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Effect of Micronutrient Metal Ions on the Inhibiton Efficiency of Citric Acid Towards the Mineralisation of Urinary Stone Forming Minerals

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Effect of micronutrient metal ions, *viz.*, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+} on the inhibition efficiency of citric acid towards the mineralisation of urinary stone forming minerals, viz., calcium phosphate, calcium oxalate or calcium carbonate has been studied in an experimetal model. Utility of the results in urolithiasis inhibition has been discussed.

INTRODUCTION

Citric acid is one of the important inhibitors of calculogenesis in the urinary tract[1]. Hypocitraturia is one of the risk factors of urolithiasis[2]. Mechanism of stone inhibition activity of citric acid is not yet clear; however, active chelation of calcium ions by the former is supposed to play a role[3]. Presence of other coordinating metal ions in the urinary milieu might affect the inhibition efficiency of citric acid. With this view in mind we have presently studied the effect of some micronutrient metal ions, *viz.*, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+} on the inhibition efficiency of citric acid towards the mineralisation of urinary stone forming minerals, *viz.*, calcium phosphate, calcium oxalate or calcium carbonate in an experimental model in aqueous and urinary milieu.

EXPERIMENTAL

Crystalloid forming solutions, *viz.*, solution of calcium chloride, trisodium phosphate, disodium oxalate and sodium carbonate of 0.01 M concentration were prepared in distilled water. Urine sample of a healthy 35 year old male was collected in sterilised plastic container. A 24 h urine output was collected and a bit of camphor was added as a preservative. It was used out in minimum possible time after collection. The micronutrient metal salts used were $Cr_2(SO_4)_3 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $FeSo_4 \cdot 7H_2O$, $CoSO_4 \cdot 7H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$ or $ZnSO_4 \cdot 7H_2O$.

To study the inhibition efficiency an experimental model was designed in which the two crystalloid forming solutions, *e.g.*, sodium phosphate and calcium chloride (for calcium phosphate) were taken in two separate burette (50 mL) and were allowed to fall simultaneously and slowly (dropwise) with equal speed into a 250 mL beaker containing 50 mL of inhibitor (citric acid solution, micronutrient metal ion treated citric acid solution, urine, citric acid solution in urine or micronutrient treated citric acid solution in urine). The whole operation took about 30 min. At the end the contents of beaker were digested in a hot water bath for 10 min, cooled to room temperature and the precipitate was collected into a

pre-weighed centrifuge tube by centrifuging small volumes at a time and rejecting the supernatant liquid. Next, the tube with the precipitate was dried in an air oven at 120°C, cooled to room temperature and weighed till constant weight. Weight of the precipitate was determined.

Various inhibitor solutions used were 0.001 M citric acid solution in water, 0.001 M citric acid solution in urine, micronutrient metal ion treated citric acid solution (0.001 M in water or urine). The quantity of micronutrient metal salt treated was such that the molarity (in 50 mL inhibitor solution) remains at 0.0003 M.

Simultaneous blank experiments with water in place of inhibitor were also carried out for evaluating the inhibition efficiency of inhibitors compared to water. All experiments were conducted at room temperature (20–25°C).

RESULTS AND DISCUSSION

Percentage efficiency of inhibition by the inhibitor was calculated using the formula,

$$\% \text{ inhibition} = \frac{\text{wt. of ppt. in blank set} - \text{wt. of ppt. in exptl. set}}{\text{wt. of ppt. in blank set}} \times 100$$

Increase or decrease of inhibition by the micronutrient metal ions as compared to that of citric acid in aqueous as well as urinary milieu has also been calculated out. Effect of micro nutrient metal ions on the inhibition efficiency of citric acid towards the mineralisation of calcium phosphate, oxalate or carbonate in aqueous and urinary media are recorded in Table-1 and Table-2 respectively.

TABLE-1
EFFECT OF MICRO-NUTRIENT METAL IONS ON THE INHIBITION EFFICIENCY OF CITRIC ACID IN AQUEOUS MEDIUM

Inhibitor (50 mL 0.001 M Soln.)	Micro-nutrient metal ion (0.0003 M with respect to inhibitor soln.)	Mineralisation inhibition (%) of			Increase (+) or decrease (–) of inhibition (%) over citric acid		
		Cal. Phos.	Cal. Ox.	Cal. Carb.	Cal. Phos.	Cal. Ox.	Cal. Carb.
Citric acid	–	50	23	92	–	–	–
Citric acid	Cr^{3+}	50	33	67	0.0	+10	–25
Citric acid	Mn^{2+}	50	28	100	0.0	+5	+8
Citric acid	Fe^{2+}	50	0	89	0.0	–23	–3
Citric acid	Co^{2+}	47	0	100	–3	–23	+8
Citric acid	Ni^{2+}	58	44	78	+8	+21	–14
Citric acid	Cu^{2+}	50	28	60	0.0	+5	–32
Citric acid	Zn^{2+}	50	28	89	0.0	+5	–3

TABLE-2
EFFECT OF MICRO-NUTRIENT METAL IONS ON THE INHIBITION EFFICIENCY OF CITRIC ACID IN URINE MEDIUM

Inhibitor (50 mL 0.001 M Soln. in urine)	Micro-nutrient metal ion (0.0003 M with respect to inhibitor soln.)	Mineralisation inhibition (%) of			Increase (+) or decrease (–) of inhibition (%) over citric acid		
		Cal. Phos.	Cal. Ox.	Cal. Carb.	Cal. Phos.	Cal. Ox.	Cal. Carb.
Citric acid	–	42	11	11	–	–	–
Citric acid	Cr^{3+}	50	44	–22	+8	+33	–33
Citric acid	Mn^{2+}	38	20	11	–4	+9	0.0
Citric acid	Fe^{2+}	33	22	11	–9	+11	0.0
Citric acid	Co^{2+}	–50	33	–13	–92	+22	–24
Citric acid	Ni^{2+}	33	22	–22	–9	+11	–33
Citric acid	Cu^{2+}	33	22	–22	–9	+11	–33
Citric acid	Zn^{2+}	–8	28	11	–50	+17	0.0

Citric acid is one of the potent inhibitors of urolithiasis, present in human urine. Hypocitraturia is one of the causes of urinary calculogenesis[2]. *In-vitro* studies[3] have also shown that citric acid is an effective inhibitor of mineralisation of urinary stone forming minerals. It probably acts by effective chelation of Ca^{2+} ions.

Micronutrient metal ions, *viz.*, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+} are important for the life process. Though required in trace amounts they are essential for various enzymatic processes. They form part of urinary system. All of them have high coordinating abilities. Their complexing tendency towards the calcium precipitating/dissolving ligands present in the urinary system might effect the mineralisation-inhibition efficiency of complexons. Citric acid is a potent chelating agent. If the micronutrient metal ions preferentially complex with citric acid it would effect the chelation of Ca^{2+} by the citric acid, and hence would decrease the latter's inhibition efficiency towards the mineralisation of insoluble calcium salts, *viz.*, phosphate, oxalate or carbonate. On the other hand, inhibition efficiency would increase if the above trace metal ions preferably complex with calcium precipitating ligands such as oxalate, phosphate or carbonate, in the form of soluble complexes and making them less available for Ca^{2+} ions for precipitation. Thus the entire chemical equilibria in the urinary tract would decide the effectivity of any of the inhibitors of calculogenesis.

A study of Table-1 and Table-2 suggests that the trace metal ions increase the inhibition efficiency of citric acid more towards oxalate mineralisation as compared to phosphate and carbonate. This might be due to better chelating ability of oxalate towards trace metal ions as compared to phoshate or carbonate. In urinary milieu there is mostly a negative effect on inhibition. This may perhaps

be due to increased solute load in the urine; it may also be due to insoluble complexation of trace metal ions by other urinary ligands such as urea, uric acid, etc. In general, Ni^{2+} and Cu^{2+} seem to be better enhancers of citric acid's inhibition efficiency. Fe^{2+} and Co^{2+} seem to prefer citric acid chelation over oxalate, thus causing a decrease of inhibition efficiency.

Our present study, in general, indicates that the presence of trace metal ions vis-a-vis citric acid in the urinary system would mostly decrease the inhibitory power of citric acid towards the mineralisation of urinary stone forming minerals with some exceptions in case of oxalate.

The present results can not be conclusive but only indicate a trend because the exact inhibitory effect, particularly in urine medium, would depend on the chemical composition of the individual urine and its solute load.

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