HgCl₂ with (●) or without (○) Na₂MoO₄ Pretreatment](image1)

![Fig. 2. Mercury Content in the Liver (Left) and Kidney (Right) of Rats Given HgCl₂ with (●) or without (○) Na₂MoO₄ Pretreatment](image2)

![Fig. 3. Dose-Response Study on Renal Nuclear Accumulation of Mercury](image3)
Lactate content in the kidney of rats given HgCl₂ with or without Na₂MoO₄ was measured to clarify the mechanism by which Na₂MoO₄ enhances urinary excretion of mercury. Excretory functions of kidney for mercury are affected directly or indirectly by the changes in renal blood flow.³¹ Mercury reduces renal blood flow.¹³ A rise in lactate level is observed in the kidney subjected to hypoxia owing to reduction of renal blood flow.¹⁴ Therefore lactate would become a good marker to assess the change in renal blood flow. The results are shown in Fig. 4. HgCl₂-alone rats showed 1.5-fold higher lactate content than the control at 0 time at as early as 4h after exposure to HgCl₂, then about a 3-fold higher value. Na₂MoO₄-pretreated rats had consistently lower concentrations of the acid than HgCl₂-alone rats throughout this experiment. These results suggest that Na₂MoO₄ prevented a drop in renal blood flow induced by mercury. Therefore, it might be concluded that the stimulative effect of Na₂MoO₄ on urinary excretion of mercury is attributable to better renal hemodynamics arising from the pretreatment with the metal.

We next examined urinary excretion of phenolsulfonphthalein to determine how Na₂MoO₄ affects tubular secretion. The experiment was done between 0 and 4h after exposure to HgCl₂. This agent is excreted in urine by proximal tubular secretion. The results are shown in Table II. The excretion of the agent was significantly decreased only in HgCl₂-alone rats when compared to the control. This suggests that in Na₂MoO₄-pretreated rats, renal tubular secretion had worked almost normally. On the other hand, creatinine clearance is a good indicator for glomerular filtration, although in rat, a part of endogenous creatinine is excreted in urine by tubular secretion.¹⁵ We compared the clearance rate between HgCl₂-alone and Na₂MoO₄-pretreated rats 12h after exposure to HgCl₂. The clearance rate was larger in the latter than in the former. The rate per 5 rats was 30.1 ± 5.0 (ml/min) for the former and 98.9 ± 4.2 for the latter.

The above results indicate that renal blood flow significantly affects urinary excretion of mercury. The mechanism by which mercury reduces renal blood flow is not clear, although there is some evidence supporting the participation of the renin-angiotensin system.¹⁶a,b,¹⁹ Interestingly, it is known that vanadate reduces renal blood flow.¹⁷ This action of vanadate seems to be related to extracellular calcium level, because thyroparathyroidectomy-induced decrease in extracellular calcium blunts the vanadate action but re-establishment of serum calcium toward normal level by addition of CaCl₂ into the infusion medium elicits the vanadate action.¹⁰ This suggests the importance of extracellular calcium for regulation of renal blood flow.

In addition, some molybdenum compounds seem to affect calcium metabolism in bone. We next measured calcium concentration in serum and urine obtained from Na₂MoO₄-alone rats 24h after the final i.p. injection of this metal. The results are shown in Table III. Na₂MoO₄ significantly reduced serum calcium but did not affect urinary calcium. The reason why Na₂MoO₄ reduces serum calcium is unknown at present, but one possibility is that Na₂MoO₄-induced hypocalcemia is closely related to the preventive effect of this metal against the decrease of renal blood flow caused by mercury.

The present study has demonstrated that Na₂MoO₄ alleviated HgCl₂-induced acute renal failure by decreasing...
<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum (mg/dl)</td>
</tr>
<tr>
<td>Control</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td>Na₂MoO₄ (1.24 mmol/kg)</td>
<td>8.9 ± 0.1</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. from 5 rats. a) p < 0.05, significantly different from the control.

tissue accumulation of mercury through enhancement of urinary excretion of the metal. This stimulative action of Na₂MoO₄ on urinary excretion of mercury seems to be attributable to better renal hemodynamics arising from the pretreatment with the metal. This facilitates the urinary excretion of mercury through normal renal functions such as glomerular filtration and tubular secretion. On the other hand, Na₂MoO₄ may also enhance urinary excretion of mercury by inhibiting reabsorption of the metal from the tubular lumen. Na₂MoO₄ increases urinary amino acids (unpublished data). This is probably connected with the adverse effect of Na₂MoO₄ on renal energy metabolism.¹ [x]

There is a little evidence suggesting that mercury is reabsorbed from the renal tubular lumen in an energy-dependent manner.

In summary, Na₂MoO₄ enhances urinary excretion of mercury by preventing mercury-induced reduction of renal blood flow, alleviating the acute mercury-induced renal failure.

References