POLYANIONIC INHIBITORS OF CALCIUM OXALATE CRYSTAL AGGLOMERATION IN URINE

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Summary

The excretions and relative potencies of various macromolecular inhibitors of the crystallisation of calcium oxalate (CaOx) were measured in the urines of idiopathic calcium stone-formers and normal subjects. The stone-formers excreted significantly less polyanionic macromolecules in their urine than did the normals, the difference being attributable to the lower excretions of glycosaminoglycans (GAGS), ribonucleic acid (RNA) and Tamm-Horsfall mucoprotein (THM), all of which are precipitable with alcian blue.

The relative potencies of the various inhibitors measured under ‘whole urine equivalent’ conditions using a batch crystallisation system, showed that the order of inhibitory activity towards CaOx crystal agglomeration was RNA > GAGS > THM > pyrophosphate (PPi). This paralleled the order of ability of these inhibitors to produce a high negative zeta potential on the surface of CaOx crystals.

Introduction

There have been many articles in recent years on the subject of urinary inhibitors of calcium oxalate (CaOx) crystallisation [1–6]. In general, these have shown that certain polyanions present in urine are important as true inhibitors of crystallisation i.e. they act at low concentration by adsorbing to the surfaces of CaOx crystals where they alter the crystal growth and agglomeration properties of the crystals [7]. The polyanions of main interest in this respect are macromolecules including ribonucleic acid (RNA), glycosaminoglycans (GAGS) and non-polymerised Tamm-Horsfall mucoprotein (THM). The only low molecular weight polyanion of any note so far found in urine is pyrophosphate (PPi). Other small ions, such as citrate and magnesium, may retard the observed growth rate of CaOx crystals but do so mainly by reducing the degree of supersaturation of urine with respect to CaOx through the complexing of calcium and oxalate ions respectively in urine. This paper concerns itself only with the first type of
inhibitor.

The study consists of a comparison of the excretions of the main polyanionic inhibitors in the urines of idiopathic calcium stone-formers and their controls and the effect of these inhibitors on the degree of crystal agglomeration in a batch crystalliser in which CaOx crystals are generated in situ at degrees of supersaturation approximating those in the urines of recurrent CaOx stone-formers [8].

Methods

Twenty-four hour urine samples were collected in sterilised containers without preservative from 30 normal men, aged 23–57 (mean 34.1 years), and 21 recurrent, idiopathic calcium stone-formers, aged 28–74 (mean 39.5 years). The urines were analysed for alcian blue precipitable polyanions (ABPP) using a modification of the method of Whiteman, glycosaminoglycans by measurement of uronic acids, Tamm-Horsfall mucoprotein by protein assay and RNA by a modified orcinol method for measuring ribosyl groups [9].

In a separate study, the effects of various concentrations of RNA, GAGS and non-polymerised THM were measured on the rates of agglomeration of CaOx crystals generated spontaneously in a batch crystalliser [9]. Pyrophosphate was also studied as an example of a low molecular weight polyanion. The zeta potential produced by the inhibitors on the surface of CaOx crystals was measured using a Zeta Meter (Zeta Meter Inc., New York).

Results and discussion

Figure 1 shows the mean values (± 1 SEM) of the daily excretion of the ABPP and their constituent GAG, THM and RNA fractions in the urines of stone-formers and normals. The overall urinary excretion of polyanions (ABPP) was reduced in the stone-formers compared with the normals confirming our earlier finding [10]. The lower excretions of ABPP in the stone-formers was attributable to a reduction in the excretion of all three constituent polyanions but the reason for the reduction in the excretion of these structurally unrelated compounds is not yet clear. It may be that, for some reason, polymerisation of these macromolecules is promoted in the urines of the stone-formers and this inactivates them as inhibitors or even causes them to act as mild promoters of crystallisation as noted in an earlier paper [9].

Figure 2 shows the measured inhibition of agglomeration produced by a number of inhibitors in a high supersaturation batch crystallisation system. In terms of molar concentration, the most active inhibitor tested was RNA. The order of inhibitory activity towards agglomeration of CaOx crystals was found to be RNA > GAGS > THM > PP<sub>1</sub>. The last-mentioned was more active at pH 6.5 than 5.5 presumably because of its greater dissociation and higher negative charge at the more alkaline pH. Other studies, not reported here, show that the ability to inhibit crystal growth was in the order RNA > GAGS > PP<sub>1</sub> > THM, the last-mentioned having virtually no effect on this process within the urinary range of concentration.
Figure 1. The 24-hour excretions of alcian blue precipitable polyanions (ABPP), glycosaminoglycans (GAGS), Tamm-Horsfall mucoprotein (THM) and ribonucleic acid (RNA) in the cetyl trimethyl ammonium bromide (CTAB) extracts of urines from normals (N) and recurrent stone-formers (RSF) (mean ± SEM)

Figure 2. The percentage inhibition of agglomeration of calcium oxalate crystals produced by various inhibitors in a batch crystalliser at high supersaturation in relation to the concentration of inhibitor in solution
Figure 3. The percentage inhibition of agglomeration of calcium oxalate crystals in a batch crystalliser in relation to the zeta potential on the crystal surface produced by different concentrations of various polyanionic inhibitors and macromolecular extracts from urine.

Figure 3 shows the inhibitory effect of various polyanions on the agglomeration of CaOx in relation to the zeta potential produced by these inhibitors on the crystal surface. This figure brings together a wide variety of polyanionic inhibitors of different structures, potencies and concentrations. Although other factors such as Van der Waal’s forces and steric effects also play a role in determining the likelihood of agglomeration of crystals, clearly zeta potential must dominate the extent to which CaOx crystals will agglomerate in this system.

In conclusion, it would appear that, of the various urinary inhibitors of crystallisation so far suggested to be of possible importance in protecting the individual against calcium oxalate stone-formation, only the macromolecular polyanions significantly inhibit agglomeration within the urinary range of concentrations of these ions and these are the inhibitors which are reduced in stone-formers’ urine. Further studies are required to establish the cause of this reduction, or apparent reduction, in the excretion of these polyanions.

References
1 Robertson WG, Peacock M, Nordin BEC. Clin Chim Acta 1973; 43: 31
5 Randolph AD, Drach GW. J Crystal Growth 1981; 53: 195
6 Sheehan ME, Nancollas GH. Invest Urol 1980; 17: 446
8  Robertson WG, Peacock M, Nordin BEC. *Clin Sci* 1971; 40: 365