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Inhibtion of Mineralization of Urinary Stone Forming Minerals by Some Low Molecular Weight Natural Urinary Inhibitors under Different Chemical Miliew

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Abstract

Biological importance of magnesium and zinc and also with a view to understand their applicability as inhibitors in urolithiasis, we have presently studied the inhibition efficiency of magnesium and zinc ions towards the mineralization of urinary stone forming minerals viz., calcium oxalate and phosphate, in aqueous as well as urinary media. An attempt has been made to unfold, tentatively, the mechanism of inhibition by these inhibitors. Methods: It was Observational Study. The participants in the study included 10 calculi patients with CaOx stones urinary stones. The Study Conducted in the surgical department of M.G.M. Medical College & L.S.K. Hospital, Period between January 2018- December 2019 and all participants provided informed consent. An experimental model was designed in which the two salt forming solutions, the whole operation took about 40 min. At the end the contents of beaker were digested in a hot water bath for 10 min, cooled to room temperature and centrifuged in small volumes. The total centrifugate was collected. Next, the calcium content of the centrifugates in case of calcium phosphate mineralization experiments and oxalate content of the centrifugate in case of calcium oxalate mineralization experiments were determined. Result: Magnesium sulphate has a moderate inhibition efficiency towards calcium oxalate mineralization. At 0.02 M concentration, MgSO4 has a net inhibition of 8.04 % which is 5 % more than that by water (blank). Compared to water the percentage inhibition increased by 164.47 %. With decreasing concentrations of MgSO4 the inhibition efficiency decreases. At very low concentration (0.001 M) its inhibition is only slightly higher than that of water. In urinary medium MgSO4 seems to function as a better inhibitor of oxalate mineralization. Conclusion: it is observed that magnesium sulphate and zinc sulphate solution, under different concentrations, exhibit moderate to good efficiency of inhibition towards mineralization of urinary stone forming minerals viz., calcium oxalate and calcium phosphate, in aqueous as well as urinary milieu. Mg++ has proved to be a comparatively better inhibitor for calcium phosphate mineralization, while Zn++ proved out to be a comparatively better inhibitor of calcium oxalate mineralization.

1. Introduction

The urinary stone formation is related to the level of inhibitors of calculo- genesis in urine1. Human urine is known to contain some low as well as high molecular weight inhibitors. These are citrate, pyrophosphate, nephrocalcin, glycosaminoglycans, magnesium and zinc. However, the mechanism of action of inhibitors has not yet been clearly established. The anions like citrate and pyrophosphate have been speculated to act by soluble-chelation of calcium ions. So far as the mechanism of action of inhibitor cations like magnesium and zinc ions are concerned, it is not yet well unraveled. Effect of magnesium and zinc on urolithiasis risk factors have been studied2-4. Attempts to correlate urinary zinc levels to urolithiasis have been made3,4. However, magnesium and zinc's inhibitory capacities towards lithogenesis in the urinary tract have not yet been quantified and the corresponding chemical mechanisms have not been elucidated. A quest in this direction would be of applied value. Magnesium and zinc are nutritionally essential for the body. Magnesium is a macronutrient while zinc is a micronutrient. Magnesium is seen both in intracellular and extracellular fluids. Its requirement is about 300 mg/day. Normal serum level of Mg++ is 2-3 mg/dl (1-1.5 mmol/L). Mg++ is the activator of many enzymes requiring ATP5. Normal serum level of zinc is 100 µg/dl (15 µmol/L). Requirement of zinc for adults is 1-1.5 mg/day; in pregnancy and lactation, 1.5-2 mg/day. More than 300 enzymes are zinc dependent6.

2. Methodology

It was Observational Study. The participants in the study included 10 calculi patients with CaOx stones urinary stones. The Study Conducted in the surgical department of M.G.M. Medical College & L.S.K. Hospital, Period between January 2018-

December 2019 and all participants provided informed consent. An experimental model was designed in which the two salt forming solutions, the whole operation took about 40 min. At the end the contents of beaker were digested in a hot water bath for 10 min, cooled to room temperature and centrifuged in small volumes. The total centrifugate was collected. Next, the calcium content of the centrifugates in case of calcium phosphate mineralization experiments and oxalate content of the centrifugate in case of calcium oxalate mineralization experiments were determined. 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). Estimations and calculations: Calcium was estimated by complexometric method using standard disodium EDTA solution7. Oxalate was estimated by permaganatometry using standard KMnO4 solution8. While calculating the calcium contents of centrifugate, an EDTA titre value, equivalent to the total inhibitor solution (Mg++ or Zn++ solution) was deducted from the total titre value (equivalent to the centrifugate). This was done because Mg++ or Zn++ that is present in the centrifugate, would also consume some EDTA. While calculating the oxalate content of the centrifugate in case of experiments with inhibitor solution in urinary media, a permanganate titre value equivalent to 50 mL urine was deducted from the total titre value (equivalent to the centrifugate). This was done because urine itself would consume some permanganate due to its own oxalate and probably other reducing substances. Inhibition efficiency of the inhibitor solutions (including that of water/urine) was calculated using the formula

$$\text{Inhibition efficiency (\% inhibition)} = \frac{Ca^{++} \text{ or oxalate in centrifugate}}{\text{Total } Ca^{++} \text{ or oxalate in experiment}} \times 100$$

Table:1. Inhibition of mineralization of calcium oxalate by magnesium and zinc ions in aqueous media

Inhibitor	Strength of inhibitor solution (M)	Oxalate in solution (mg)	Oxalate precipitated (mg)	Inhibition	Increase of Inhibition over blank (%)	Increase of Inhibition relative to blank blank (%)
Water (Blank)	–	1.34	42.66	3.04	–	–
$MgSO_4$	0.020	3.54	40.46	8.04	5.00	164.47
$MgSO_4$	0.010	2.22	41.78	5.05	2.01	66.12
$MgSO_4$	0.002	1.66	42.34	3.77	0.73	24.01
$MgSO_4$	0.001	1.54	42.46	3.49	0.45	14.80
$ZnSO_4$	0.020	8.85	35.15	20.11	17.07	561.51
$ZnSO_4$	0.010	5.53	38.47	12.56	9.52	313.15
$ZnSO_4$	0.002	4.65	39.35	10.57	7.53	247.69
$ZnSO_4$	0.001	4.20	39.80	9.54	6.50	213.81

Table: 2.Inhibition of mineralization of calcium oxalate by magnesium and zinc ions in urinary media

Inhibitor	Strength of inhibitor solution (M)	Oxalate in solution (mg)	Oxalate precipitated (mg)	Inhibition	Increase of Inhibition over blank (%)	Increase of Inhibition relative to blank blank (%)

Urine (Blank)	–	6.10	37.90	13.86	–	–
$MgSO_4$	0.020	28.26	15.74	64.22	50.36	363.34
$MgSO_4$	0.010	22.72	21.28	51.63	37.77	272.51
$MgSO_4$	0.002	12.75	31.25	28.97	15.11	109.02
$MgSO_4$	0.001	6.10	37.90	13.86	0.00	0.00
$ZnSO_4$	0.020	21.64	22.36	49.18	35.32	254.83
$ZnSO_4$	0.010	14.96	29.04	34.00	20.14	145.31
$ZnSO_4$	0.002	10.53	33.47	23.93	10.07	72.65
$ZnSO_4$	0.001	6.10	37.90	13.86	0.00	0.00

Table: 3. Inhibition of mineralization of calcium phosphate by magnesium and zinc ions in aqueous media.

Inhibitor	Strength of inhibitor solution (M)	Oxalate in solution (mg)	Oxalate precipitated (mg)	Inhibition	Increase of Inhibition over blank (%)	Increase of Inhibition relative to blank blank (%)
Water (Blank)	–	5.88	19.00	23.63	–	–
$MgSO_4$	0.020	13.50	11.38	54.26	30.63	129.62
$MgSO_4$	0.010	14.73	10.15	52.20	28.57	120.90
$MgSO_4$	0.002	14.01	10.87	51.52	27.89	118.02
$MgSO_4$	0.001	11.50	13.38	46.22	22.59	95.60
$ZnSO_4$	0.020	19.74	5.14	79.34	55.71	235.76
$ZnSO_4$	0.010	16.62	8.26	66.79	43.16	182.65
$ZnSO_4$	0.002	14.91	9.97	59.91	36.28	153.53
$ZnSO_4$	0.001	12.26	12.62	49.26	25.63	108.46

Table:4. Inhibition of mineralization of calcium phosphate by magnesium and zinc ions in urinary media.

Inhibitor	Strength of inhibitor solution (M)	Oxalate in solution (mg)	Oxalate precipitated (mg)	Inhibition	Increase of Inhibition over blank (%)	Increase of Inhibition relative to blank blank (%)
Urine (Blank)	–	10.80	14.08	43.40	–	–
$MgSO_4$	0.020	18.18	6.70	73.07	29.67	68.36
$MgSO_4$	0.010	17.49	7.39	70.29	26.89	61.96
$MgSO_4$	0.002	15.28	9.60	61.41	18.01	41.49
$MgSO_4$	0.001	12.95	11.93	52.04	8.64	19.90
$ZnSO_4$	0.020	14.16	10.72	56.90	13.50	31.10
$ZnSO_4$	0.010	12.56	12.32	50.47	7.07	16.29
$ZnSO_4$	0.002	11.69	13.19	46.97	3.57	8.22
$ZnSO_4$	0.001	11.67	13.21	46.89	3.49	8.04

3. Discussion

A study of Table-1 suggests that magnesium sulphate has a moderate inhibition efficiency towards calcium oxalate mineralization. At 0.02 M concentration, MgSO4 has a net inhibition of 8.04 % which is 5 % more than that by water (blank). Compared to water the percentage inhibition increased by 164.47 %. With decreasing concentrations of MgSO4 the inhibition efficiency decreases. At very low concentration (0.001 M) its inhibition is only slightly higher than that of water. In urinary medium (Table-2) MgSO4 seems to function as a better inhibitor of oxalate mineralization. At 0.02 M concentration the net inhibition is as high as 64.22 %, which comes to 61.18 % higher than that of water and 50.36 % higher than that of urine. Urine itself has inhibited up to 13.86 % which is 10.82 % higher than that of water. This inhibition efficiency of pure urine might be due to its natural inhibitors like citrate, pyrophosphate, etc. With decreasing concentration of Mg++, the inhibition has been found to gradually decrease and becomes equal to that of pure urine at 0.001 M strength. Thus only up to 0.002 M strength Mg++ retains its own inhibition power. Calcium oxalate is the most frequently occurring constituent of urinary calculi9. It is also the most stubborn constituent. A moderate inhibition of oxalate by Mg++ up to as low as 0.002 M concentration, particularly in urinary medium, suggests that this metal ion would be a useful inhibitor of stone formation in the urinary tract. So far as calcium phosphate inhibition is concerned, the Mg++ shows a good inhibition in aqueous media (Table-3). The net inhibition at 0.02 M being 54.26 %. However, since phosphate has relatively better solubility than oxalate, water itself has shown 23.63 % inhibition. As such, compared to water it is only 30 % increase of inhibition by 0.02 M MgSO4. With decrease of concentrations of Mg++, the phosphate inhibition decreases only slightly. Even at 0.001 M, the inhibition is 22.59 % higher than water, which comes to a 95 % increase over blank. This shows that Mg++ is a good sequesterant of calcium phosphate even at very low concentration. In urinary media (Table-4) too, Mg++ shows more or less similar trend of inhibition. So far as calcium phosphate mineralization is concerned, ZnSO4 exhibits a good inhibition in aqueous media (Table-3). Compared to water (blank), 0.02 M Zn++ shows an increase of 55.71 % inhibition. With the decrease in concentration of inhibitor (Zn++), the inhibition efficiency has been found to decrease only slightly. In urinary media, ZnSO4 does not show up as a good inhibitor for calcium phosphate (Table-4). The net inhibition by Zn++ at 0.02 M concentration is only 56.9 % which is just 13.5 % above that of the urine (blank). With a decrease of concentration the net inhibition decreases slightly and at very low concentration (0.001 M), Zn++ has an inhibition almost equal to pure urine i.e., with almost no additional increase over blank. On the whole, it looks that Mg++ is a uniformly good inhibitor of calcium phosphate mineralization, whereas, Zn++ is a better inhibitor of calcium oxalate mineralization. The underlying mechanism behind inhibition might be the soluble complexation of phosphate/oxalate by Mg++/ Zn++ (inhibitor). It looks Zn++ forms a soluble stable complex with oxalate ions and, in turn, screens the later (oxalate) from Ca++. Oxalate precipitation is, thus, inhibited. Mg++, on the other hand, seem to have comparatively (compared to Zn++) less affinity for oxalate, hence unable to sequesterate much oxalate in solution. Calcium oxalate precipitation is thus only slightly inhibited, However, at least some inhibition by Mg++ points to the fact that a relatively more soluble (compared to calcium oxalate) magnesium oxalate complex of some stoichio- metry participates in the entire solution equilibria in the experiment.

For calcium phosphate inhibition, on the other hand, slightly reverse is the trend. Mg++ proved to be a better inhibitor than Zn++. Relative affinities between the cation (Mg++ on Zn++) and anion (PO4---) seem to play role, once again. Due to poor chelating ability of phosphate ion, Zn++ fails to stabilize the former in solution. Magnesium ion, however, might be forming a relatively more covalent (Mg++ has a higher ionic potential) and less aggregating salt. Metal oxalates are known to exhibit C=O stretching vibration in wide variation8; 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 centrifugate of the reaction mixture of calcium chloride, sodium oxalate and magnesium sulphate, showed C=O stretching vibration at 1630 cm-1. The crystals from the centrifugate of the reaction mixture of calcium chloride, sodium oxalate and zinc sulphate exhibited C=O stretching vibration at 1624 cm-1. A quite low position of v(C=O) band in these crystals suggest that the C=O groups are not free but are rather bridged ones8,9. Most probably the C=O groups might be bridging the Ca++ and Mg++/Zn++ (inhibitor) ions. The low position of C=O vibration might also be due to a chelation of oxalate with the Mg++/Zn++ (inhibitor ion) resulting ion in coordinated C=O groups and consequent polymeric association through C=O bridging. Thus, in any case, soluble chelation of oxalate by the inhibitor cation seems to be the mechanism behind inhibition of calcium oxalate

mineralization by the inhibitors (Mg++/Zn++). The phosphate ion (PO4---) has a tetrahedral (Td) symmetry and shows 4 infrared absorption modes10. These are symmetric P-O stretching (v1) asymmetric P-O stretching (v3) and the two O-P-O bending modes (v2 and v4). In a non-equivalent force field around the phosphate ion, however, there occurs distortion from the tetrahedral symmetry10,11. In case of ionic phosphate, the totally symmetric stretching mode (v1) is Raman active, but in coordinated phosphates this band becomes IR active12. Presently the infrared spectra of the crystals, obtained from the centrifugate of reaction mixture of calcium chloride, sodium phosphate and magnesium sulphate, showed a band of medium intensity at 1094 cm-1. This band may be assigned to asymmetric P-O stretch (v3). The symmetric P-O stretch (v1) showed rather low at 915 cm-1. Weak bands at 680 and 604 may be assigned to the two split components of O-P-O bending mode (v4). Relatively low position of v3 band coupled with split of v4 band suggests a coordinated nature of phosphate in the crystals12 In the infrared spectra of the crystals from the centrifugate of calcium chloride, sodium phosphate and zinc sulphate, the v3 band showed at 1155 cm-1 as a double headed peak. The v1 band has been observed just as a shoulder at ca. 950 cm-1. The v4 band, however, has been found to split into two, showing at 671 and 602 cm-1. Split of v3 and v4 bands suggests that the phosphate in the crystals is not ionic but is rather in some coordinated state. Thus, it seems, Mg++ or Zn++ inhibits calcium phosphate mineralization through sequestering-complexation of phosphate.

5. Conclusion

Presently, it is observed that magnesium sulphate and zinc sulphate solution, under different concentrations, exhibit moderate to good efficiency of inhibition towards mineralization of urinary stone forming minerals viz., calcium oxalate and calcium phosphate, in aqueous as well as urinary milieu. Mg++ has proved to be a comparatively better inhibitor for calcium phosphate mineralization, while Zn++ proved out to be a comparatively better inhibitor of calcium oxalate mineralization. Infrared studies suggested that the magnesium or zinc ions inhibit calcium phosphate or calcium oxalate mineralization by sequestering complexation (soluble chelation) of phosphate or oxalate. Calcium oxalate forms a stubborn constituent of urinary calculi. Its inhibition by Zn++, particularly in urinary medium, would be of applied value in the prevention and control of urolithiasis.

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