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Effect of chelating biomolecules on solubility of calcium oxalate: An *in vitro* study

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ABSTRACT

Urinary citrate appears to be an important factor in the crystallization process of calcium oxalate and calcium phosphate. Oxaluria can be reduced by means of reducing intestinal absorption of oxalate through feeding a mixture of freeze-dried lactic acid bacteria. Chelation therapy is now a days expanding its applications in various treatments. Calcium chelation by citric acid and lactic acid can play a major role in chelation therapy of naphrolithiasis. So, in present study, we analyzed the effect and prospect of chelating agents in urological calcium oxalate calculi dissolution as around 70% calculi are of calcium oxalate type. Present study concluded that as the concentration of citric acid and lactic acid increases the calcium ion concentration in soluble form increases. Statistical analysis of data with F test shows calculated F value is larger than tabulated F, a significant difference in response to dissolution with citric acid and lactic acid at df =1 between sample and df = 10 within samples, p = 0.05

Keywords: Calcium oxalate, Citric acid, Lactic acid, Chelation therapy.

INTRODUCTION

Urinary citrate appears to be an important factor in the crystallization process of calcium oxalate and calcium phosphate. The urinary excretion of citrate was found to be significantly lower in patients with calcium oxalate stone disease as compared with normal subjects, and about 30 per cent of the calcium stone formers can be considered as hypocitraturic [1]. Citric acid irrigation was used in treat ment of struvite stone [2]. Consumption of fruit juices advised to the patients of kidney stone.[3,4,5] Hyperoxaluria is a major risk factor for renal stones, and in most cases, it

appears to be sustained by increased dietary load or increased intestinal absorption. Previous studies have shown that components of the endogenous digestive microflora, in particular *Oxalobacter formigenes*, utilize oxalate in the gut, thus limiting its absorption. Oxaluria can be reduced by means of reducing intestinal absorption through feeding a mixture of freeze-dried lactic acid bacteria [6].

Chelation therapy is now a days expanding its applications in various treatments. Iron chelating therapy used for treatment of thalassemia [7]. Metal chelation therapy in Alzheimer's disease [8,9] and in atherosclerosis [10,11]. Calcium chelation citric acid and lactic acid can play a major role in chelation therapy of naphrolithiasis. So, in this study, we analyze the effect and prospect of chelating agents in urological calcium oxalate calculi dissolution.

EXPERIMENTAL SECTION

Material

All the chemicals used were of A.R. Grade.

Instruments and apparatus used

Remi magnetic stirrer
Systronics Flame Photometer
Class A glassware

Method

In two sets one of citric acid and one of lactic acid 0.1M citric acid and 0.1M lactic acid prepared in artificial urine and artificial urine were mixed as given in the table 1. 500mg calcium oxalate was added to each. The mixture is stirred using magnetic stirrer for 15 minutes at 37° C. The suspension was filtered with Whatman filter paper no.40. Calcium ion content was measured using flame photometer. The change in calcium ion concentration was determined relative to control. Difference in calcium ion concentration in μg/ ml was plotted against increase in molar concentration.

Statistical analysis

One way ANOVA was performed to test variation between samples and variation within the samples.

RESULTS AND DISCUSSION

As the concentration of citric acid and lactic acid increases the Calcium ion concentration in soluble form increases (Fig.1) (Table 2 and 3). This study shows that citric acid and lactic acid increase the solubility of calcium oxalate crystals. Comparison of both the plot Calcium ion conc. Vs molar conc. of citric acid and of lactic acid shows that lactic acid increases the solubility very much more than the citric acid. Statistical analysis of data with F test shows calculated F value is larger than tabulated F, a significant difference in response to dissolution with citric acid and lactic acid at df =1 between sample and df = 10 within samples, P = 0.05

Citric acid is an organic and natural component of many fruits and fruit juices. It is also a good chelating agent which produces soluble complex calcium citrate by reacting with calcium. Calcium citrate can be easily removed from body while accumulation of calcium oxalate forms kidney stone. This chelating property can be used to dissolve kidney stone.

Hyperoxaluria is a major risk factor for renal stones, and in most cases, it appears to be sustained by increased dietary load or increased intestinal absorption. Previous studies have shown that components of the endogenous digestive microflora, in particular *Oxalobacter formigenes*, utilize oxalate in the gut, thus limiting its absorption.

Lactic acid is a good chelating agent which produces soluble complex calcium lactate by reacting with food calcium. Calcium lactate a soluble complex of calcium can easily be removed from body. This chelating property can be used to dissolve kidney stone. Lactic acid is the main constituent of butter milk.

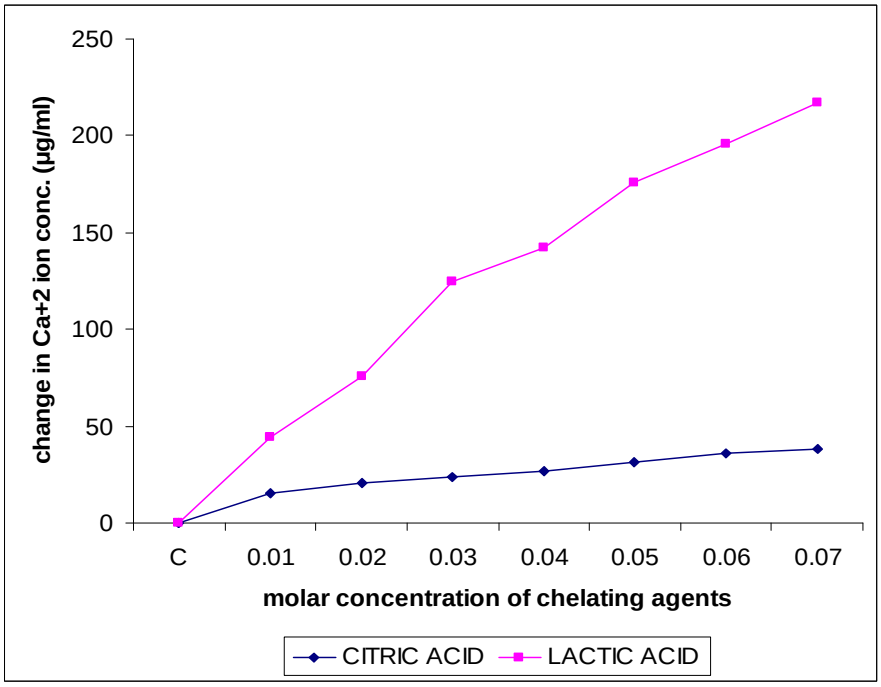

Figure 1 The solubility of CaOx crystals was measured by calcium ion concentration in filtrate, in presence of increasing concentrations of chelating argents citric acid and lactic acid. $F_C > F_t$, a significant difference in response to dissolution with citric acid and lactic acid at df =1 between sample and df = 10 within samples, P = 0.05

The effect of lactic acid is quite better than citric acid which can be seen with the figure 1. It is the normal constituent of our routine food habit. We can include both of these natural chelating agents in our routine food habits. That by making complex with calcium ion make soluble complexes and prevent the formation of calcium oxalate in the kidney.

Table 1

Test tube no.	Set 1		Set 2	
	AU (ml) added	0.1 M Citric acid solution prepared in AU (ml) added	AU (ml) added	0.1 M Lactic acid solution prepared in AU (ml) addded
C	25	0	25	0
1 (0.01M)	22.5	2.5	22.5	2.5
2 (0.02M)	20	5	20	5
3 (0.03M)	17.5	7.5	17.5	7.5
4 (0.04M)	15	10	15	10
5 (0.05M)	12.5	12.5	12.5	12.5
6 (0.06M)	10	15	10	15
7 (0.07M)	7.5	17.5	7.5	17.5

Table 2 Calcium ion concentration (µg/ml) in the filtrate, measured using flame photometer in the set of Citric acid of different molar concentrations (n=6).

Sr. no.	Molar Concentration of Citric acid	Mean[a] Calcium ion concentration (µg/ml)	± Standard deviation[b]
1	0	63.33	3.4448
2	0.01	77.33	3.3266
3	0.02	84.17	3.7638
4	0.03	87.17	5.1153
5	0.04	89.83	4.3089
6	0.05	94.17	2.9268
7	0.06	98.67	2.6583
8	0.07	101.33	3.6696

a, mean value of six determinations; b, standard deviation; n, number of repetitions

Table 3 Calcium ion concentration (µg/ml) in the filtrate, measured using flame photometer in the set of Lactic acid of different molar concentrations (n=6).

Sr. no.	Molar Concentration of Lactic acid	Mean[a] Calcium ion concentration (µg/ml)	± Standard deviation[b]
1	0	224.33	3.6147
2	0.01	285.67	4.1311
3	0.02	299.67	4.0331
4	0.03	349.00	2.8284
5	0.04	365.83	4.1190
6	0.05	400.00	6.0663
7	0.06	420.33	5.0859
8	0.07	440.83	3.5449

a, mean value of six determinations; b, standard deviation; n, number of repetitions

CONCLUSION

Citrate fruits are already used in the treatment/ prevention of renal calculi. Lactic acid or lactate salts can be an alternative treatment for dissolution of renal calculi and may prove better than citric acid.

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