

A COMPARISON BETWEEN EFFECTS OF PYRUVATE AND HERB MEDICINES IN PREVENTING EXPERIMENTAL OXALATE UROLITHIASIS IN RATS

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Sodium pyruvate, choreito (a herbal preparation), and urajirogashi (a herb) were added to a calcium-oxalate lithogenic diet (a glycolic-acid diet) to determine their effects in preventing lithogenicity. Male Wistar-strain rats which had been fed the glycolic-acid diet developed marked urinary calculi within 4 weeks. Rats in the groups fed a pyruvate diet had, however, almost no stones in the urinary system. The choreito and urajirogashi were slightly less effective than the pyruvate. Urinary oxalate excretion was high in all the groups during the experiment, especially in the pyruvate and the glycolic-acid groups, but, it was relatively lowered in the herb groups, especially towards the end of the experiment ($p < 0.05$). Urinary citrate excretion was high in the pyruvate group, but it was significantly low in the other groups. In the choreito group, remarkable increases in urinary volume and magnesium excretion were observed; however, they were statistically non-significant and urinary calcium excretion was higher than in the glycolic-acid group during the experiment.

Therefore, it can be concluded that choreito and urajirogashi may have some beneficial effect though any such effect is inferior to that of pyruvate, in preventing calculi formation, partly by decreasing the urinary oxalate excretion; increased urine volume and magnesium excretion may also have some other, additional effects in the choreito group.

Key words: Pyruvate, Choreito, Urajirogashi, Urolithiasis, Rat

INTRODUCTION

In clinical settings, various treatments have been employed for preventing calcium-oxalate calculi. They may basically be classified into two types. One type of treatment is to administer inhibitory substances for calcium-oxalate precipitation in order to decrease the urinary saturation of calcium oxalate by increasing the formation-product ratio. The other is to reduce the hypercalciuric or hyperoxaluric condition by inhibiting metabolic action. Herb medicines, which have been proved to be effective in promoting the spontaneous passage of urinary calculi, have been widely used for the treatment of urinary calculi. Their mechanism can be explained in terms of their having a diuretic effect or an effect

on the peristaltic activity of the collecting system. Even if their precise mechanism has not yet been clarified, they may have some effect in expelling small, visible urinary calculi; therefore, their effect can be expected to be demonstrated under experimental conditions. Based on clinical observation, herb medicines are suspected also to work in preventing calculi formation in experimental rats.

A study was conducted to investigate whether or not choreito and urajirogashi have any preventive effect and to compare their potencies with that of pyruvate, which is a well-established inhibitory substance in calcium-oxalate calculi formation¹⁾.

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 rats (187.6 ± 4.7 g, mean \pm SE). The calcium-oxalate lithogenic diet (a glycolic-acid diet) was MM-1 (Funabashi Farms, Japan) containing 3% glycolic acid. Sodium pyruvate, a choreito extract, and a urajirogashi extract were added to the lithogenic diet in concentrations of 5, 1 and 2% respectively. All the diets were fed in powder form, and the rats were allowed tap water *ad libitum*. The rats were weighed

weekly. Pooled 24-hour urine samples from each group were collected weekly by using metabolic cages housing two or three rats. Urine samples were collected in flasks containing 100 μ l of 20% chlorohexidine gluconate,²⁾ and their volumes were measured; they were then acidified so as to make the pH lower than 2.0 and subsequently stored at -40°C until analysis. At the end of the fourth experimental week, all the rats were killed, and the urinary tracts were examined for calculi-formation. Renal cal-

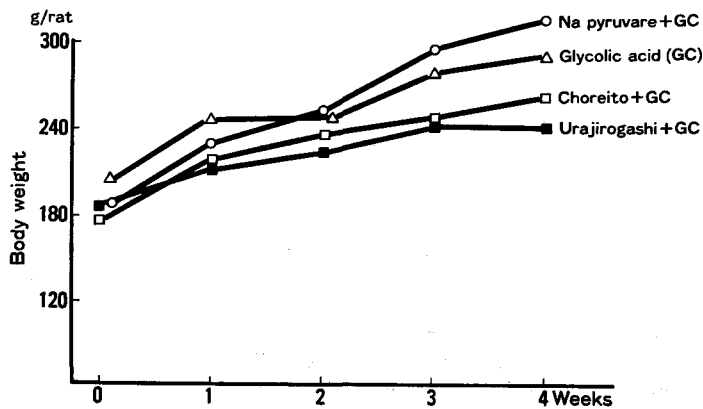


Fig. 1. Body-weight change in rats in 4 groups. The addition of pyruvate to the glycolic-acid diet increased bodyweight significantly, while that of herbs retarded body-weight gain, compared with the glycolic-acid group.

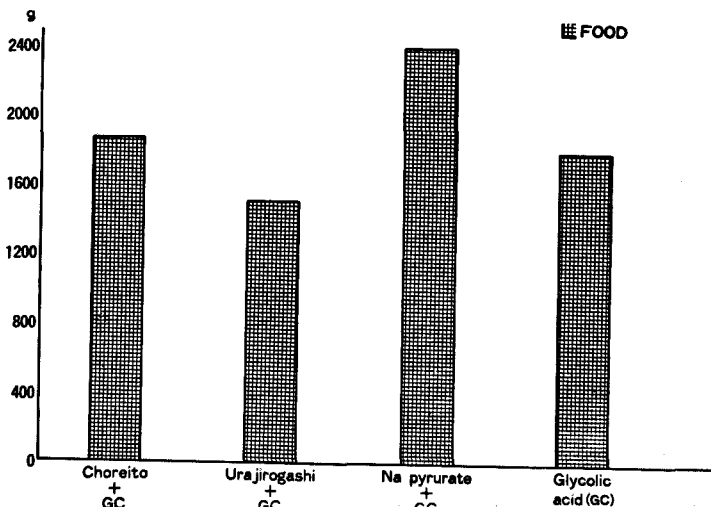


Fig. 2. Total amounts of overall food consumption in each group in 4 weeks. A similar trend was observed between the body-weight gain and the overall food consumption. Food intake was low in the urajirogashi and glycolic-acid groups.

culi formation was expressed in 3 grades in accordance with the criteria of Hasegawa et al³⁾. 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 oxalate deter-

mination by means of ion chromatography. The urine samples were also analyzed for oxalate and citrate by means of ion chromatography after a 100-fold dilution^{4,5)} and for calcium and magnesium by means of atomic absorption spectroscopy after 100-fold and 1,000-fold dilutions respectively. The Bonferroni method was used to test the statistical difference between the groups,

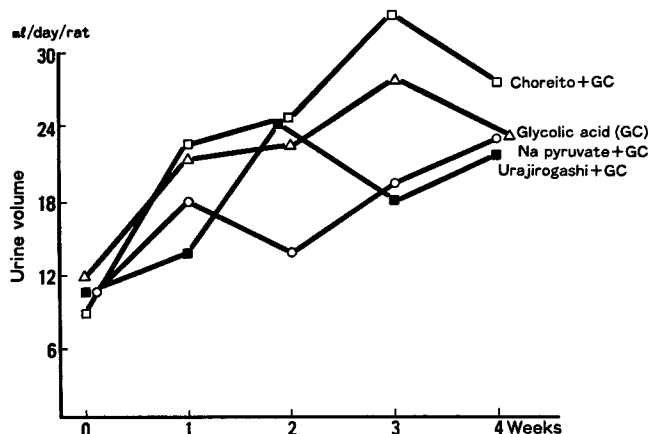


Fig. 3. 24-hour urinary volume per rat in 4 groups. Urinary volume was high in the choreito group during the experiment; however, there was no significant difference between groups.

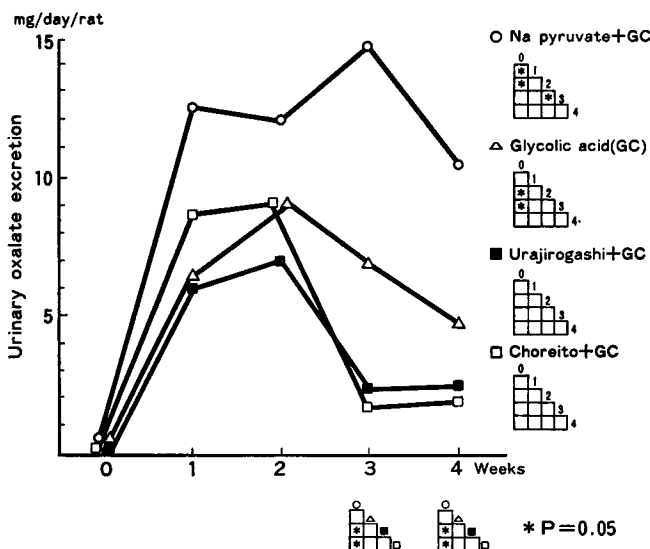


Fig. 4. 24-hour urinary oxalate excretion per rat in 4 groups. During the experiment, a high excretion of urinary oxalate was observed in all 4 groups. Urinary oxalate excretion was consistently highest in the pyruvate group. In the other 3 groups, it was highest in the second week and thereafter decreased until the fourth experimental week. Urinary oxalate excretion showed a marked decrease in the herb groups at the third and fourth experimental weeks ($p < 0.05$).

while paired t tests were used to test the response of the same animals to each treatment over time⁶².

The choreito extract was a gift from Tsumura-Juntendo, and the urajirogashi extract (urocalun) was a gift from Nihon-Shinyaku, both pharmaceutical companies.

RESULTS

The addition of pyruvate to the glycolic-

acid diet increased body-weight gain (120.0 ± 5.2 g, mean \pm SE) significantly ($p < 0.01$), while that of urajirogashi decreased body-weight gain (46.6 ± 10.0 g) significantly ($p < 0.05$) and that of choreito decreased body-weight gain slightly (77 ± 16.7 g), compared with the glycolic-acid group (84.0 ± 8.9 g) (Fig. 1). The total amount of food consumption (Y) in each group was parallel to the body-weight gain (X) ($Y = 2.4X$

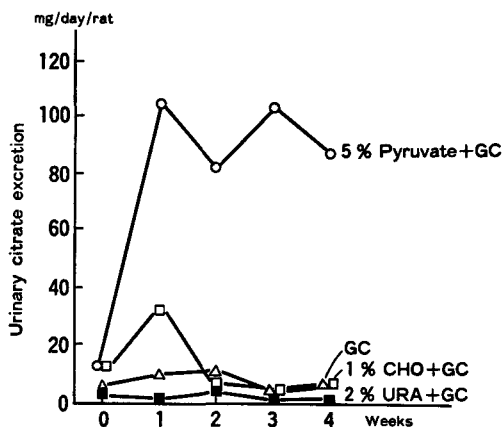


Fig. 5. 24-hour urinary citrate excretion per rat in 4 groups. In the pyruvate group, urinary citrate excretion increased markedly. Urinary citrate excretion did not increase in the other 3 groups.

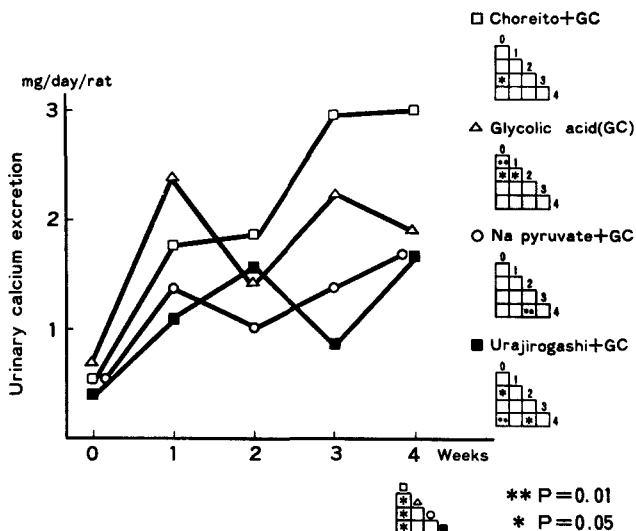


Fig. 6. 24-hour urinary calcium excretion per rat in 4 groups. Urinary calcium excretion increased markedly in all 4 groups; it was, however, higher in the choreito group and lower in the pyruvate and glycolic-acid groups.

+184.5, $r=0.977$, $p<0.05$) (Fig. 2). The urinary volume was highest in the choreito group during the experiment, but there was no significant difference between the groups (Fig. 3). A high excretion of urinary oxalate was observed in all 4 groups during the experiment, but it was most remarkable in the pyruvate group. It was highest in the second or third week, and it then decreased toward the fourth experimental week; this decreasing trend was marked in the herb groups (Fig. 4). In the pyruvate group, urinary citrate excretion increased markedly. Urinary citrate excretion did not increase in the other 3 groups (Fig. 5). Urinary calcium excretion increased markedly in all groups, but was higher in the herb groups and lower in the pyruvate and glycolic-acid groups (Fig. 6). Urinary magnesium excretion also increased markedly in all the groups during the experiment. It was higher in the choreito group and lower in the urajirogashi group than in the glycolic-acid group (Fig. 7). The 3% glycolic-acid diet was a potent calculi-former; heavy calculi deposits were observed in the kidneys of all 5 rats in the glycolic acid group.

The calculi deposited were identified by means of infrared spectrophotometry as being composed of calcium oxalate. Pyruvate and herbs both reduced renal-calculi deposits, though in slightly different ways. Almost no gross calculi were observed in the pyruvate group, although there were a few in the herb groups (Table 1). Fig. 8 shows the oxalate concentrations in the kidney tissue. At the level of the administered dosage, the inhibitory activity of urajirogashi was slightly less than that of choreito.

DISCUSSION

Chow et al. reported that 5% pyruvate, added to a 3% glycolic-acid diet, completely prevented the formation of calcium-oxalate deposits in the kidneys and the ureters. They suspected that pyruvate was an analog of glycolate and might act as an enzymic inhibitor in the oxidation of glyoxylate to oxalate¹². Our recent results demonstrate that pyruvate administration increases urinary citrate excretion and also urinary oxalate excretion; therefore, pyruvate can be concluded to inhibit urinary calculi formation by increasing urinary cit-

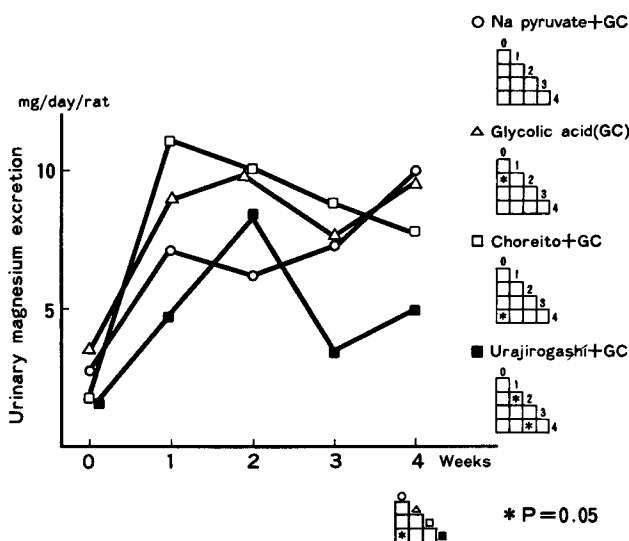


Fig. 7. 24-hour urinary magnesium excretion per rat in 4 groups. Urinary magnesium increased markedly in all the groups during the experiment. It was higher in the choreito, and lower in the urajirogashi group, than in the glycolic-acid group; it was lower in the pyruvate group in the early weeks, but increased over time to become the highest at the end of the experiment.

Table 1. Effects of pyruvate and herb medicines on incidence of urolithiasis. Almost no gross calculi were observed in the pyruvate group, although there were a few in the herb groups. Marked calculi deposits were observed in the glycolic-acid group. The ridit analysis was used to test for the differences in renal calculi deposition.

EXPERIMENT GROUP	(rats)	DEGREE OF NEPHROCALCINOSIS					mean of ridit	UROLITHIASIS IN			
		3+	2+	+	-	PELVIS		URETER	BLADDER		
							+	+	-	+	
Glycolic acid (GC)	(5)	4	6	0	0	.860	5	0	10	5	
Na pyruvate+GC	(5)	0	0	0	10	.175	1	0	10	0	
Choreito+GC	(5)	0	0	8	2	.455	0	0	10	0	
Urajirogashi+GC	(5)	0	2	6	2	.510	2	2	8	5	

**P<0.01

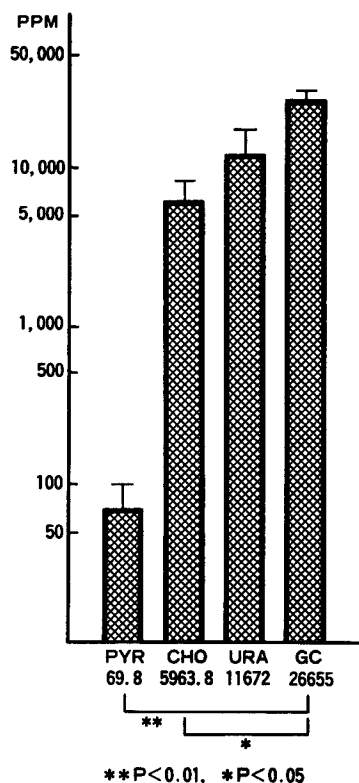


Fig. 8. Mean oxalate concentrations in the renal tissues at the end of the experiment. There were significant differences between the pyruvate group and the glycolic-acid group ($p<0.01$), and between the choreito group and the glycolic acid group ($p<0.05$). The Bonferroni method was used to test for the differences in the renal oxalate concentration between groups.

rate excretion⁷⁾. Using this well-developed experimental model of stone formation inhibition, pyruvate's inhibitory effects on stone formation were compared with those of herb medicines. Glycolic acid induces hyperoxaluria, which causes the development of urolithiasis in rat. Urinary oxalate exhibited a high excretion in the first and second weeks and a gradual decrease thereafter in all groups. The mechanism of this is not clear, but urinary oxalate excretion showed a marked decrease in the herb groups. At present, we can not say that it is simply because of the formation of a glycolate-metabolizing enzyme induced by the aid of the herb in order to normalize the disordered metabolism. It is also not clear how much the decrease in urinary oxalate excretion affects stone formation. In the herb groups, urinary citrate excretion did not increase; therefore, the possibility of inhibition by an increasing citrate excretion can be discarded. In the choreito group, the increases in the urinary volume, magnesium, and calcium excretions were remarkable; the former two factors may work to inhibit stone formation, but the latter promotes stone formation. In the urajirogashi group, the urinary volume was similar to that in the glycolic-acid group, urinary magnesium excretion was slightly lower, and urinary calcium excretion was higher than that in the glycolic-acid group. These two herb medicines showed similar inhibitory effects in stone

formation, although their precise mechanisms in stone inhibition were slightly different. Clinically, choreito and urajirogashi are believed to work to promote the spontaneous passage of urinary calculi; therefore, they may work to eliminate crystallites by increasing the peristaltic activity of the urinary collecting system. More studies will be required to disclose the complete mechanisms involved in the prevention of calculi formation.

In summary, our present results show that choreito and urajirogashi inhibit urinary calculi formation, partly by decreasing the urinary oxalate excretion; the choreito group also showed an increased urinary volume and magnesium excretion, though these increases were not observed in the urajirogashi group.

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和文抄録

ラット実験的尿酸結石症におけるピルビン酸塩と漢方製剤の結石形成抑制作用の比較

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実験的尿酸カルシウム結石食 (グリコール酸食) をウイスター系ラットに投与すると4週後には尿路結石の高度の沈着をみる。結石形成抑制作用を比較する目的で、ピルビン酸ナトリウムと漢方製剤である猪苓湯と裏白樗をそれぞれこの結石形成食に添加した。結石形成食群では高度の尿路結石症が認められたのに対し、ピルビン酸塩群では結石形成はほとんど認められなかった。猪苓湯群及び裏白樗群でも結石形成抑制が認められた。尿中尿酸の排泄はすべての群で高かったが、ピルビン酸群とグリコール酸群では著明であり、

4週に近づくに従い低下し、その減少傾向は漢方群では著しかった。ピルビン酸群のみに尿中クエン酸排泄の増加が見られた。猪苓湯群ではグリコール酸群に比し、尿量と尿中マグネシウム及びカルシウムの排泄が多い傾向を示した。

以上の結果より、ピルビン酸塩の強い結石形成抑制作用と比較すると、漢方製剤は5%ピルビン酸塩添加の抑制作用には及ばなかった。漢方群では3週以後尿中尿酸排泄減少が認められ、更に猪苓湯群では尿量と尿中マグネシウムの排泄増加傾向なども認められた。