Urol Res (1984) 12:271–273

Urological Research

Relationship Between Experimentally Induced Crystalluria and Relative Supersaturation of Various Stone Salts in Rats

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Accepted: January 11, 1984

Summary. Calcium oxalate crystalluria was induced in laboratory rats by subcutaneous implantation of potassium oxalate containing mini-osmotic pumps in their intercapsular region. Concentrations of major urinary ions were measured and urinary supersaturations of various urinary salts were calculated using a computer programme. The urines of experimental animals that received oxalate had calcium oxalate crystals and higher supersaturations for calcium oxalate compared to their controls. Oxalate levels of the urines of experimental animals were higher than their controls and this increase was proportional to the increase in urinary supersaturation of calcium oxalate. No significant difference was found in the calcium levels of urines from experimental and control animals.

Key words: Urinary supersaturation, Calcium oxalate, Crystalluria, Mini-osmotic pumps.

Introduction

It is well documented that urinary supersaturation with stone forming salts is necessary for stone initiation, that the presence of crystalluria is an indication of previous urinary supersaturation, and that the crystalluria of human stone patients is different from that of normal individuals. Therefore, any experimental study of urinary stone disease must consider the phenomenon of crystalluria and its correltation with urinary supersaturation of crystal salts. With this in mind we have developed a relatively non-toxic experimental model of crystalluria wherein crystalluria is induced in rats by implantation of mini-osmotic pumps filled with a saturated solution of potassium oxalate. Various crystal morphologies have been studied, urinary ions and pH have been measured, and 24 h urinary volume has been determined to calculate supersaturation of calcium oxalate, calcium phosphate (hydroxyapatite), and magnesium ammonium phosphate. The results of morphological studies and details of the experimental methods have already been described [8]. The present paper describes the results of the measurement of major urinary ion concentrations and calculations of urinary supersaturations of various urinary salts.

The purpose of our experiments has been to induce crystalluria lasting many weeks. Our initial method involved repetitive abdominal operations to replace the pumps. This intraperitoneal approach presented problems because the operations placed severe stress on the animals and the pumps adhered to the intestines making their replacement difficult. Also, local deposits of calcium oxalate appeared in the peritoneum and at the heads of the mini-osmotic pumps. Therefore, we repeated the experiment with potassium oxalate-containing pumps planted subcutaneously. The animlas tolerated this implantation better, there was minimal subcutaneous crystal deposition, and pump adhesion with the tissue did not occur. The results described here are from animals which were subcutaneously implanted with potassium oxalate (KOx)-containing mini-osmotic pumps.

Materials and Methods

Except for changing the implantation site of the mini-osmotic pumps, no other changes were made in methodology [8]. Male Sprague-Dawley rats weighing between 150–200 g were used. The mini-osmotic pumps were model #2002.

Minni-osmotic pumps were fully loaded with approximately 225 μl of a 1.2 M solution of KOx and were implanted subcutaneously in the intercapsular region of the animals; four pumps in each of the five animals. On the tenth day these discharged pumps were replaced with fully loaded new pumps and the experiment was terminated on the twentieth day. On the 3rd, 8th, 13th, and 18th day of the experiment 3 h urine collections were made, and on 5th and 15th day 24 h urine collections were made.

Urine collections were done in metabolic cages. Freshly voided 3 h urine samples were used for counting crystals and to study crystal morphology by light microscopy (LM) and scanning electron microscopy (SEM). The urinary crystals were counted and categorized using Neubauer counting chamber [6]. The pH was determined from urine freshly voided when a few drops of ether were dropped on the animal's back. 24 h urine samples were collected

Table 1. Mean (±SD) of the concentrations of various ions and relative supersaturations (RS) of various salts in the urines of experimental and control animals (*NS*, Not significant; *AP*, Activity product; *MAP*, Magnesium ammonium phosphate; *MPP*, Magnesium potassium phosphate)

Urinary values	Experimental ($n = 9$)	Controls ($n = 9$)	P
Sodium (mM)	167.77 ± 61.69	174.33 ± 48.83	NS
Potassium (mM)	222.77 ± 48.61	224.77 ± 59.97	NS
Calcium (mM)	3.81 ± 1.36	3.55 ± 1.79	NS
Magnesium (mM)	13.68 ± 3.44	16.97 ± 7.61	NS
Phosphate (mM)	8.42 ± 2.57	7.72 ± 2.91	NS
Sulphate (mM)	38.33 ± 4.55	36.44 ± 6.98	NS
Ammonia (mM)	15.43 ± 3.00	16.00 ± 5.03	NS
Citrate (mM)	8.40 ± 4.06	10.88 ± 4.77	NS
Oxalate (mM)	1.98 ± 0.47	0.84 ± 0.2	< 0.001
pH	7.75 ± 0.54	7.68 ± 0.33	NS
#CaOx crystals (per mm^3)	455.56 ± 528.00	0	< 0.001
RS CaOx	18.46 ± 7.37	7.94 ± 3.83	0.002
#Struvite-type crystals (per mm^3)	35.00 ± 44.9	20.55 ± 28.98	NS
RS Struvite	7.59 ± 5.67	9.62 ± 9.10	NS
Ln RS Hydroxyapatite	28.64 ± 4.54	30.47 ± 4.00	NS
Ln sum of AP of MAP and MPP	−26.04 ± 1.12	−25.85 ± 1.08	NS

with 2 ml of 4 N HCl in collection tubes. Total urinary volume was measured for each 24 h collection and urine was aliquoted for chemical determination of various ions. Ammonia was measured by the method of Chaney et al. [2], sulphates according to Bergland et al. [1], citrate according to Natelson et al. [9], oxalate according to Hodgkinson and Williams [7], phosphorus according to Chen et al. [3], and calcium, magnesium, sodium, potassium according to the Perkin Elmer procedure manual using a model #306 atomic absorption spectrophotometer. From these values the relative supersaturations of hydroxyapatite, struvite, and calcium oxalate were calculated using a computer model [4]. Our energy dispersive x-ray microanalysis and x-ray diffraction of struvite-type crystals showed them to be a mixture of magnesium ammonium phosphate (MAP) and magnesium potassium phosphate (MPP). Therefore we also calculated the activity products of MAP and MKP.

At the end of the experiment all animals were autopsied. Bladder urine was aspirated for SEM, pH measurement, and bacterial culture. Various tissues were processed for pathological examination [8]. Pump surfaces and implantation sites were examined for crystal deposition and identification.

Control rats were implanted with pumps containing 1.2 M potassium chloride solution, and analyses were performed on their urines and varoius tissues, identical to those on experimental animals.

Results

The implantation site in control animals contained no free fluids or crystals and no crystals were found on the surface of osmotic pumps extracted from it. There was a moderate amount of CaOx monohydrate crystal deposition around the heads of osmotic pumps extracted from experimental animals but considerably less than with the intraperitoneal method. A small amount of fluid was also present at the implantation site. Tissue specimens other than implantation sites from both groups of animals were free of any calcification or any other abnormality.

The crystalluria of these animals was similar to that described earlier [8] for intraperitoneally implanted animals. Both normal and experimental animals had amorphous calcium phosphate, hydroxyapatite, and struvite-type crystals. No CaOx crystals were found in the urine of control animals. Urine from both normal and experimental animals had casts. Calcium oxalate crystals present in the urine of experimental animals were mostly dipyramidal CaOx dihydrate ranging in size from 2 μ–12 μ. Smaller dihydrate crystals were generally found associated with casts or large struvite-type crystals. Single as well as multiple interpenetrating twins of dihydrate crystals were also common. Some of these twins were upto 50 μ in their largest dimension.

The results of our measurements of various urinary ions, pH, volume, and number of crystals, and our computer calculations of supersaturations and activity products of major urinary salts are given in the Table 1. It is clear that urines of experimental animals had significantly higher oxalate levels and higher CaOx supersaturations than their control counterparts. On average the urine of experimental animals contained 2.36 times more total oxalate than the urine of controls, and the relative supersaturation of CaOx in the experimental animals' urine averaged 2.33 times higher than in control urines. Relative supersaturation of CaOx had a low positive correlation ($R = 0.302$) with number of CaOx crystals in the urine and a low negative correlation ($R = -0.178$) with urinary pH. Relative supersaturation of struvite had a high positive correlation with urinary pH ($R = 0.893$) and a negative correlation with number of struvite-type crystals ($R = -0.552$). Correlation between the log of the sum of activity products of magnesium ammonium phosphate and magnesium potassium phosphate and number of struvite-type crystals was also negative ($R = -0.619$).

Discussion

The results of our experiments show correlations between crystalluria and urinary supersaturation, albeit not strong. All urines were supersaturated with regard to struvites and hydroxyapatites and all of them had struvite-type crystals as well as crystals of hydroxyapatite. The urines of experimental animals that received oxalate through implanted pumps had higher urinary supersaturation of CaOx than the control animals and were the only ones that had crystals of CaOx. However, crystal numbers did not correlate well with the degree of supersaturation. This may be due to our method of crystal counting since we cannot see crystals less than 4 μ across and the space between coverslip and slide will not admit crystals greater than 100 μ. Scanning electron microscopic examination of the urine revealed numerous calcium oxalate crystals less than 4 μ across and

struvite-type crystals greater than 100 μ. This may explain the low positive as well as negative correlation between crystal number and supersaturation. It is interesting to note that Werness et al. [12] had somewhat similar results during a study of human crystalluria. They found that although urines supersaturated for a particular salt showed crystals of that salt, the relative number of each crystal phase did not correlate well with the degree of supersaturation. They suggested that some factors such as inhibitors which are not taken into account in the calculation of supersaturations were responsible for their observations.

The main factors determining saturation of urine with CaOx are urinary concentrations of calcium and oxalate. Although no consensus exists as to which of the two is more important in stone disease [11], elementary ion equilibria theory leaves no question about the matter. It has been proposed that an increase in the urinary concentration of oxalate, even to the upper limit of normal, will have a greater effect on CaOx supersaturation, and is more likely to produce crystalluria, than will an equivalent increase in the urinary concentration of calcium, even well into hypercalciuric range [4, 5, 10, 11]. One of the major reasons for this greater effect of an oxalate increment on the relative supersaturation of urine with CaOx is the high calcium to oxalate ratio in human urine [11], and the formation of poorly soluble complex between calcium and oxalate. Analysis of the equilibrium Ca + Ox = CaOx shows that as calcium increases the total oxalate to calcium oxalate concentration ratio asymptomatically decreases to 1. Therefore the higher the calcium concentration, the less is the effect of an increment in calcium on calcium oxalate concentration. A similar statement can be made for oxalate. Therefore, incrementing the ion present in the higher concentration will have less effect than incrementing the ion present in the lower concentration. Simply stated, an increase in the urinary concentration of any one of the two, calcium or oxalate, whichever is present in the lesser quantity than the other would result in the greater production of supersaturation of urine with calcium oxalate. The greater the difference in concentration between the two ions the greater will be the difference in the effect on supersaturation and the ion present in the lower concentration will be more or less a limiting factor.

Although our results show that an increase in urinary oxalate results in an increase in the supersaturation of urine with CaOx, there was no significant difference in the urinary calcium levels of control rats and experimental rats. We are therefore unable to give a practical demonstration of the effect of calcium concentration variation on the relative supersaturation of calcium oxalate. However, the positive effect of an increase in the urinary oxalate level on CaOx supersaturation is evident from the fact that the relative supersaturation of CaOx was much higher in the urines of experimental animals than in urine of control animals and the increase was proprtional to the increase in urinary oxalate levels.

Acknowledgements. The authors thank Dr. Henry C. Aldrich of the Department of Microbiology and Cell Science, UF, for the use of his EM facilities. Ecxellent technical assistance was provided by Mr. John Konicek and Mrs. Martha Tilden. This work was supported by a National Institute of Health grant #20586–06.

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