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

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In order to gain further insight into the protective action of Na_2MoO_4 pretreatment (1.24 mmol/kg, once a day, i.p.) against the acute toxicity of $HgCl_2$ (30 μmmol/mmol/kg, once, s.c.), changes of renal function, tissue accumulation of mercury, and urinary excretions of mercury and phenolsulfonphthalein after exposure to $HgCl_2$ were investigated. Lactate content in the kidney and serum calcium were also measured.

Na_2MoO_4 pretreatment enhanced urinary excretion of mercury. Renal function of Na_2MoO_4-pretreated rats was better maintained as compared to that of the rats given $HgCl_2$ 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 $HgCl_2$-alone rats. This pretreatment prevented the rise in lactate content in the kidney and the reduction of urinary excretion of phenolsulfonphthalein caused by $HgCl_2$. Na_2MoO_4 reduced serum calcium. These results suggest that Na_2MoO_4 prevented mercury-induced acute renal failure by decreasing tissue accumulation of the metal through urinary excretion of mercury. Better renal hemodynamics attributable to hypocalcalcemia 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 Na_2MoO_4 protected them against the acute toxicity of $HgCl_2$, and the protective action of Na_2MoO_4 was exerted by enhancing mercury-induced renal metallothionein induction and urinary excretion of mercury.[1a-e] It has been presumed that the latter action of Na_2MoO_4 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.[2] 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 $HgCl_2$ were evaluated in relation to urinary excretion of mercury. The mechanism of stimulative action of Na_2MoO_4 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 Na_2MoO_4 dissolved in saline once a day for 3 d at a dose of 1.24 mmol/kg and was given one s.c. injection of $HgCl_2$ dissolved in saline at a dose of 30 μmol/kg 24 h after the final i.p. injection of Na_2MoO_4. The other was treatd with saline instead of Na_2MoO_4 for 3 d and received the same dose of $HgCl_2$ as Na_2MoO_4-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 $HgCl_2$. Five rats from each group were killed by cervical dislocation 4, 8 and 12 h after exposure to $HgCl_2$ 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.[1a]

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 $HgCl_2$ 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 Na_2MoO_4 as in the above experiment and were given one s.c. injection of $HgCl_2$ at a dose of 30 μmol/kg 24 h after the final i.p. injection of Na_2MoO_4. All the rats in all the experimental groups were killed by cervical dislocation 12 h after exposure to $HgCl_2$ and the kidneys were processed and removed as in the above experiment. Renal nuclear fraction was prepared according to the method of Hogeboom.[3] Mercury in the crude nuclei was analyzed as described above.

Renal Function after Exposure to $HgCl_2$ 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 $HgCl_2$ either 15 or 30 μmol/kg 24 h after the final i.p. injection of saline. Group 4 rats were pretreated with Na_2MoO_4 as in the above experiment and received one s.c. injection of $HgCl_2$ at a dose of 30 μmol/kg 24 h after the final i.p. injection of Na_2MoO_4. The rats were killed by exsanguination by cardiac puncture 12 h after exposure to $HgCl_2$. 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 Hare[4] and Kitamura and Nishina,[5] 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 Na_2MoO_4 for 3 d as in the above experiment. Group 3 rats received saline for 3 d, then were given 30 μmol/kg of $HgCl_2$ (s.c.). Group 4 rats were pretreated with Na_2MoO_4, then one s.c. injection of $HgCl_2$ as in the above experiment. Group 1 and 2 rats were killed by cervical dislocation 0 h after exposure to $HgCl_2$ and the kidneys were analyzed for lactate. Five rats from groups 3 and 4 were killed 4, 8 and 12 h after exposure to $HgCl_2$ and the kidneys were removed. Lactate was determined by the method of Gutmann and Wahlefeld.[6]

Urinary Excretion of Phenolsulfonphthalein in Rats Given $HgCl_2$ with or without Na_2MoO_4 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 Na_2MoO_4 or $HgCl_2$ or both Na_2MoO_4 and $HgCl_2$ 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 $HgCl_2$. Urine was collected in a ice bath from 0 to 4 h after mercury treatment. Phenolsulfonphthalein in the urine was measured by the method of Ishii.[7]

Calcium Concentration in Serum and Urine of Rats after Pretreatment with Na_2MoO_4 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 Na_2MoO_4 as in the above experiment. Urine was collected in an ice bath from 20 to 24 h after the final i.p. injection of Na_2MoO_4. Blood was collected by cardiac puncture 24 h after the final i.p. injection of Na_2MoO_4. 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.[8a-c] However, it is expected that such excretory functions of the kidney for mercury will be increasingly impaired after exposure to $HgCl_2$ because of the development of mercury toxicity. Actually, many renal cells are found in the urine of mercury-treated rats.[9] Considering that in our previous study,[1a] urine samples were collected every 24 h after exposure to $HgCl_2$, these results suggest that Na_2MoO_4 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.[1a] Figure 1 shows urinary excretion of mercury up to 12 h after exposure to $HgCl_2$, $HgCl_2$-alone rats excreted only a total of 20 μg of mercury up to 12 h whereas Na_2MoO_4-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 $HgCl_2$ was 12-fold higher in the former than the latter (not shown in this paper). These results suggest that the stimulative effect of Na_2MoO_4 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. Na_2MoO_4 did not cause any significant difference of 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 Na_2MoO_4 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 Na_2MoO_4 reduced tissue accumulation of mercury. The kidney is a target organ of the toxicity after administration of an acute dose of mercury.[10] Mercury taken up by the kidney is mainly found in nuclei and cytosol.[1b] 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 $HgCl_2$ at either dose as indicated in the figure and Na_2MoO_4-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 Na_2MoO_4-pretreated rats was almost identical to that from the rats treated with 15 μmol/kg of $HgCl_2$ alone, even though they had received 30 μmol/kg.

Renal function of Na_2MoO_4-pretreated rats was compared to that of the rats given 15 μmol/kg of $HgCl_2$ to see whether Na_2MoO_4 could alleviate renal toxicity of mercury through enhancement of its urinary excretion. The results shown in Table I suggest that the renal function of Na_2MoO_4-pretreated rats was better maintained as compared to that in rats given 15 μmol/kg of $HgCl_2$, although there was no difference in mercury content of the kidney between the two groups. The above data clearly suggest that Na_2MoO_4 could alleviate renal toxicity of $HgCl_2$ by decreasing tissue accumulation of mercury through enhancement of urinary excretion of the metal.

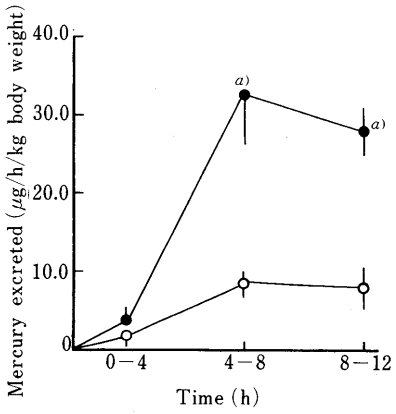

Fig. 1. Urinary Excretion of Mercury in Rats Given $HgCl_2$ with (●) or without (○) Na_2MoO_4 Pretreatment

Rats were pretreated with saline or Na_2MoO_4 as described in Materials and Methods, then were given 30 μmol/kg of $HgCl_2$. Urine was collected every 4 h up to 12 h after exposure to $HgCl_2$. Each value represents the mean ± S.E. from 5 rats. *a)* A significant difference ($p<0.05$) from $HgCl_2$-alone rats at corresponding time points.

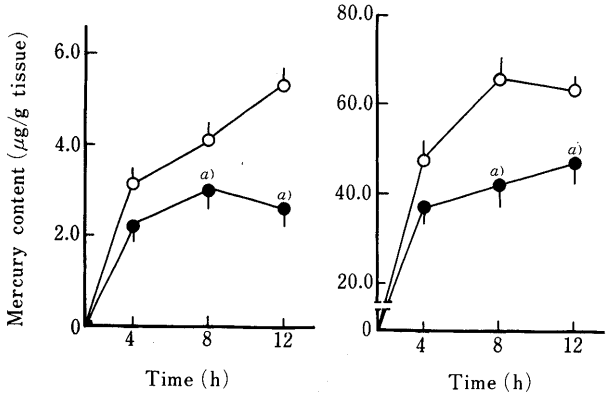

Fig. 2. Mercury Content in the Liver (Left) and Kidney (Right) of Rats Given $HgCl_2$ with (●) or without (○) Na_2MoO_4 Pretreatment

Liver and kidneys were sampled from rats examined for urinary excretion of mercury (Fig. 1) at the time points indicated in the figure. Each value represents the mean ± S.E. from 5 rats. *a)* A significant difference ($p<0.05$) from $HgCl_2$-alone rats at corresponding time points.

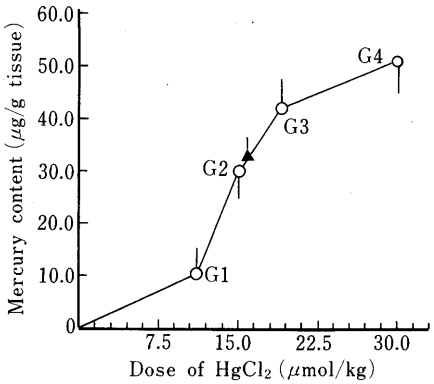

Fig. 3. Dose–Response Study on Renal Nuclear Accumulation of Mercury

Rats were divided into 5 groups. Groups 1—4 (G1—G4) received i.p. injection of saline once a day for 3 d, then were given one s.c. injection of $HgCl_2$ at 11, 15, 19 or 30 μmol/kg. The remaining group was pretreated with Na_2MoO_4 on a day for 3 d as described in Materials and Methods, then received one s.c. injection of $HgCl_2$ at a dose of 30 μmol/kg. All the rats in all the groups were killed 12 h after exposure to $HgCl_2$. The open circles and the closed triangle show mercury content in the renal nuclear fraction prepared from G1—4 rats and Na_2MoO_4 pretreated rats, respectively. The data represent the mean ± S.E. from 5 rats.

TABLE I. Renal Function of Rats Given $HgCl_2$ with or without Na_2MoO_4 Pretreatment

Experimental groups	Mercury content in the kidney (μg/g tissue)	Blood urea nitrogen (mg/dl serum)	Serum creatinine (mg/dl serum)
Control	ND	19.29 ± 1.89	0.54 ± 0.03
$HgCl_2$ (15 μmol/kg)	$48.42 \pm 0.59^{b)}$	$49.17 \pm 4.97^{a)}$	$2.62 \pm 0.09^{a)}$
$HgCl_2$ (30 μmol/kg)	63.22 ± 1.59	$71.63 \pm 6.81^{a)}$	$2.49 \pm 0.26^{a)}$
Na_2MoO_4 (1.24 mmol/kg) + $HgCl_2$ (30 μmmol/kg)	$47.20 \pm 2.94^{b)}$	$33.40 \pm 4.12^{a,c)}$	$1.69 \pm 0.15^{a,c)}$

Serum and kidney samples were obtained 12 h after exposure to $HgCl_2$. Each value represents the mean $\pm$ S.E. from 5 rats. *a)* $p < 0.05$, significantly different from the control. *b)* $p < 0.05$, significantly different from the rats given $HgCl_2$ at a dose of 30 μmol/kg. *c)* $p < 0.05$, significantly different from the rats given $HgCl_2$ at either 15 or 30 μmol/kg.

Lactate content in the kidney of rats given $HgCl_2$ with or without Na_2MoO_4 was measured to clarify the mechanism by which Na_2MoO_4 enhances urinary excretion of mercury. Excretory functions of kidney for mercury are affected directly or indirectly by the changes in renal blood flow.[11] Mercury reduces renal blood flow.[12] A rise in lactate level is observed in the kidney subjected to hypoxia owing to reduction of renal blood flow.[13] Therefore lactate would become a good marker to assess the change in renal blood flow. The results are shown in Fig. 4. $HgCl_2$-alone rats showed 1.5-fold higher lactate content than the control at 0 time at as early as 4 h after exposure to $HgCl_2$, then about a 3-fold higher value. Na_2MoO_4-pretreated rats had consistently lower concentrations of the acid than $HgCl_2$-alone rats throughout this experiment. These results suggest that Na_2MoO_4 prevented a drop in renal blood flow induced by mercury. Therefore, it might be concluded that the stimulative effect of Na_2MoO_4 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 Na_2MoO_4 affects tubular secretion. The experiment was done between 0 and 4 h after exposure to $HgCl_2$. 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 $HgCl_2$-alone rats when compared to the control. This suggests that in Na_2MoO_4-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 $HgCl_2$-alone and Na_2MoO_4-pretreated rats 12 h after exposure to $HgCl_2$. 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 suggesting the participation of the renin-angiotensin system.[16a,b] Interestingly, it is known that vanadate reduces renal blood flow.[17] This action of vanadate seem 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 $CaCl_2$ into the infusion medium elicits the vanadate action.[18] This suggests the importance of extracellular calcium for regulation of renal blood flow.

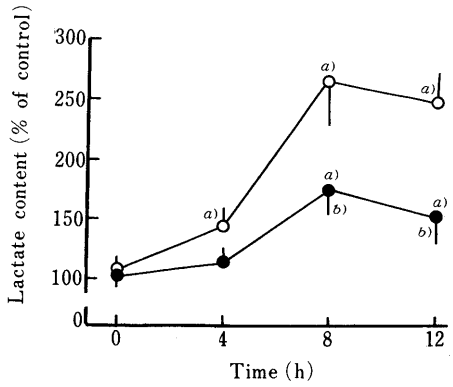

Fig. 4. Lactate Content in the Kidney of Rats Given $HgCl_2$ with (●) or without (○) Na_2MoO_4 Pretreatment.

The data show the mean of lactate content from 5 rats expressed as % of the control (0.51 ± 0.04 μmol/g wet tissue) at 0 time after exposure to $HgCl_2$, with the S.E. *a)* $p < 0.05$, significantly different from the control. *b)* $p < 0.05$, significantly different from $HgCl_2$-alone rats.

TABLE II. Urinary Excretion of Phenolsulfonphthalein in Rats Given $HgCl_2$ with or without Na_2MoO_4 Pretreatment

Experimental groups	Phenolsulfonphthalein excreted (% of administered dose)
Control	44.0 ± 3.7
Na_2MoO_4 (1.24 mmol/kg)	40.6 ± 3.6
$HgCl_2$ (30 μmol/kg)	$13.6 \pm 4.3^{a)}$
Na_2MoO_4 (1.24 mmol/kg) + $HgCl_2$ (30 μmol/kg)	$41.9 \pm 0.8^{b)}$

Urine samples were collected 2 to 4 h after simultaneous injection of mercuric chloride and phenolsulfonphthalein. Each value represents the mean $\pm$ S.E. from 5 rats. *a)* $p < 0.05$, significantly different from the control. *b)* $p < 0.05$, significantly different from the rats given $HgCl_2$ alone.

In addition, some molybdenum compounds seem to affect calcium metabolism in bone. We next measured calcium concentration in serum and urine obtained from Na_2MoO_4-alone rats 24 h after the final i.p. injection of this metal. The results are shown in Table III. Na_2MoO_4 significantly reduced serum calcium but did not affect urinary calcium. The reason why Na_2MoO_4 reduces serum calcium is unknown at present, but one possibility is that Na_2MoO_4-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 Na_2MoO_4 alleviated $HgCl_2$-induced acute renal failure by decreasing

TABLE III. Calcium Concentration in Serum and Urine of Rats after Pretreatment with Na_2MoO_4

Experimental groups	Calcium	
	Serum (mg/dl)	Urine (μg/h/kg body weight)
Control	10.0±0.2	22.4± 8.0
Na_2MoO_4 (1.24 mmol/kg)	8.9±0.1[a)]	43.2±17.7

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 Na_2MoO_4 on urinary excretion of mercury seem 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, Na_2MoO_4 may also enhance urinary excretion of mercury by inhibiting reabsorption of the metal from the tubular lumen. Na_2MoO_4 increases urinary amino acids (unpublished data). This is probably connected with the adverse effect of Na_2MoO_4 on renal energy metabolism.[1e)] There is a little evidence suggesting that mercury is reabsorbed from the renal tubular lumen in an energy-dependent manner.

In summary, Na_2MoO_4 enhances urinary excretion of mercury by preventing mercury-induced reduction of renal blood flow, alleviating the acute mercury-induced renal failure.

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