

Decrease of Calcium Concentration in Urine of Rats treated with Stannous Chloride

MASAYOSHI YAMAGUCHI, HIDEJI SATO, and TAKEO YAMAMOTO

*Shizuoka College of Pharmacy*¹⁾

(Received March 18, 1976)

Effect of stannous chloride on the urinary calcium concentration was examined in rats intraperitoneally administered with stannous chloride. The concentration of calcium in urine was significantly decreased by the administration of stannous chloride (Sn 3.0 mg/100 g), while the concentration of inorganic phosphorus was not altered markedly. The reduction of calcium concentration in the urine, induced by stannous chloride, was not restored by the injection of calcium in rats treated with stannous chloride. The present study indicates that the administration of stannous chloride inhibited the excretion of calcium into urine of rats.

Several cases of tin intoxication have been observed after intake of canned food containing a high level of tin.²⁾ However, the mechanism of toxic action of tin has not been fully resolved. Recently, Benoy *et al.*³⁾ reported that the concentration of tin in the rats ingesting orange juice (tin content, 540 ppm) for 7 to 36 days was mainly found in the gastrointestinal tract and kidneys, and that tin was found in a large amount in kidney of mice subcutaneously administered with tin citrate. These facts suggest that tin absorbed into the body is accumulated specifically in the kidneys. It is possible that tin induces disturbance of the kidneys in tin poisoning.

We had examined the effect of stannous chloride on the calcium metabolism in order to investigate the mechanism of toxic action of tin, and found that the administration of stannous chloride induced accumulation of calcium in the kidneys and decrease of calcium in serum.⁴⁾ Previously, we reported that the reduced serum calcium caused by tin is a result of kidney disturbance from calcium accumulation in the kidney.⁵⁾ Therefore, the present studies were undertaken to examine whether the calcium concentration in urine is influenced by the administration of stannous chloride and it was found that the urinary excretion of calcium is markedly decreased by the administration of stannous chloride.

Methods

Male Wistar strain rats, each weighing approximately 120 g, were used in this experiment. The animals were kept in a room temperature of $25^{\circ} \pm 1^{\circ}$ and fed on lab chow and tap water freely.

SnCl₂, freshly dissolved in HCl solution (pH 1.8) was diluted with distilled water to a final concentration of 3 mg/ml as Sn. This solution was given as a single intraperitoneal injection (1.0 ml/100 g body weight) to the rats and controls were injected with HCl solution (pH 1.8). The animals were fixed and then urine was directly collected from penis into a test tube in order to prevent contamination of the feces. Collection of urine was started 24 or 72 hr after the administration of SnCl₂, continued for a period of 2 hr, and then the animals were subcutaneously injected with the solution of CaCl₂ (2.0 mg/100 g as Ca) 26 or 74 hr after SnCl₂ treatment and again the urine was collected for a period of 2 hr. The animals were given the diet and water during collection of urine.

Determination of calcium and inorganic phosphorus was made on 0.2-ml aliquot of the urine. The urinary calcium was determined by the atomic absorption spectrophotometry (Perkin-Elmer, Model 303) after

- 1) Location: 2-1, Oshika 2-chome, Shizuoka, 422, Japan.
- 2) S. Warburton, W. Ulder, R.M. Ewert, and W.S. Haynes, *Public Health Rep.*, 77, 798 (1962).
- 3) C.J. Benoy, P.A. Hooper, and R. Schneider, *Food Cosmet. Toxicol.*, 9, 645 (1971).
- 4) T. Yamamoto, M. Yamaguchi, and H. Sato, *J. Toxicol. Environ. Health.*, 1, 749 (1976)
- 5) M. Yamaguchi, H. Sato, and T. Yamamoto, *Jap. J. Hyg.*, 30, 536 (1975).

precipitation of the protein with 10% trichloroacetic acid.⁶⁾ The urinary inorganic phosphorus was determined by the method of Taussky and Shon.⁷⁾

Results

The effect of stannous chloride on the urinary inorganic phosphorus and calcium concentrations after a single intraperitoneal administration of this compound in rats is shown in Table I. The collection of urine was started 24 hr after the administration of stannous chloride and continued for a period of 2 hr. Then, the concentration of inorganic phosphorus in urine (mg/100 ml and $\mu\text{g/hr}$) increased significantly compared with the control rats, while the level of calcium in urine (mg/100 ml and $\mu\text{g/hr}$) decreased significantly. Subsequently, the treated or control rats were given calcium chloride as a single subcutaneous injection after the collection of urine for a period of 2 hr, and then the urine was further collected for a period of 2 hr. The elevation of urinary inorganic phosphorus level produced by the treatment of stannous chloride was markedly prevented by the injection of calcium chloride, while the concentration of calcium was not inhibited significantly.

On the other hand, the same experiment was examined 72 hr after the administration of stannous chloride. The urinary inorganic phosphorus level did not show a significant alteration in the stannous chloride-treated rats, with or without calcium chloride injection compared with the control rats, while the urinary calcium concentration (mg/100 ml and $\mu\text{g/hr}$) decreased strikingly. Meanwhile, a significant ($p < 0.01$) increase in the urinary calcium concentration (mg/100 ml) occurred following the injection of calcium chloride to the control rats when compared with the values obtained from untreated rats. However, the injection of calcium chloride in the stannous chloride-treated rats did not affect the urinary calcium concentration. Thus, the administration of stannous chloride to rats caused the inhibition of urinary calcium excretion.

TABLE I. The Concentration of Inorganic Phosphorus and Calcium in Urine of Rats treated with Stannous Chloride

Treatment	Urine volume ^{a)} (ml/hr)	Inorganic phosphorus		Calcium		
		(mg/100 ml)	($\mu\text{g/hr}$)	(mg/100 ml)	($\mu\text{g/hr}$)	
24 hr after tin administration						
None	Control	0.64 ± 0.19	46.1 ± 14.2	269 ± 84.4	30.4 ± 9.7	173 ± 52.9
	SnCl ₂	0.59 ± 0.05	84.5 ± 5.1 ^{b)}	487 ± 54.9 ^{b)}	7.8 ± 2.7 ^{c)}	45.1 ± 14.9 ^{c)}
Calcium	Control	0.47 ± 0.10	103 ± 15.9	399 ± 18.7	35.2 ± 10.8	132 ± 24.3
	SnCl ₂	0.43 ± 0.08	100 ± 3.0	430 ± 83.4	12.7 ± 3.5 ^{d)}	58.2 ± 22.6 ^{d)}
72 hr after tin administration						
None	Control	0.62 ± 0.12	74.2 ± 18.5	369 ± 86.2	12.8 ± 3.1	74.1 ± 16.8
	SnCl ₂	0.45 ± 0.10	78.0 ± 15.0	302 ± 52.6	3.3 ± 0.6 ^{d)}	16.9 ± 5.4 ^{d)}
Calcium	Control	0.27 ± 0.03	118 ± 24.8	306 ± 74.1	35.5 ± 6.7	102 ± 27.8
	SnCl ₂	0.39 ± 0.09	96.4 ± 28.8	330 ± 97.7	3.1 ± 0.6 ^{e)}	11.2 ± 3.6 ^{b)}

a) mean ± SEM for 7 or 10 animals

b) significance from the control $p < 0.05$ (Student's t-test)

c) significance from the control $p < 0.02$

d) significance from the control $p < 0.01$

Discussion

In the previous study, we found that the decrease in serum calcium caused by the administration of stannous chloride is associated with the accumulation of calcium in the kidneys.⁴⁾

6) J.B. Willis, *Nature* (London), **16**, 249 (1960).

7) H.H. Taussky, and E. Shon, *J. Biol. Chem.*, **202**, 675 (1953).

Administration of vitamin D₃ (25 µg/100 g) to normal rats significantly increases the concentration of calcium in serum, while injection of vitamin D₃ to rats treated with stannous chloride does not enhance the concentration of calcium in serum. Also, the predominant increase in kidney calcium concentration induced by the administration of stannous chloride is significantly suppressed by the injection of sodium citrate to the treated rats and the amount of tin accumulated in the kidney of stannous chloride treated rats is not altered. The decreased serum calcium concentration produced by the administration of stannous chloride is significantly enhanced by the injection of sodium citrate, and the hypercalcemia produced by vitamin D₃ in the stannous chloride-treated rats is approximately 50% of the value obtained from normal rats. This fact seems to be consistent with the view that the reduction of serum calcium induced by the administration of stannous chloride is caused by the disturbance of kidney as a result of accumulation of calcium in the kidneys.⁵⁾ However, it is possible that the decreased serum calcium caused by stannous chloride originates at least partly, from the accelerated excretion of calcium into urine by this treatment.

In the present study, we examined the effect of stannous chloride on the urinary calcium concentration. The data obtained from the present experiment clearly demonstrate that the urinary calcium level is markedly reduced by a single intraperitoneal administration of stannous chloride. Accordingly, the reduced serum calcium concentration described above does not originate from the inhibition of the reabsorption of calcium by the kidney cortex. Presumably, the hypercalcemic effect of vitamin D₃, which is converted into the active form, 1,25-dihydroxycholecalciferol, in the kidney is not able to be exhibited in rats administered with stannous chloride and this fact suggests that the stannous chloride-treated rats may have a lowered ability to absorb calcium from the intestine by the action of 1,25-dihydroxycholecalciferol.⁸⁾ It is known that the active metabolite of vitamin D₃ not only stimulates the mobilization of calcium from bone mineral⁹⁾ but also amplifies the action of bone absorption of a parathyroid hormone.¹⁰⁾ This suggests that the release of calcium into blood from the bone is decreased by the administration of stannous chloride. The present result further supports the idea that the decreased serum calcium concentration produced by the administration of stannous chloride is mainly caused by the disturbance of vitamin D₃ metabolism in the kidneys induced as a result of the accumulation of calcium in the kidneys.

8) J.F. Myrtle, and A.W. Norman, *Science* **171**, 79 (1971).

9) Y. Tanaka, and H.F. DeLuca, *Arch. Biochem. Biophys.*, **146**, 574 (1971).

10) A. Mahgoub, and H. Sheppard, *Biochem. Biophys. Res. Commun.*, **62**, 901 (1975).