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COMPARATIVE STUDY OF THE EFFECTS OF PYRUVATE AND CG-120 IN PREVENTING EXPERIMENTAL OXALATE UROLITHIASIS IN RATS

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Male Wistar-strain rats which had been fed a calcium-oxalate lithogenic diet (a glycolate diet) developed urinary calculi in 4 weeks. Sodium pyruvate or CG-120 (a mixture of citrate salts) had been added to this diet to determine its effect in preventing lithogenicity. Rats in the group fed a pyruvate diet had, however, almost no stones in the urinary system. Rats in the CG-120 group showed results somewhat similar to those in the pyruvate group. Increased urinary citrate excretion was observed in both groups and could be implicated as the main inhibitory factor in stone formation. Therefore, it can be concluded that CG-120 exerts a beneficial effect close to that of pyruvate in preventing calculi formation and that both substances cause a high citrate excretion in urine.

Key words: Pyruvate, CG-120, Oxalate urolithiasis, Rat

INTRODUCTION

Citrate has been reported to be an *in vitro* inhibitor of the crystallization of stone-forming calcium salts[1~3]. The clinical usefulness of potassium citrate for renal-stone disease has also been reported[4,5]. However, there have been few reports on its effect in animal (*in vivo*) experiments. Our preliminary experiment demonstrated that potassium citrate, when added to a calcium-oxalate lithogenic diet (a 3% glycolate diet) so as to make the citrate level 2%, inhibited stone formation. This inhibitory effect on stone formation was suspected to be attained at a 2% citrate level of the glycolate diet. Therefore, we examined whether or not CG-120 has any preventive effect in stone formation and, if any, to compare its potency with that of pyruvate, which is a well-established inhibitory substance in calcium-oxalate-calculi formation[6].

MATERIALS AND METHODS

Male Wistar-strain rats (ca 150 g) were acclimated 1 week and then randomly divided into three groups, each group consisting of 5 rats. The calcium-oxalate lithogenic diet (a glycolate diet) was MM-1 (Funabashi Farms, Japan) containing 3% glycolic acid. Sodium pyruvate or CG-120 (K and Na-citrate) was added to the glycolate diet up to the 5% or 2% level respectively. The CG-120 (sodium and potassium citrate) was a gift from Nippon Chemiphar Co., Ltd. All diets were fed in powder form. 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. Urine samples were collected in flasks containing 100 μl of 20% chlorohexidine gluconate, and their volumes measured ; they were then acidified so as to make the pH lower than 2.0 and subsequently

stored at −40°C until analyzed. At the end of the fourth experimental week, all the rats were sacrificed. All the urinary tracts were examined for calculi-formation, which was expressed in 3 grades in accordance with the criteria of Hasegawa et al[7]. Half of the right kidneys were weighed and homogenized in 5 ml of 2N 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 or 1000-fold with deionized distilled water for the oxalate determination by means of ion-chromatography. Urine samples were also analyzed for oxalate and citrate by means of ion-chromatography after a 100-fold dilution[8,9].

RESULTS

The addition of pyruvate or CG-120 to the glycolate diet did not significantly affect body-weight gain or urinary volume (Fig. 1, 2). The effects of pyruvate and CG-120 on urinary oxalate excretion are shown in Fig. 3. During the experiment, a high excretion of urinary oxalate was observed in all three groups. The addition of pyruvate somewhat increased the urinary oxalate excretion in comparison with the other two groups. The excretion of urinary oxalate was highest at the third week in the pyruvate and glycolate groups and then decreased toward the fourth experimental week. A 3 % glycolate diet was a potent calculi-former ; calculi deposits were observed in the kidneys of all 5 rats in the glycolate group. The calculi deposited were identified by infrared spectrophotometry as being composed of calcium oxalate. Pyruvate and CG-120 both eliminated renal calculi deposits, though in slightly different ways. Almost no calculi were observed in the pyruvate group, although there were a few in the CG-120 group (Table 1). The oxalate concentrations in the kidney tissue are shown in Fig. 4, which depicts the degree of the calculi deposit in each group. Under these experimental conditions, the inhibitory activity of CG-120 in stone formation was a little less than that of pyruvate, but still it was beneficial. The urinary citrate excretion in the three

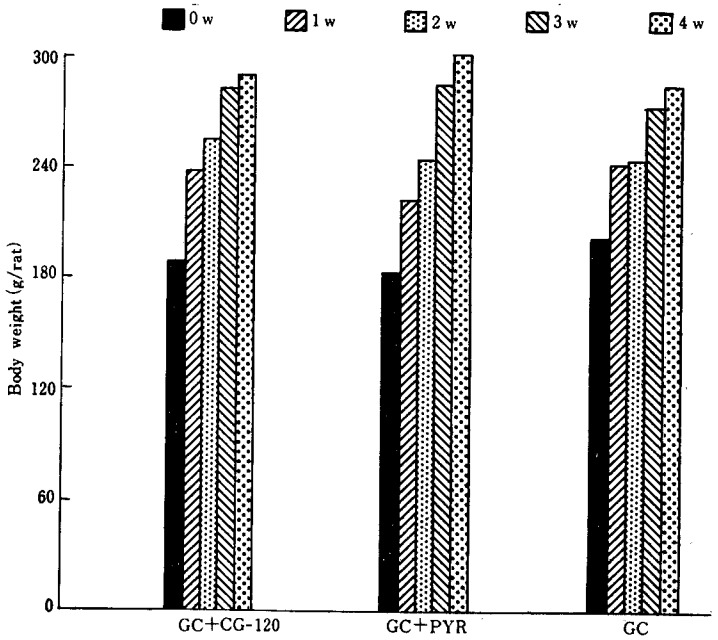

Fig. 1 Body-weight change in rats in 3 groups. The addition of pyruvate or CG-120 to the glycolate diet did not significantly affect body-weight gain. Growth was slightly retarded in the glycolate-group.

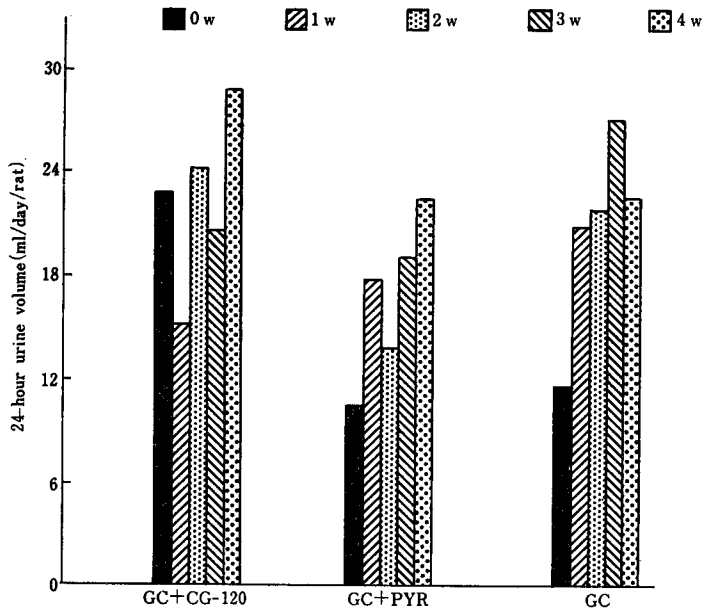

Fig. 2 24-hour urinary volume in 3 groups. No remarkable change was observed in urine volume of the same week among the three groups.

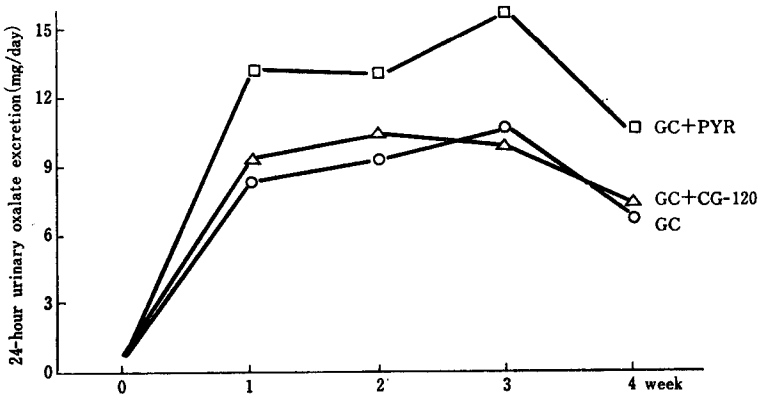

Fig. 3 24-hour urine oxalate excretion in 3 groups. During the experiment, a high excretion of urinary oxalate was observed in all three groups. The urinary oxalate excretion was highest in the pyruvate group throughout the experiment, and in both the glycolate and pyruvate groups excretion was highest in the third week.

groups is summarized in Fig. 5. In both the CG-120 and pyruvate groups, urinary citrate excretion increased markedly. However, urinary citrate excretion decreased gradually week-by-week in the CG-120 group, while it was unchanged in the pyruvate group.

DISCUSSION

Citrate is generally accepted as an inhibitor of stone formation and has been shown to reduce the saturation of calcium salts by producing a soluble complex with calcium[1~3]. Moreover, citrate may work as an inhibitor of the crystal growth of calcium oxalate[10]. Recently, a trace metal-citrate complex was postulated to play an important part in inhibiting the crystal growth of calcium salts[11,12]. Our preliminary study revealed that calcium

Table 1. Effects of CG-120 and pyruvate on incidence of urolithiasis. Almost no calculi were observed in the pyruvate group, although there were a few in the CG-120 group. Pyruvate and CG-120 were shown to be effective in preventing stone formation by means of ridit analysis for the degrees of nephrocalcinosis among the three groups. There was also a high incidence of calculi in the collecting system in the glycolate group compared with that in other two groups.

Experiment Group	Rats	Degree of Nephrocalcinosis				Mean of ridit		Urolithiasis in		
		−	+	++	+++			Pelvis +	Ureter +	Bladder +
GC	5	0	0	6	4	0.833	**	4	0	5
GC+Na pyruvate	5	10	0	0	0	0.267	**	1	0	0
GC+CG-120	5	6	4	0	0	0.400		2	0	2

GC : MMI containing 3% glycolic acid
Na pyruvate : 5% added to GC diet
CG-120 : 2% added to GC diet

** : P<0.01

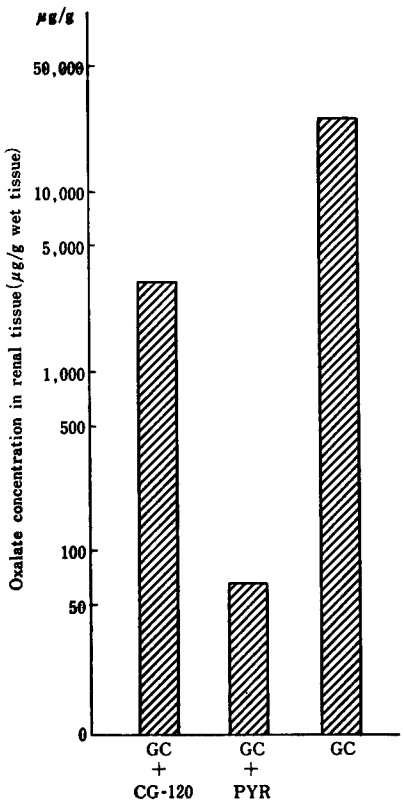

Fig. 4 Oxalate concentration of renal tissue in 3 groups. Oxalate concentration of renal tissue in the pyruvate group was close to normal. Pyruvate and CG-120 both eliminated renal calcium oxalate deposits, though the former was more effective.

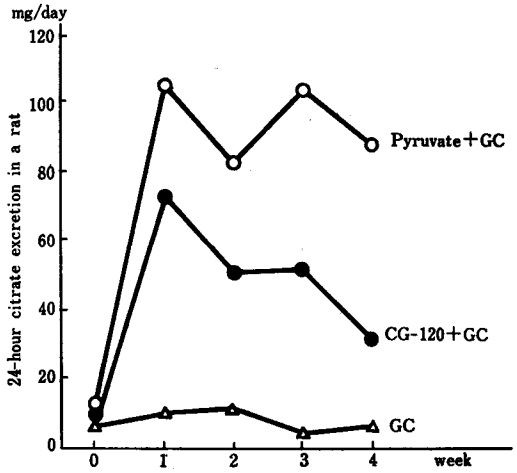

Fig. 5 Citrate excretion in the 3 groups. In both the CG-120 and pyruvate groups, urinary citrate excretion increased markedly. High citrate excretion was sustained in the pyruvate group. However, it decreased gradually in the CG-120 group towards the end of the experiment.

oxalate lithogenicity is not inhibited adequately by the administration of citric acid alone, but by the administration of sodium or potassium citrate. Sakhaee and his associates, after comparing different citrate salts, reported that potassium citrate is superior to sodium citrate in clinical application[4]. In clinical experience, a high prevalence of hypocitraturia in nephrolithiasis has also been reported[13]. However, there is no adequate explanation of this hypocitraturia. Therefore, a more extensive study of citrate is needed.

Pyruvate was suspected by Chow (1978) to reduce the formation of oxalate from glyoxalate[6]. Similarly, Murphy et al. recently reported that pyruvate feeding inhibits glycolate oxidase, thereby decreasing oxalate biosynthesis[14]. Our present results are, however, different ; urinary oxalate excretion did not decrease in the pyruvate group in comparison with that in the glycolate group. The inhibitory activity of pyruvate in stone formation was also confirmed in our experiment.

There has been almost no report concerning the relation between pyruvate and citrate. Our results showed a marked increase in urinary citrate excretion ; a high urinary citrate excretion was observed in all experimental weeks. However, there is not yet any adequate explanation of the phenomenon that the pyruvate administration increases urinary citrate excretion. It is generally accepted that a systemic acid-base change has striking effects on citrate clearance and metabolism. The effect of metabolic alkalosis on renal citrate handling arises from the inhibition of citrate metabolism in cells of the renal cortex rather than from the stimulation of the intracellular synthesis of citrate[15]. Studies with labeled citrate precursors have demonstrated that, when metabolic precursors of citrate are infused, citrate synthesis by the kidney from these compounds increases[16]. As for pyruvate, a metabolic precursor and alkaline, there are two possible mechanisms to increase urinary citrate excretion ; one is that it works as an alkali to cause metabolic alkalosis, and the other is that it works as a metabolic precursor to increase citrate synthesis by the kidney and the liver.

The urinary excretion of citrate in the pyruvate group was high compared with that in the CG-120 group, especially toward the end of the experiment. One possible explanation for this phenomenon is probably a slight impairment of renal tubular function due to hyperoxaluria and mechanical obstruction by renal calculi in the CG-120 group. The calculi deposition in the pyruvate group is less than that in CG-120 ; therefore, there may be no apparent renal tubular dysfunction. One difference is that pyruvate was administered at the 5% level, while CG-120 was administered at the 2% level. Therefore, it was not fair to compare the inhibitory potency under these experimental conditions. In our experiment, however, the pyruvate group was used as a positive stone-inhibitory control. There is another possible explanation, which has already been put forward by Pak et al., that oral citrate is short-acting in increasing urinary citrate and that the circadian rhythm of urinary citrate plays an important role in inhibiting stone formation[17]. However, there is not yet any proof that pyruvate is long-acting in increasing urinary citrate.

In summary, the present results of our last experiment show that pyruvate administration increases urinary citrate excretion, but does not decrease urinary oxalate excretion. Therefore, it is suspected that pyruvate works to inhibit calculi formation by increasing urinary citrate rather than by decreasing oxalate synthesis and excretion.

In conclusion, the addition of either pyruvate or CG-120 reduces calculi deposits in the experimental rat kidney. Both substances increase urinary citrate excretion and are suspected, therefore, to work to inhibit stone formation.

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和文抄録

ラット実験的蓚酸カルシウム結石症におけるピルビン酸塩と CG-120 の結石形成抑制作用

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諸角誠人

実験的蓚酸カルシウム結石食（3％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形成を見る．クエン酸塩製剤である CG-120 とピルビン酸ナトリウムの結石形成抑制作用を比較する目的で，上記の結石形成食にこれらを添加した．結石形成食のみの投与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（5％ピルビン酸ナトリウム添加）では結石形成はほとんど認められなかった．CG-120 群（2％ CG-120 添加）でも結石形成抑制が認められた．この両群において，尿中クエン酸排泄の著しい増加を見た．各群間の尿中蓚酸の排泄の差は明らかでなかった．4週後の腎組織への蓚酸カルシウムの沈着はピルビン酸群ではほとんど認められず，CG-120 群では軽度認められた．以上の結果よりピルビン酸塩は尿中クエン酸排泄を増加させ，結石形成抑制作用は強い．これと比較し CG-120 も同様に尿中クエン酸排泄を増加させるが，2％の添加では5％ピルビン酸塩添加にはその抑制作用は及ばなかった．