

EFFECTS OF SODIUM CITRATE, POTASSIUM CITRATE, AND CITRIC ACID IN PREVENTING EXPERIMENTAL CALCIUM OXALATE UROLITHIASIS IN RATS

Yoshihide OGAWA, Tohru TANAKA, Kazumi YAMAGUCHI,
Makoto MOROZUMI and Ryuichi KITAGAWA

*From the Department of Urology, School of Medicine, Juntendo University
(Director: Prof. R. Kitagawa)*

Male Wistar-strain rats which had been fed a glycolic-acid diet developed severe nephrocalcinosis with urinary calculi within 4 weeks. Rats fed the same diet with citrate salts added had, however, either slight or no nephrocalcinosis without any stones in the urinary system. Nephrocalcinosis intermediate between those in the citrate groups and the glycolic-acid group, with some urinary calculi, was observed in the citric-acid group. During the experiment, the urinary oxalate concentration increased markedly and was higher in the citrate and citric-acid than in the glycolic-acid group. The urinary citrate concentration was significantly higher in the citrate groups and lower in the citric acid and glycolic-acid groups.

Therefore, citrate salts can be concluded to inhibit nephrocalcinosis and calculi formation as a result of decreased urinary saturation by means of increase in urinary citrate, in spite of a slight increase in the urinary oxalate.

Key words: Calcium-oxalate urolithiasis, Sodium citrate, Potassium citrate, Citric acid

INTRODUCTION

Citrate has been reported to be an *in vitro* inhibitor of the crystallization of stone-forming calcium salts¹. The clinical usefulness of potassium citrate for renal stone-disease has also been reported², but there have been few reports on its effect in animal (non-human) experiments³. Previously, we demonstrated that a 5% pyruvate salt-containing glycolic-acid diet (calcium-oxalate lithogenic diet) inhibited nephrocalcinosis and stone formation⁴. Adequate inhibitory activity of citrate would be obtained at a similar concentration. Therefore, we investigated whether or not potassium citrate is superior to sodium citrate and whether or not citric acid has any effect in preventing nephrocalcinosis and stone-formation.

MATERIALS AND METHODS

Male Wistar-strain rats (ca 150 g) were

acclimated 1 week and then randomly divided into 4 groups, each group consisting of 5~6 rats; the calcium-oxalate lithogenic diet (a glycolic-acid diet) was MM-1 (Funabashi Farms, Japan) containing 3% glycolic acid (GC group)⁵, and the glycolic acid diet containing 5% sodium citrate (Na citrate+GC group) or an equimolar level of potassium citrate (K citrate+GC group) or citric acid (citric acid+GC group). All the diets were fed in powder form, and the rats had access to drinking water *ad libitum*. Rats were weighed weekly. Pooled 24-hour urine samples from each group were collected weekly by using metabolic cages in two-some and three-some fashion; they were collected at 0 week from rats on the basal diet (MM1). Immediately thereafter the experimental diets were started. They were collected in flasks containing 100 μ l of 20% chlorohexidine gluconate⁶, and their volumes and pH were measured; they were then acidified

to a pH lower than 2.0⁷⁾ and subsequently stored at -40°C until analysis. At the end of the fourth experimental week, all the rats were killed. All the urinary tracts were examined for calculi-formation and nephrocalcinosis, the latter of which was expressed in 4 grades (nil-, slight +, moderate ++, and severe +++) in accordance with the criteria of Hasegawa et al.⁸⁾ Half of the right kidneys were weighed and homogenized in 5 ml of 2 N HCl by means of a disintegrator (Bio-mixer). The supernatant was deproteinized with an equal portion of 6% sulfosalicylic acid and then diluted 100-fold with deionized distilled water for the oxalate determination by ion-chromatography⁹⁾. The urine samples were also analyzed for oxalate and citrate by ion-chromatography after a 100-fold dilution and¹⁰⁾ for calcium and magnesium by atomic-absorption spectrophotometry after 100-fold and 1,000-fold dilutions respectively. The Bonferroni method was used to test the statistical significance between groups, while paired t tests were used to test the responses of the same animals to each treatment over time¹¹⁾.

RESULTS

Within four weeks, the body weight increased in the sodium-citrate and potas-

sium-citrate groups (60.1 ± 24.1 g, mean \pm SE, and 106.0 ± 8.7 g respectively); growth was retarded somewhat in the glycolic-acid group (25.2 ± 9.5 g) and was retarded markedly in the citric-acid group (-3 ± 9.5 g). The urinary volume was higher in the glycolic-acid and sodium-citrate groups than in the potassium-citrate and citric-acid groups. Throughout the experiment, the 24-hour urinary excretion of oxalate was extremely high in all 4 groups compared with that reported in a basal diet group. The 24-hour urinary excretion of oxalate was highest in the first or second week in all but the potassium citrate group, and then decreased toward the fourth experimental week. The 24-hour urinary oxalate concentration (Fig. 1) was persistently higher in the citrate groups and was significantly higher in the potassium citrate-group than in the glycolic-acid group. In the citrate groups, the 24-hour urinary excretion of citrate increased markedly; the 24-hour urinary excretion of citrate stayed low in the glycolic-acid group and decreased in the citric-acid group. The 24-hour urinary citrate concentration (Fig. 2) was consistently high in the citrate groups (being highest at the fourth week) and was consistently lowest in the glycolic-acid and citric-acid groups.

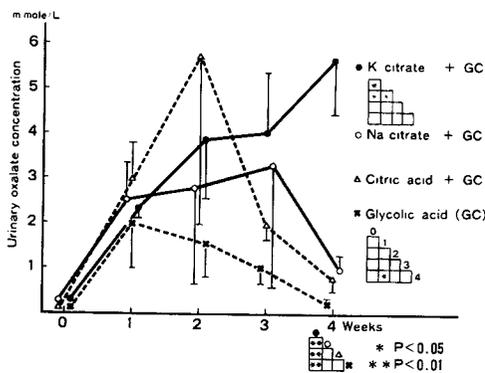


Fig. 1. The 24-hour urinary oxalate concentration (mean \pm SE) was high in all groups during experiment. It increased over time in the potassium-citrate group and was significantly higher in the potassium citrate group than in the other three groups ($p < 0.01$). It was highest at the first, second, or third experimental week and decreased at the fourth week in the latter three groups.

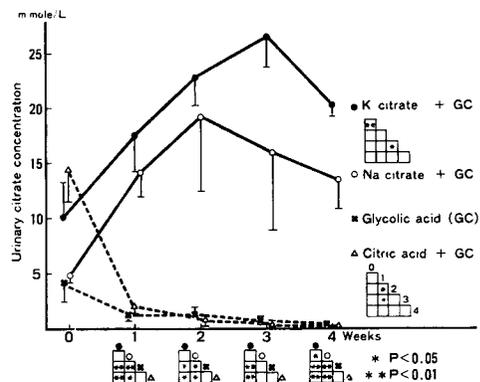


Fig. 2. The 24-hour urinary citrate concentration (mean \pm SE) was high in the citrate-salt groups throughout experiment. It was consistently high in the citrate groups ($p < 0.05$), was highest at the second or third week, and then decreased at the fourth week. It was consistently lowest in the citric-acid and glycolic-acid groups ($p < 0.05$).

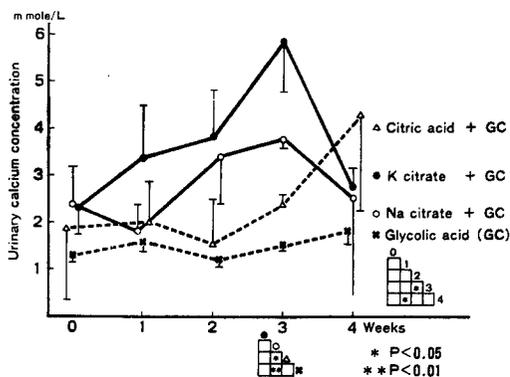


Fig. 3. The 24-hour urinary calcium concentration (mean±SE) was highest in the potassium-citrate group and was lowest in the glycolic-acid group during experiment, but differences were not statistically significant.

The 24-hour urinary excretion of calcium was higher in the citrate groups than in the citric-acid and glycolic-acid groups; the 24-hour urinary calcium concentration was also higher in the citrate groups than in the citric-acid and glycolic-acid groups (Fig. 3). However, there was no statistically significant difference between any two groups in urinary calcium concentration; therefore, the urinary calcium concentration was not affected by the various diets. There was no significant difference between any two groups in the 24-hour urinary excretion of magnesium. There was no significant difference in the 24-hour urinary magnesium concentration in (Fig. 4) between any two groups. The 24-hour urinary pH (Fig. 5) was consistently

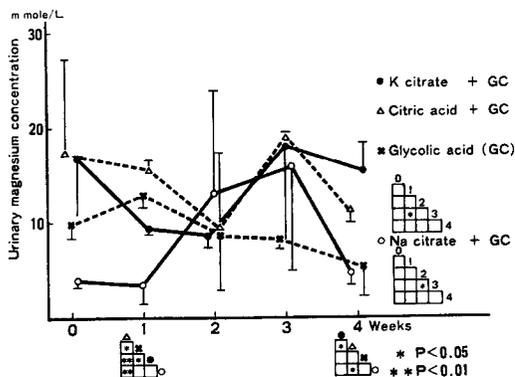


Fig. 4. The 24-hour urinary magnesium concentration (mean±SE) was highest at the first week in the citric-acid group ($p < 0.05$). Overall, there was no definite trend in the urinary magnesium concentration during experiment.

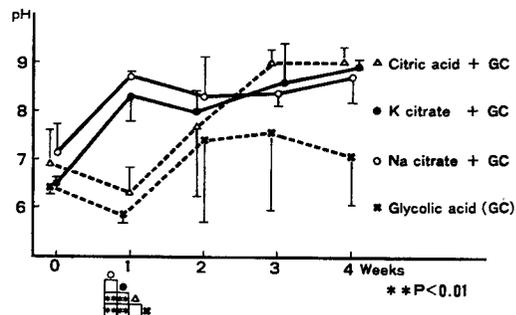


Fig. 5. The 24-hour urinary pH (mean±SE) was consistently lowest in the glycolic-acid group. It was significantly higher in the citrate-salt groups than in the acid groups at the first experimental week ($p < 0.01$). Although it increased toward the end of the experiment in the citric-acid group, this trend was not statistically significant.

EXPERIMENT GROUP	DEGREE OF NEPHROCALCINOSIS (rats)				Mean of ridit	UROLITHIASIS IN PELVIS URETER BLADDER						
	3+	2+	+	0		+	-	+	-	+	-	
Glycolic acid (GC) (5)	4	4	2	0	0.18**	6	4	2	8	0	1	4
Na citrate + GC (5)	0	0	0	10	0.76**	0	10	0	10	0	0	5
K citrate + GC (5)	0	0	2	8	0.69**	0	10	0	10	0	0	5
Citric acid + GC (5)	0	4	5	1	0.36**	3	7	2	8	2	2	1

** $P < 0.01$

Fig. 6. Effects of sodium citrate, potassium citrate, and citric acid on incidence of urolithiasis. Almost no gross calculi were observed in the citrate groups, although there were a few in the citric-acid group. Marked calculi deposits were observed in the glycolic-acid group.

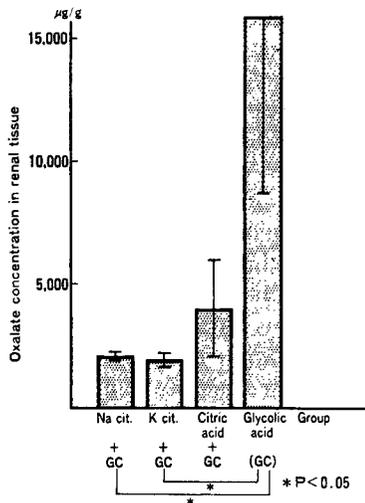


Fig. 7. Mean oxalate concentrations (mean \pm SE) of the renal tissue at the fourth experimental week. There were significant differences in renal oxalate concentration between the glycolic-acid group and the alkali salt groups ($p < 0.05$). There was no significant difference between the sodium-citrate and potassium-citrate groups.

lower in the glycolic-acid and citric-acid groups, and there was a significant difference between the citrate groups and the acid groups. The 3% glycolic-acid diet was a potent calculi-former; nephrocalcinosis and urinary calculi were observed in the kidneys of all 5 rats in the glycolic-acid group (Fig. 6). The urinary calculi were identified by means of infrared-spectrophotometry as being composed of calcium oxalate. Sodium and potassium citrate eliminated nephrocalcinosis and urinary calculi to a similar extent. The oxalate concentrations of the kidney tissue (Fig. 7) reflected the degree of nephrocalcinosis. At the same equimolar level, the inhibitory activity of potassium citrate was the same as that of sodium citrate ($p < 0.05$), based on the oxalate tissue level and the degree of nephrocalcinosis. There was a significant difference in the renal oxalate level and the degree of nephrocalcinosis between, on the one hand, the sodium citrate group and the potassium citrate group and, on the other hand, the glycolic-acid group ($p < 0.05$).

DISCUSSION

Citrate has been shown *in vitro* to inhibit the crystallization of calcium oxalate and calcium phosphate¹²⁾, the two of which substances together constitute more than 70% of urinary-tract stones. In urine, citrate complexes calcium, reducing the free calcium-ion activity. This effect is pH-dependent, with maximum complex formation at or above pH 6.5. By decreasing the free calcium-ion activity, the state of urinary saturation for both the calcium-oxalate and calcium-phosphate-crystal systems is reduced. The second role of citrate is as a specific inhibitor of crystal growth and aggregation, although aggregation has been less well studied. Citrate is one of the major inhibitors of calcium-phosphate-crystal growth. In the calcium oxalate system, its effect is thought to be small and pH-dependent. Although phosphocitrate and citrate-metal complexes are potent inhibitors of the calcium-oxalate¹³⁾ and calcium phosphate-crystal-growth systems, none of these substances have been demonstrated to occur in urine, so their biological importance is not established. Based on these theories, Pak and his associates conducted a long-term clinical trial with potassium citrate¹⁴⁾. This treatment produced a significant increase in urinary pH and citrate and also a significant clinical improvement for hypocitraturic calcium nephrolithiasis and uric acid lithiasis with or without calcium stones. Our present results, however, are not in complete agreement with their clinical results: citrate salts inhibit experimental urolithiasis in the rat by increasing the urinary citrate concentration, with a slight increase in the urinary oxalate concentration; potassium citrate would seem to be advantageous over sodium citrate in increasing the urinary citrate concentration, although the differences in inhibitory activity were not significant. More studies are needed to clarify the latter problem. In our experimental model, the inhibitory activity is defined on the bases of the renal tissue level of oxalate and the de-

gree of nephrocalcinosis. Tissue oxalate probably involves multiple factors, but it may correlate with the degree of nephrocalcinosis and calculi formation. Moreover, the doses of the inhibitory substances administered to rats were 100 times as high as those administered to humans. Therefore, it is difficult to relate the findings obtained in animal studies to clinical conditions. However, any substances which eliminate nephrocalcinosis and stone formation in rats may possibly inhibit stone formation in humans.

Increased urinary oxalate concentration during long-term treatment with potassium citrate was reported not to be significant by Pak and his associates. Our present experiment in the rat has confirmed that urinary oxalate is increased significantly at the 4th week, much as in the case of pyruvate salts. Even if this phenomenon reflects the true feature of citrate-salt treatment, the urinary calcium-oxalate saturation decreases, therefore, nephrocalcinosis and stone formation are inhibited.

Urinary calcium was reported by Sakhae and his associates to decrease during short-term treatment with potassium citrate¹⁵⁾. However, Pak and his associates reported that urinary calcium did not change significantly on a long-term basis¹⁴⁾. Our present findings have also confirmed that there are no significant differences in urinary calcium between potassium-citrate and sodium-citrate treatments.

Citric acid has been shown by our present experiment to have no inhibitory effect on lithogenicity nor to increase urinary citrate. This suggests that there is no advantage in administering citrate in an acid form in order to increase urinary citrate. We also reported this previously for pyruvic acid. Recent advancements in renal physiology have demonstrated that systemic acid-base changes cause striking changes in citrate clearance and metabolism. Any organic acid, causing acidosis in rats, naturally decreases urinary citrate excretion and does not actively inhibit stone formation.

In summary, our present experiment

using glycolate-induced calcium-oxalate lithiasis has shown that citrate administration increases the urinary citrate concentration and also the urinary oxalate concentration, without exerting any significant effect on the urinary concentrations of calcium and magnesium. Therefore, sodium and potassium citrate salts can be concluded to inhibit nephrocalcinosis and urinary calculi formation in rat experimental lithiasis as a result of the reduced urinary saturation caused by the urinary citrate increase, in spite of a slight increase in the urinary oxalate.

ACKNOWLEDGEMENTS

The authors wish to thank Miss Y. Seki for her skillful technical assistance and Mr. Yukio Fukuda for his advice on the statistical calculations.

REFERENCES

- 1) Shorr E, Almy TP, Sloan M, Taussky Hand Toscani V: The relation of citric acid and calcium; its implications for urinary calcium stone formation. *Science* **96**: 587~588, 1942
- 2) Preminger GM, Harvey JA, and Pak CYC: Comparative efficacy of specific potassium citrate therapy versus conservative management in nephrolithiasis of mild to moderate severity. *J Urol* **134**: 658~661, 1985
- 3) Ebisuno S, Morimoto S, Fukatani T, Miyazaki Y, Yasukawa S, Sawada Y and Ohkawa T: Studies of experimental calcium oxalate stones. 2. The preventive effects of phytin and citrate on renal deposition of calcium oxalate induced by ethylene glycol administration. *Jpn J Urol* **76**: 843~849, 1985
- 4) Ogawa Y, Yamaguchi K, Tanaka T and Morozumi M: Effects of pyruvate salts, pyruvic acid, and bicarbonate salts in preventing experimental oxalate urolithiasis in rats. *J Urol* **135**: 1057~1060, 1986
- 5) Chow FC, Hamar DW, Boulay JP and Lewis LD: Prevention of oxalate urolithiasis by some compounds. *Invest Urol* **15**: 493~495, 1978
- 6) Griffith DP and Dunn D: Collection and preservation of urine for biochemical analyses. *Invest Urol* **15**: 459~461, 1978
- 7) Hodgkinson A: Sampling errors in the determination of urine calcium and oxalate: solubility of calcium oxalate in HCl-urine

- mixtures. *Clin Chim Acta* **109**: 239~244, 1981
- 8) Hasegawa M, Kitada H, Toda T, Kakinoki K, Tanaka S, Hara N and Doi S: The effects of rowatin on experimental calcium-oxalate stone formation in the kidney. *Basic Pharmacol Therap* **7**: 2577~2586, 1979
- 9) Ogawa Y and Kitagawa R: Determination of urinary oxalate by ion chromatography: some modifications. *Acta Urol Jpn* **30**: 147~152, 1984
- 10) Ogawa Y, Morozumi M, Tanaka T and Yamaguchi K: Determination of urinary citrate by ion chromatography. *J Urol* **135**: 178~181, 1986
- 11) Wallenstein S, Zucker CL and Fleiss JL: Some statistical methods useful in circulation research. *Circ Res* **47**: 1~9, 1980
- 12) Hastings AB, McLean FC, Eichelberger L, Hall JL and Costa ED: The ionization of calcium, magnesium and strontium citrates. *J Biol Chem* **107**: 351~370, 1934
- 13) Meyer JL and Thomas WC Jr: Trace metal-citric acid complexes as inhibitors of calcification and crystal growth II. Effects of Fe(III), Cr(III) and Al(III) complexes on calcium oxalate crystal growth. *J Urol* **128**: 1376~1378, 1982
- 14) Pak CYC, Fuller C, Sakhaee K, Preminger GM and Eritton F: Long-term treatment of calcium nephrolithiasis with potassium citrate. *J Urol* **134**: 11~19, 1985
- 15) Sakhaee K, Nicari M, Hill K and Pak CYC: Contrasting effects of potassium citrate and sodium citrate therapies on urinary chemistries and crystallization of stone-forming salts. *Kidney Int* **24**: 348~352, 1983
- (Accepted for publication November 14, 1986)

和文抄録

ラット実験的蓚酸カルシウム結石症におけるクエン酸塩、 クエン酸の結石形成抑制作用

順天堂大学医学部泌尿器科学教室 (主任: 北川龍一教授)
小川由英・田中徹・山口千美
諸角誠入・北川龍一

グリコール酸食をウィスター系ラットに4週間投与すると腎石灰化症および尿路結石を著明に認める。この食事にクエン酸塩を添加すると結石形成が抑制される。クエン酸のみの添加ではこの抑制作用がほとんど認められなかった。実験食投与中は尿中蓚酸濃度はグリコール酸のみの対照群に比して、クエン酸およびその塩投与群では高かった。尿中クエン酸濃度はクエン

酸塩投与群では高く、対照群とクエン酸投与群では低かった。尿中のカルシウムおよびマグネシウム濃度は各群間に有意差は認められなかった。したがって、クエン酸塩投与は尿中の蓚酸濃度を多少増加させるが、尿中のクエン酸濃度を著しく増加させるため尿の過飽和状態が低下し、腎石灰化症および尿路結石形成を抑制する。