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Inhibition of Mineralisation of Urinary Stone forming Minerals by L-Ascorbic Acid and Effect of Micronutrient Metal Ions on the Inhibition in Aqueous and Urinary Milieu

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L-Ascorbic acid has been studied as inhibitor in the mineralisation of urinary stone forming minerals, *viz.*, calcium phosphate, oxalate or carbonate. Inhibition efficiency has been studied in different experimental models. 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 L-ascorbic acid has also been investigated in aqueous and urinary milieu. Utility of the results in urolithiasis inhibition has been discussed.

INTRODUCTION

Urinary stones contain both crystalloid and colloid components. The crystalloids are mainly calcium oxalate, calcium phosphate, calcium carbonate, magnesium ammonium phosphate, uric acid and cysteine.[1] Stone formation is apparently related to level of urinary crystalloid and also to the level of inhibitors of calculogenesis in urine.[2–4] Calcium chelating agents might form suitable inhibitors in urolithiasis. Presence of other coordinating metal ions in the urinary milieu might also affect the inhibition efficiency of the inhibitor. As a part of our systematic study on inhibitors of urinary calculogenesis, we are presently reporting on the inhibition efficiency of L-ascorbic acid on the mineralisation of calcium phosphate, oxalate or carbonate in different experimental models. 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 has also been studied 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 $Cr_2(SO_4)_3 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot H_2O$ or $ZnSO_4 \cdot 7H_2O$.

To study the inhibition efficiency, four experimental models, namely, 'simul-

taneous flow static model' (SSM), 'simultaneous flow dynamic model' (SDM), 'reservoir static model' (RSM) and 'reservoir dynamic model' (RDM) were designed. In the SSM model the two salt forming solutions, *e.g.*, calcium chloride and sodium phosphate (for calcium phosphate) and the inhibitor (solution of ascorbic acid) were taken in three separate burettes (50 mL each) and were allowed to fall simultaneously into a 250 mL beaker in a slow (dropwise) and equal speed. The whole operation took about 40 min. At the end the mixture was digested in a hot water bath for 10 min, cooled to room temperature and the precipitate was collected in 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.

In the SD model, the process was same except that the reaction mixture in the beaker was continuously stirred on a magnetic stirrer during the flow of salt forming solutions and the inhibitor. In the RS model, the whole amount of inhibitor solution (50 mL) was placed in the beaker in the beginning itself and the two salt forming solutions were allowed to run into it dropwise through burettes. Thus, a reservoir of inhibitor was created into which the salt forming solutions ran down. Rest of the operation was same as in other models. In the RD model the process was same as RS model except that the reaction mixture was stirred continuously on a magnetic stirrer during the experiment.

The effect of micronutrient metal ions on the inhibition efficiency was studied in the reservoir dynamic model (RDM). In the inhibitor reservoir (50 mL, 0.001 M ascorbic acid solution in water or urine), calculated quantity of solid metal salts were added so that the concentration of metal salt was 0.0003 M in the reservoir. Rest of the process was same as in other RD models.

Simultaneous blank experiments with water in place of inhibitor were also carried out for evaluating the inhibition efficiency of inhibitiors 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,

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

Inhibition efficiency of L-ascorbic acid towards the mineralisation of calcium phosphate, oxalate and carbonate are recorded in Table-1. Effect of micronutrient metal ions on the inhibition efficiency of ascorbic acid towards the mineralisation of calcium phosphate, oxalate or carbonate in aquous and urinary media are recorded in Table-2 and Table-3 respectively.

TABLE-1
INHIBITION OF MINERALISATION OF CALCIUM PHOSPHATE, CALCIUM OXALATE OR CALCIUM CARBONATE BY L-ASCORBIC ACID IN AQUEOUS MEDIUM

Inhibitor (Concn.)	Inhibition Efficiency (%)											
	Calcium phosphate				Calcium oxalate				Calcium carbonate			
	SSM	SDM	RSM	RDM	SSM	SDM	RSM	RDM	SSM	SDM	RSM	RDM
Ascorbic acid (0.01 M)	100	100	100	100	4	5	6	6	100	100	100	100
Ascorbic acid (0.001 M)	70	71	73	75	0	0	2	2	62	64	66	67

Study of Table-1 suggests that L-ascorbic acid (vitamin-C) is moderate to good inhibitor of calcium phosphate and carbonate mineralisation; for oxalate, however, it seems to be a poor inhibitor. Relative specificities of ascorbate, phosphate, carbonate and oxalate for calcium ions seem to decide the inhibition efficiencies. Sequestering of these insoluble calcium salts by the ascorbic acid might be due to effective single or mixed ligand chelation, stabilised by effective hydrogen-bonding through the —OH groups. It is observed that the inhibitory capacity decreases with a decrease in the strength of inhibitor solution. Mass effect might be playing a role here. As the concentration of inhibitor decreases the equilibrium might be favouring the precipitation of insoluble salts. A comparative study of different models indicates that the reservoir dynamic model is the most effective one in the inhibition of mineralisation. This may also be due to the 'ma effect'. An *ab-initio* presence of large concentration of inhibitor (in the reservoir) coupled with continuous stirring might be effectively chelating the Ca^{2+} and screening from precipitating anions like phosphate, oxalate or carbonate.

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

Inhibitor (50 mL 0.001 M soln.)	Micronutrient metal ion (0.0003 M with respect to inhibitor soln.)	Mineralisation inhibition (%) of			Increase (+) Decrease (–) Inhibition (%) over ascorbic acid		
		$Ca_3(PO_4)_2$	CaC_2O_4	$CaCO_3$	$Ca_3(PO_4)_2$	CaC_2O_4	$CaCO_3$
Ascorbic acid	–	75	02	67	–	–	–
Ascorbic acid	Cr^{3+}	42	39	44	–33	+37	–23
Ascorbic acid	Mn^{2+}	53	33	78	–22	+31	+11
Ascorbic acid	Fe^{2+}	50	37	44	–25	+35	–23
Ascorbic acid	Co^{2+}	33	33	78	–42	+31	+11
Ascorbic acid	Ni^{2+}	33	39	80	–42	+37	+13
Ascorbic acid	Cu^{2+}	50	39	67	–25	+37	00
Ascorbic acid	Zn^{2+}	33	39	67	–42	+37	00

TABLE-3
EFFECT OF MICRO-NUTRIENT METAL IONS ON THE INHIBITION EFFICIENCY OF ASCORBIC ACID IN URINARY MEDIUM

Inhibitor (50 mL 0.001 M soln. in urine)	Micronutrient Metal ion (0.0003 M with respect to inhibitor soln.)	Mineralisation inhibition (%) of			Increase (+) Decrease (–) Inhibition (%) over ascorbic acid		
		$Ca_3(PO_4)_2$	CaC_2O_4	$CaCO_3$	$Ca_3(PO_4)_2$	CaC_2O_4	$CaCO_3$
Ascorbic acid	–	–33	28	–16	–	–	–
Ascorbic acid	Cr^{3+}	–17	28	–11	+16	00	+05
Ascorbic acid	Mn^{2+}	–17	44	11	+16	+16	+27
Ascorbic acid	Fe^{2+}	–33	33	–16	00	+05	00
Ascorbic acid	Co^{2+}	–22	22	–16	+13	–06	00
Ascorbic acid	Ni^{2+}	–25	28	–22	+08	00	–06
Ascorbic acid	Cu^{2+}	–25	33	–22	+08	+05	–06
Ascorbic acid	Zn^{2+}	50	17	–11	+83	–11	+05

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. Ascorbic acid is good chelating agent. If the micronutrient metal ions preferentially complex with ascorbic acid it would effect the chelation of Ca^{2+} by the ascorbic acid, and hence, would decrease the later's inhibition efficency towards the mineralisation of insoluble calcium salts. 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 precipitations. Thus the entire chemical equilibria in the urinary tract would decide the effectivity of any of the inhibitors of calculogenesis.

A study of Table-2 shows that the trace metal ions have a positive effect on the inhibition efficiency of ascorbic acid towards calcium oxalate mineralisation in aqueous medium. For calcium phosphate the effect is mostly negative, *i.e.*, a decrease in inhibition. For carbonate, the effect is mixed; Cr^{3+} and Fe^{2+} decrease the inhibition whereas Co^{2+}, Ni^{2+} and Mn^{2+} increase the same. A positive effect of trace metal ions on the oxalate inhibition by ascorbic acid might be due to better chelating ability of oxalate towards trace metal ions. This, perhaps, is not true, at least comparatively, with phosphate or carbonate.

A study of Table-3 suggests that inhibition efficiency of ascorbic acid decreases in case of phosphate and carbonate but increases for oxalate when the medium is urine. The negative values of net inhibition by the ascorbic acid + trace metal

ion system in case of phosphate or carbonate mineralisation might be due to increased solute load in the urine. It might also be due to insoluble complexation of trace metal ions by other urinary ligands such as urea, uric acid, etc. For calcium oxalate, the inhibition results are mostly positive and encouraging. In general, Mn^{2+} has a positive effect in most of the cases in aqueous as well as urinary milieu on the inhibition efficiency of ascorbic acid. Zn^{2+} and Cr^{3+} enhance phosphate inhibition by ascorbic acid in urinary medium. In general, it is difficult to exactly speculate the mechanism of inhibition in the urinary medium because apart from added ascorbic acid the urine too contains some inhibitors.

Ascorbic acid is a water-soluble vitamin. Urinary level of ascorbic acid is an index of its nutritional status. As indicated by our present studies, that ascorbic acid can inhibit the mineralisation of urinary stone forming minerals, it might find application in chemodissolution of urinary stones and prophylaxis of urolithiasis. So far as the effect of trace metal ions on the inhibition efficiency of ascorbic acid is concerned, our present results cannot 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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