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Chemoinhibition of Mineralization of Urinary Stone Forming Minerals by Some Inorganic and Organic Salts of Aluminium in Aqueous and Urinary Milieu

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Chemoinhibition of mineralization of urinary stone forming minerals *viz.*, calcium oxalate and calcium phosphate by some inorganic and organic salts of aluminium *viz.*, aluminium chloride, aluminium acetate and aluminium citrate, have been studied in aqueous as well as in urinary milieu. Inhibition efficiency has been studied in an experimental model. Crystals were isolated from the centrifugates of inhibited solutions in aqueous medium and their infrared spectra were studied. Results revealed that aluminium chloride and aluminium citrate show a uniformly good inhibition of calcium oxalate and calcium phosphate mineralization (35 to 53 % inhibition above blank for oxalate and 42 to 75 % inhibition above blank for phosphate) in aqueous as well as urinary media. Aluminium acetate showed a good inhibition only in aqueous media. Aluminium citrate was found to be a particularly potent inhibitor. Infrared spectra of the crystals, isolated from the centrifugates of inhibited solutions, evidenced a bridging mode of C=O of oxalate and a coordinated mode of phosphate. Thus soluble complexation seem to be the probable mechanism behind inhibition of oxalate and phosphate mineralization by aluminium salts.

Key Words: Urolithiasis, Urinary stones, Urolithology, Aluminium.

INTRODUCTION

The crystalloid components of urinary stones are mainly calcium oxalate, calcium phosphate, calcium carbonate, magnesium ammonium phosphate, uric acid and cystine[1]. Of these calcium oxalate and calcium phosphate form two usual and stubborn constituents. Urinary stone formation is related to the level of stone forming crystalloids as well as inhibitors of calculogenesis in urine[2]. Although a number of natural inhibitors *viz.*, citrate, pyrophosphate, nephrocalcin, glycosaminoglycans, magnesium and zinc are known, some novel and efficient inhibitors might be searched for. Metal ions, that are widely used in food and pharmaceuticals, should also be investigated for their role in urolithiasis. Aluminium is one such metal that is widely used in food and pharmaceuticals industries. Powdered aluminium is variously used as a protective in treating ulcer and fissures[3]. It has also been used in treating silicosis. Many antacids contain aluminium hydroxide gel. Aluminium also finds use in food packaging materials. A number of

aluminium salts are used as food additives[4]. Despite being third most abundant element in the earth's crust, aluminium is a non-nutrient metal in biological system. Aluminium occurs in the blood of animals[5] in the range of 0.05-0.1 mg/100 mL. Recent studies indicate that most individuals, on an average, consume about 1-10 mg Al/d from different natural sources. Only one per cent of the daily intake of aluminium enters the blood stream, while the vast majority is excreted by the kidney in urine and partially with feaces[3]. Since Al^{3+} can be excreted through urine, its stone inhibitory role in the urinary tract would form an interesting matter of investigation.

Thus, in view of importance of aluminium *vis-a-vis* our quest for novel inhibitors of urinary calculogenesis, some inorganic and organic salts of aluminium are studied as *in vitro* inhibitors of mineralization of urinary stone forming minerals *viz.*, calcium oxalate and calcium phosphate. The crystals were also isolated from the centrifugates of inhibited solutions and studied their infrared spectra in an apparent attempt to study the mode of oxalate and phosphate bonding in inhibited solutions. This would throw some light on the mechanism behind inhibiton.

EXPERIMENTAL

Crystalloid forming solutions *viz.*, solution of calcium chloride, trisodium phosphate and disodium oxalate of 0.01 M concentration were prepared in distilled water. Inhibitor solutions *viz.*, 0.01M aluminium chloride, 0.01 M aluminium acetate and 0.01 M aluminium citrate were prepared separately in distilled water as well as in urine. Aluminium citrate was actually prepared *in situ*. Calculated quantities of aluminium hydroxide and citric acid monohydrate were mixed in 1:1 mol ratio in aqueous/urinary media. The reaction mixture was stirred well till a clear solution was obtained. The solution was then quantitatively transferred and diluted to the calculated volume in a measuring flask so as to get a 0.01 M solution of aluminium citrate. The urine sample for preparing the inhibitor solutions, was collected from a healthy 35 year old male in a sterlized 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.

Inhibition experiments: An experimental model was designed in which the two salt forming solutions *e.g.* sodium phosphate and calcium chloride (for calcium phosphate) were taken in two separate burrets (50 mL) and were allowed to fall simultaneously and slowly (drop wise) with equal speed into a 250 mL beaker containing 50 mL of inhibitor (aluminium salt solution). The whole operation took about 40 min. At the end, the contents of beaker were digested on a hot water bath for 10 min, cooled to room temperature and centrifused in small volumes. The total centrifugate was collected.

Simultaneous blank experiments with water/urine in place of inhibitor solution were also carried out for evaluating the inhibition efficiency of inhibitors compared to water/urine. All experiments were conducted at room temperature (20-25 ºC).

Calcium content of the centrifugate was determined by complexometric method using standard disodium EDTA solution[6]. While calculating the calcium content of centrifugate, a titre value of EDTA *vs.* corresponding total inhibitor solution was deducted from the total titre value (equivalent to the centrifugate). This was done because Al^{3+}, present in the centrifugate, would also consume some EDTA.

Inhibition efficiency of the inhibitor solutions (including that of water/urine) was calculated using the following formula:

$$\text{Inhibition efficiency (\% inhibition)} = \frac{Ca^{2+} \text{ in centrifugate}}{\text{Total } Ca^{2+} \text{ in expt.}} \times 100$$

Total Ca^{2+} in expt. means the Ca^{2+} content of 50 mL 0.01 M $CaCl_2$ solution (used in the experiment), which was determined separately.

From the percentage inhibition values, the increase of inhibition efficiency of inhibitors over blank (water/urine) were calculated out. Percentage increase of inhibition efficiency of inhibitors relative to blank (water/urine) were also calculated out using the following formula:

$$\text{Percentage increase of inhibition efficiency relative to blank} = \frac{\text{Increase of \% inhibition over blank}}{\text{\% Inhibition by blank}} \times 100$$

Isolation of crystals from the centrifugates of inhibition experiments in aqueous media: Separate inhibition experiments were conducted in the above experimental model using 50 mL each of 0.02 M concentration of salt forming solutions (calcium chloride and sodium phosphate/oxalate solutions) and 50 mL of 0.02 M inhibitor solutions (solution of aluminium salt) in aqueous medium.

The centrifugate from these inhibition experiments (after removing precipitates) were subjected to crystallization by evaporating off to a small volume. The crystals, so obtained, were filtered off from the mother liquor and dried at 110 ºC in an air oven and preserved over fused calcium chloride. Infrared spectra of these crystals were recorded in the range 4000-500 cm^{-1} in KBr phase on a Shimadzu 8201 PC infrared specrophotometer.

RESULTS AND DISCUSSION

Inhibition data of aluminium salts towards calcium oxalate mineralization in aqueous and urinary media are recorded in Tables 1 and 2, respectively. Inhibition data of aluminium salts towards calcium phosphate Mineralization in aqueous and urinary media are recorded in Tables 3 and 4, respectively.

TABLE-1
INHIBITION OF MINERALIZATION OF CALCIUM OXALATE BY ALUMINIUM SALTS IN AQUEOUS MEDIA

Inhibitor	Strength of inhibitor (M)	Ca^{2+} in solution (mg)	Ca^{2+} Precipitated (mg)	% Inhibition	Increase of inhibition over blank (%)	% Increase of inhibition relative to blank (%)
Water (Blank)	–	0.61	24.27	2.45	–	–
Aluminium chloride	0.01	10.80	14.08	43.41	40.96	1671.84
Aluminium acetate	0.01	13.85	11.03	55.67	53.22	2172.24
Aluminium citrate	0.01	13.90	10.98	55.87	53.42	2188.41

TABLE-2
INHIBITION OF MINERALIZATION OF CALCIUM OXALATE BY ALUMINIUM SALTS IN URINARY MEDIA

Inhibitor	Strength of inhibitor (M)	Ca^{2+} in solution (mg)	Ca^{2+} Precipitated (mg)	% Inhibition	Increase of inhibition over blank (%)	% Increase of inhibition relative to blank (%)
Urine (Blank)	–	2.77	21.67	11.13	–	–
Aluminium chloride	0.01	12.45	12.43	50.04	38.91	349.59
Aluminium acetate	0.01	2.87	22.01	11.53	00.40	3.59
Aluminium citrate	0.01	11.68	13.08	46.94	35.81	321.74

TABLE-3
INHIBITION OF MINERALIZATION OF CALCIUM PHOSPHATE BY ALUMINIUM SALTS IN AQUEOUS MEDIA

Inhibitor	Strength of inhibitor (M)	Ca^{2+} in solution (mg)	Ca^{2+} Precipitated (mg)	% Inhibition	Increase of inhibition over blank (%)	% Increase of inhibition relative to blank (%)
Water (Blank)	–	5.88	19.00	23.63	–	–
Aluminium chloride	0.01	16.80	8.08	67.52	43.89	185.74
Aluminium acetate	0.01	19.00	5.88	76.37	52.67	222.89
Aluminium citrate	0.01	24.70	0.18	99.28	75.65	320.14

Table-1 suggests that the aluminium salts are moderate to good inhibitors of calcium oxalate mineralization. Organic salts of aluminium (acetate and citrate) particularly seem to be better inhibitors. The inhibition

TABLE-4
INHIBITION OF MINERALIZATION OF CALCIUM PHOSPHATE BY ALUMINIUM SALTS IN URINARY MEDIA

Inhibitor	Strength of inhibitor (M)	Ca^{2+} in solution (mg)	Ca^{2+} Precipitated (mg)	% Inhibition	Increase of inhibition over blank (%)	% Increase of inhibition relative to blank (%)
Urine (Blank)	–	10.80	19.63	43.41	–	–
Aluminium chloride	0.01	21.15	3.73	85.01	42.00	96.75
Aluminium acetate	0.01	5.98	18.90	24.03	-19.38	-44.64
Aluminium citrate	0.01	21.70	3.18	87.22	43.81	100.92

efficiency of aluminium citrate came to be as high as 55.87 %. This amount to an increase of 53.43 % over blank (water). In urinary media (Table-2), aluminium chloride and citrate have shown a moderate inhibition of oxalate. Aluminium acetate has shown an inhibition of only 11.53 % proving thus, to be a poor inhibitor in this case. Urine itself has shown an inhibition of 11.13 % of oxalate. Inhibition by urine might be vested in its natural inhibitors like citrate, pyrophosphate, *etc*. Compared to the blank, the increase of inhibition by aluminium chloride and citrate are just 38.91 and 35.81 %, respectively. Both these values are comparatively less than their corresponding values in aqueous media. This might be due to the solute load of urine which has a depressing action on the inhibition.

So far as calcium phosphate inhibiton is concerned, all the three aluminium salts showed uniformly good inhibition in aqueous medium (Table-3). Aluminium citrate proved out to be an exceptionally good inhibitor in this case; the inhibition efficiency coming to as high as 99.28 %. Compared to the blank (water), the increase of inhibition by aluminium citrate is 75.65 %; the corresponding percentage increase of inhibition relative to blank comes to 320.14 %. In urinary media too (Table-4), aluminium citrate showed as high as 87.22 % inhibition of calcium phosphate precipitation; the increase over corresponding blank value is 43.18 %. Urinary solute load seems to have depressed some inhibition. However, urine's own inhibition of phosphate by 43.41 % made up much of depression caused by its solute load. Aluminium chloride too showed a good inhibition, almost comparable to that of aluminium citrate. Aluminium acetate, somehow, failed in this case also just like that for oxalate in urine medium. Compared to the blank (urine), aluminium acetate rather showed a decrease of inhibition by 19.38 %.

A study of inhibition data also suggests that the counter anions of aluminium salts have some role in inhibition by Al^{3+} ions. With acetate as anion, Al^{3+} almost failed in the urinary media for either of the minerals (oxalate and phosphate). On the other hand, with citrate as counter anion, Al^{3+} showed very good inhibition in almost all the cases. With chloride as counter anion, the inhibitions by Al^{3+} are slightly less than that of aluminium citrate in almost all cases, with one exception. The exception is in case of oxalate mineralization in urinary media, wherein, the value with aluminium chloride is slightly higher. Thus, on the whole, a definite trend can not be drawn about the effect of counter anion on the inhibition efficiency of aluminium ions but some latent role is envisaged. Aluminium citrate's exceptionally good performance can also be explained by the fact that citrate anion itself is a known potent inhibitor of urinary stone formation. It seems, in solution, aluminium and citrate ions independently sequesterate the oxalate/phosphate and Ca^{2+}, respectively. The stability of calcium-citrate and aluminium-oxalate/phosphate chelates seem to be higher than calcium-oxalate and aluminium-citrate chelates, respectively. Thus, perhaps owing to a low stability of aluminium citrate chelate coupled with greater affinity of citrate for Ca^{2+}, the Al^{3+} and citrate ions do not cancel each other's inhibition efficiencies, rather they work cohesively to exhibit a very good inhibition of calcium oxalate and calcium phosphate mineralization.

Infrared studies of crystals isolated from the centrifugate of reaction mixtures of calcium chloride, sodium oxalate/phosphate and aluminium salt (inhibitor): One must be aware of the fact that these crystals are not likely to be pure compounds and rather contain more than one ingredients left out in the centrifugates. However, they definitely contain the sequestered (inhibited) portion of oxalate/phosphate. We are just interested in the mode of bonding of this inhibited oxalate/phosphate in the crystals.

Metal oxalates are known to exhibit C=O stretching vibration in wide variation[7]; the frequencies ranging from above 1700 cm^{-1} to as low as 1650-1600 cm^{-1}. The C=O group might be terminal or bridged ones. Presently, the crystals, isolated from the centrifugates containing the sequestered (inhibited) oxalate, showed ν(C=O) in the range 1622 to 1635 cm^{-1}. This low position of ν(C=O) band in these crystals suggest a bridging mode of oxalate in the crystals[7-9]. The bridging might be occurring through a polymeric association of inhibitor metal ion (Al^{3+})-oxalate complex or the oxalate might be bridging the aluminium and calcium ions.

The phosphate ion (PO_4^{3-})has a tetrahedral (T_d) symmetry and shows four infrared absorption modes[10]. These are symmetric P-O stretching (ν_1), asymmetric P-O stretching (ν_3) and the two O-P-O bending modes (ν_2 and ν_4). In a non-equivalent force field around the phosphate ion, however, there occurs distortion from the tetrahedral symmetry[10,11]. In case of ionic

phosphate, the totally symmetric stretching mode (ν_1) is Raman active but in coordinated phosphates this band becomes IR active[12]. Presently, the crystals, isolated from the centrifugates containing the sequestered (inhibited) phosphate, showed the ν_3 (asymmetric P-O stretch) as two bands at 1100-1160 cm^{-1}. The ν_1 band shows at *ca.* 900 cm^{-1} as a quite weak band. The O-P-O bending mode (ν_4) has also been found to occur as doubly split bands at *ca.* 650 and *ca.* 600 cm^{-1}. The split of ν_3 and ν_4 bands suggest a coordinated nature of phosphate in the crystals[12,13].

Thus, it seems the mechanism behind inhibition of precipitation of oxalate/phosphate by the aluminium salts is through their (oxalate/phosphate) complexation in solution.

Conclusion

It is observed that some aluminium salts *viz.*, aluminium chloride, aluminium acetate and aluminium citrate can inhibit mineralization of calcium oxalate and calcium phosphate in aqueous as well as in urinary media. Aluminium chloride and aluminium citrate have shown uniformly good inhibition. Aluminium citrarte, in particular, has proved out to be a very good inhibitor. Calcium oxalate is the least soluble and most stubborn constituent of urinary stones. A good inhibition of precipitation of this mineral by aluminium citrate, especially in urinary media, would be of high applied value in the prevention and control of urolithiasis.

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