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Measurement of the Risk of Calcium Phosphate Crystallization in Urine

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Summary. A method is described for analysis of the risk of calcium phosphate (CaP) crystallization in urine samples. The pH required for formation of 500 crystals in the size range 3.5 to 5 μm (pH_{CaP}) was determined in a Coulter Counter following addition of sodium hydroxide. The risk of CaP crystallization (CaP-CR) was defined as: $1/(pH_{CaP} - 5.8)$. CaP-CR was determined in 24 h urine collections from 25 patients with calcium stone disease and 26 normal subjects, each urine diluted to a creatinine concentration of 5 μmol per ml. The mean (± SD) CaP-CR was 0.71 ± 0.18 and 0.63 ± 0.14 respectively and did not differ significantly. This method might be useful for evaluations and follow-up of stone formers with respect to the risk of CaP precipitation and stone formation.

Key words: Calcium phosphate, Crystallization, pH, Urine.

Introduction

Calcium phosphate (CaP), mainly in the form of apatite, is an important component of calcium containing renal stones. Pure CaP concrements occur, but a mixture of CaP and calcium oxalate is much more common. Crystallization and growth of CaP is thought to be the net result of supersaturation with respect to CaP and activity of CaP-crystallization inhibitors [1]. Although the supersaturation of urine with CaP can be approximately determined by means of different methods to calculate the ion-activity products of different calcium phosphate salts [3], there are no simple methods available for measurement of the inhibiting activity or the total crystallization potential of undiluted urine.

Because CaP-crystallization certainly is very important for the formation and growth of calcium stones in urine, a simple method to determine the CaP crystallization risk is highly desirable.

Methods

Urine from 26 normal subjects and 25 patients with calcium stone disease was collected in bottles containing 90 mmol of hydrochloric acid and stored frozen at −20 °C until analyzed. The acidification dissolved crystals of CaP and calcium oxalate that had formed in the urinary tract or during storage.

Before the crystallization experiment the urine was thawed, carefully mixed and adjusted to pH 5.8 by addition of sodium hydroxide. In order to standardize the procedure all urine samples were diluted with distilled water to give a final creatinine concentration of 5 μmol per ml. Immediately prior to the crystallization measurement urine was passed through a Millipore filter with a pore size of 0.22 μm.

To 200 ml of urine prepared in this way was added small aliquots of sodium hydroxide (1 mol/l) at room temperature. The pH was recorded and the number of crystals measured every 0.05 pH unit in a Coulter Counter (Model ZBI) with Channelyser. The experiment was interrupted as soon as 500 particles with diameters between 3.5 and 5 μm were recorded. The pH corresponding to 500 particles in the size range 3.5 to 5 μm was determined by interpolation (pH_{CaP}). The inverted value of the difference between pH_{CaP} and the starting pH of 5.8 was used as an estimate of the crystallization risk (CaP-CR):

$$\text{CaP-CR} = 1/pH_{CaP} - 5.8)$$

Light microscopy showed that the formed crystals were amorphous.

Ten urine samples were also analyzed with respect to their content of calcium (Ca), phosphate (P), and citrate (Cit) by methods previously described [4, 5].

The ion-activity product of CaP was estimated by means of the AP(CaP)-index [3]:

$$\frac{2.7 \times 10^{-3} \times Ca^{1.07} \times P^{0.70} \times (pH - 4.5)^{6.8}}{Cit^{0.20} \times V^{1.31}}$$

Results

The crystallization of CaP in four urine samples is shown in Fig. 1. The different curves clearly demonstrate the rapid crystallization that takes place. With steep curves of this type there is a very small error in the interpolation procedure.

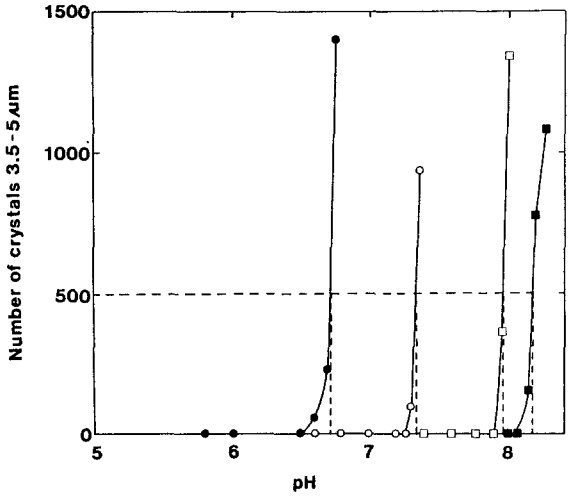

Fig. 1. Crystallization in 4 urine samples during alkalinization

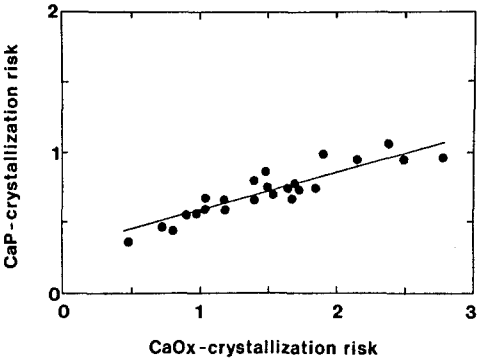

Fig. 3. Correlation between CaP-CR and CaOx-CR in urine from patients with calcium stone disease

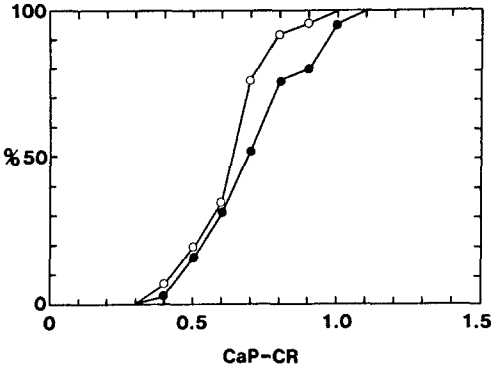

Fig. 2. Cumulative frequency distribution curves of CaP-CR in normal subjects (○) and patients with calcium stone disease (●)

There were slightly more urines with a high CaP-CR among stone formers than among normal subjects, but the cumulative frequency distribution curves are very close to each other (Fig. 2). The mean (± SD) CaP-CR was for normal subjects 0.63 ± 0.14 and for patients 0.71 ± 0.18. The difference was not statistically significant.

The mean (± SD) pH_{CaP} was in normal urine 7.32 ± 0.35 and in urine from stone formers 7.26 ± 0.38.

In order to check that already precipitated calcium salts were dissolved in the acidified urine, the calcium concentration was analysed before and after Millipore filtration. No differences in calcium concentration were recorded, indicating that the filtration procedure did not affect calcium concentration in the urine samples.

The recorded CaP-CR values in 10 urine samples were positively correlated with the AP(CaP)-index calculated at pH 5.8 (r = 0.44). There was also a positive correlation between CaP-CR and CaOx-CR [6] both in normal subjects (r = 0.86) and in patients with calcium stone disease (r = 0.89) (Fig. 3).

Although subject to considerable individual variations, the AP(CaP)-index calculated for pH_{CaP} in 20 normal subjects and 10 patients took values around 60 in both groups.

Discussion

In order to standardize the experimental conditions the CaP-CR in this investigation was referred to a fixed creatinine concentration and a pH of 5.8. However, the risk of CaP crystallization can probably be even better predicted by the difference between pH_{CaP} and the true original pH of fresh urine. In this form CaP-CR might be useful for evaluation of the crystallization potential in urine collected during shorter periods of the day when a reliable measurement of urine pH can be obtained. A low pH_{CaP} probably reflects either a high supersaturation with respect to CaP or a low activity of crystallization inhibition and patients with a pH close to pH_{CaP} will thus be at risk of CaP-crystallization.

When analysed in 24 h urine samples with a starting pH of 5.8, there were no significant differences in CaP-CR between normal subjects and stone formers. However, attributable to pH variations during the day, differences in CaP-CR during special risk periods might occur and cannot be excluded unless CaP-CR is determined in fractionated 24 h urine samples.

An AP(CaP)-index above 60 appears to indicate a particularly high risk of CaP precipitation. The crystallization in this system might be of homogenous type and thus not informative of the risk of heterogenous CaP crystallization that can be anticipated in the presence of calcium oxalate crystals or other particles.

In the method described by Nicar et al. [2] the risk of CaP crystallization was derived from the amount of calcium required for CaP (brushite) precipitation. However, at low pH levels calcium oxalate rather than CaP will precipitate, a

risk which is eliminated when urine pH is successively increased.

Measurement of CaP-CR either under standardized conditions or in freshly voided urine might be useful in the evaluation of stone formers during different forms of prophylactic treatment. A method to determine the risk of CaP crystallization is particularly valuable in patients given alkali in prevention of calcium oxalate stone formation.

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