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Studies on Crystalluria in Calcium Oxalate Stone Formers

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Summary. The excretion of calcium oxalate and calcium phosphate crystals was studied in fractionated 24 h urine from 7 men with recurrent calcium oxalate stone disease, both before and during daily administration of 5 mg bendroflumethiazide. Urinary calcium, oxalate, magnesium, citrate, phosphate, pH, and inhibition of calcium oxalate crystal growth rate were analyzed in all samples. Exclusively calcium oxalate crystals were found in 30 per cent of the samples, all with a pH below 6.25, whereas calcium phosphate was the crystal type encountered in urine with a pH above 6.50. Bendroflumethiazide decreased the volume of calcium phosphate but not of calcium oxalate crystals. During the period of observation there was no correlation between calcium oxalate supersaturation and calcium oxalate crystal volume, but a relationship was demonstrated between calcium phosphate supersaturation and calcium phosphate crystal volume.

Key words: Calcium phosphate, Calcium oxalate, crystalluria, Diurnal variation, pH, Thiazide treatment.

Introduction

Calcium oxalate crystals are common both in normal subjects and in calcium oxalate stone formers [5, 16]. Once formed these crystals will grow and aggregate as a result of supersaturation with respect to calcium oxalate [19]. The process is apparently also modified by inhibitors of crystallization [19]. Calcium oxalate crystals were shown to be more frequent and larger in stone formers than in normal subjects [5, 16, 17]. The formation of calcium oxalate-containing renal stones is thought to be the final outcome of this process.

Calcium phosphate crystals are also frequently found in urine, but nevertheless pure calcium phosphate stones are rare [8, 9]. On the other hand stones composed of a mixture of calcium oxalate and calcium phosphate are common [8].

Analysis of the effects on crystal excretion might therefore be an important tool in the evaluation of stone prophylactic regimens [5, 25]. We therefore studied the excretion of calcium containing crystals during 24 h periods in recurrent calcium oxalate stone formers before and during treatment with bendroflumethiazide.

Material and Methods

Seven men with a median age of 42 years (range 37–69 years), and with recurrent calcium oxalate stone disease were studied. From the analysis of 24 h urine composition according to principles previously described [20], 6 were found to be hypercalciuric (above 500 mmol per mol of creatinine). In one the calcium excretion was slightly below the upper normal limit, but a low magnesium excretion (below 200 mmol per mol of creatinine) resulted in a calcium/magnesium quotient above 2.0 [20]. One of them also had hyperoxaluria (above 30 mmol per mol of creatinine). Three patients had residual renal concrements.

Crystalluria and urine composition were analyzed in fractionated 24 h urine collections before and during treatment with bendroflumethiazide. Urine was always collected on an in-patient basis. Bendroflumethiazide (Salures-K, Ferrosan AB, Sweden) was administered as a single dose of 5 mg at breakfast. Each tablet contained 2.5 mg of bendroflumethiazide and 0.57 g of potassium. There was an interval of 2 to 6 weeks between the start of treatment and the second analysis. The patients were evaluated at short intervals in order to avoid seasonal variations in crystalluria and urine composition [6].

After an over-night fasting period the patients were given a standardized diet composed of ordinary Swedish food at regular intervals: breakfast at 07.30 h, lunch at 11.30 h, dinner at 16.30 h, and evening coffee at 18.30 h. The fluid intake was standardized to a volume of 2,000 ml per 24 h, because a high urine flow was necessary for measurements of both urine crystals and urine biochemical composition. The diet contained approximately 77 g of protein, 83 g of fat, 280 g of carbohydrates, 33 mmol of calcium, 12.5 mmol of magnesium, 33 mmol of phosphorus, and 1.2 mmol of oxalate. The energy content was 8.4 MJ.

Urine was collected directly into a Thermos flask every third hour from 06.00 to 21.00 h and then in one single portion during the night. All samples were immediately brought to the laboratory where pH and volumes were recorded. After an incubation period of 20 min at 37 °C the urine was examined for its content of cal-

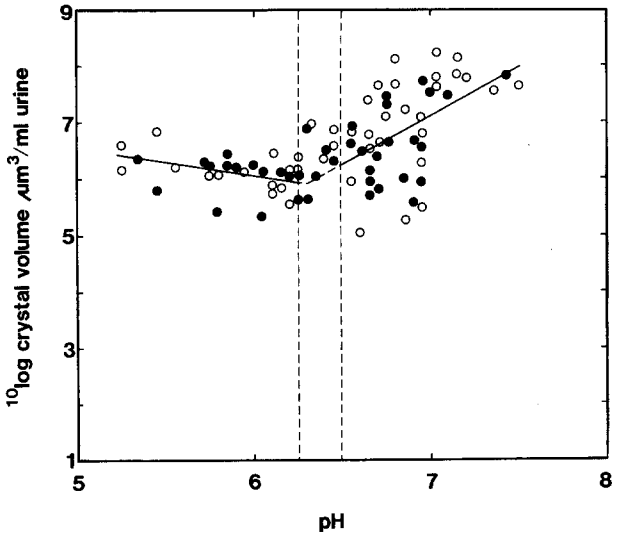

Fig. 1. Relationship between crystal volume and pH in samples of urine, before (○) and during (●) treatment with bendroflumethiazide

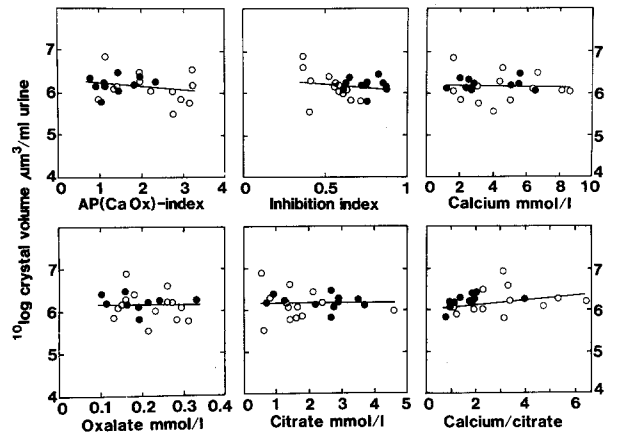

Fig. 2. Calcium oxalate crystal volume in relation to different urine variables, before (○) and during (●) treatment with bendroflumethiazide

cium crystals in a Coulter Counter (model Z_B with Channelyzer) according to principles described by Robertson [12]. Thereby it was possible to estimate the number and volume of crystals, and also their size and volume distribution. Light microscopy was used to differentiate between calcium oxalate and calcium phosphate crystals, but no attempt was made to differentiate the types of crystals within these groups. Calcium oxalate was most often seen as tetragonal bipyramids and calcium phosphate as irregular deposits.

A urine aliquot was taken from each collection for analysis of calcium, magnesium, citrate, oxalate, phosphate, and creatinine [20], and for determination of inhibition of calcium oxalate crystal growth rate in diluted urine (inhibition index) [21]. Approximate estimates of the ion-activity products of calcium oxalate (AP_{CaOx}) and calcium phosphate (AP_{CaP}) were calculated in 3-h urine collections by means of an *AP(CaOx)-index* [22]:

$$\frac{6.7 \times \text{calcium}^{0.71} \times \text{oxalate}}{\text{magnesium}^{0.14} \times \text{citrate}^{0.10} \times \text{volume}^{1.2}}$$

and an *AP(CaP)-index* [23]:

$$\frac{4.5 \times 10^{-3} \times \text{calcium}^{1.07} \times \text{phosphate}^{0.70} \times (\text{pH} - 4.5)^{6.8}}{\text{citrate}^{0.20} \times \text{volume}^{1.31}}$$

These indices approximately correspond to the ion-activity products in the following way:

$AP_{CaOx} \approx$ *AP(CaOx)-index* $\times 10^{-8}$

$AP_{CaP} \approx$ *AP(CaP)-index* $\times 10^{-13}$

Statistics: Statistical analysis was performed by means of Wilcoxons rank sum test for paired samples, and Mann-Whitney U-test for unpaired samples. The correlation coefficient (r_s) was calculated by Spearman's rank correlation method.

Results

Although the urine flow during each collection period was high, with a median flow rate of 80 ml per hour (range 20–250 ml) calcium crystals were observed in almost all samples. It was quite obvious that urinary pH was the most important determinant of calcium phosphate precipitation, and consequently such crystals were never present in samples with a pH below 6.25. At a pH above 6.50 crystals of calcium phosphate were predominant.

In some samples with pH above 6.50 calcium phosphate crystallization proceeded during the incubation period. However, attempts to acidify urine in order to measure only crystals of calcium oxalate did not result in acceptable reproducibility.

The effect of pH on crystal volume is demonstrated in Fig. 1. It is evident that the crystal volume increased with increasing pH above 6.50 ($r_s = 0.49$; $p < 0.01$). Similarly there was a slight increase in crystal volume when pH decreased in the range 6.25 to 5.25 ($r_s = -0.59$; $p < 0.01$). Apparently the lowest crystal volume was recorded at a pH somewhere between 6.0 and 6.5.

A pH below 6.25 was recorded in about 30% of our samples. This mainly occurred in early morning or night urine. In these samples, where calcium oxalate crystals predominated, crystal number, crystal volume, and mean crystal size were not affected by treatment with bendroflumethiazide, nor were the size or volume distributions altered. In most samples, no correlations were recorded between crystal volume and *AP(CaOx)-index*, inhibition index, calcium/citrate quotient, or concentrations of calcium, oxalate, and citrate (Fig. 2). However, in urine collected during the night, *AP(CaOx)-index* decreased during treatment (Fig. 3), and so did the volume of calcium oxalate crystals.

A decreased *AP(CaP)-index* was recorded during treatment (Fig. 3), and there was a positive correlation ($r_s = 0.77$, $p < 0.001$) between *AP(CaP)-index* and crystal volume (Fig. 4), which is in agreement with the findings in Fig. 1. This is certainly a result of the decreased calcium concentration, which was also shown to correlate with the crystal volume ($r_s = 0.56$, $p < 0.001$). During treatment with bendroflumethiazide, the calcium phosphate crystal volume ($p < 0.05$) and mean crystal volume ($p < 0.01$) decreased. In addition the relative number of large crystals (diameter above 11 μm) decreased ($p < 0.05$).

The concentration of different urine variables before and during treatment with bendroflumethiazide is summarized

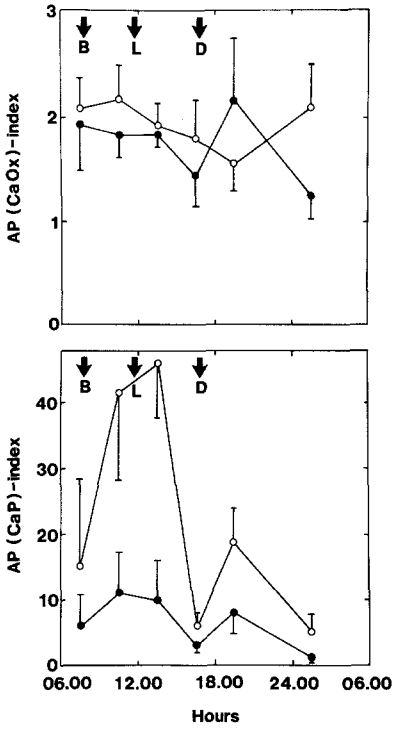

Fig. 3. Diurnal variation of *AP(CaOx)-index* and *AP(CaP)-index* (mean ± SEM), before (○) and during (●) treatment with bendroflumethiazide

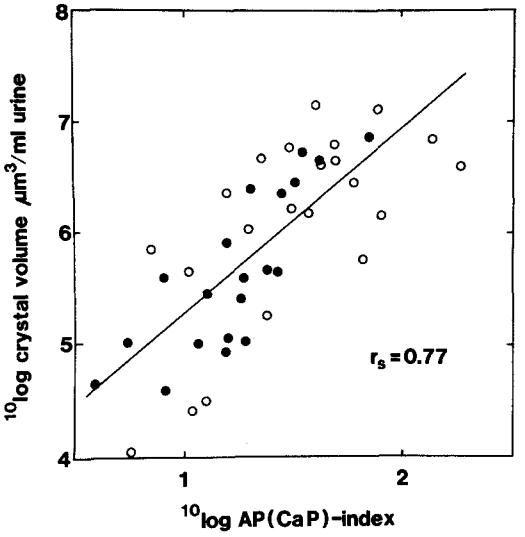

Fig. 4. Calcium phosphate crystal volume in relation to *AP(CaP)-index*, before (○) and during (●) treatment with bendroflumethiazide

in Fig. 5. Except for calcium ($p < 0.05$), urine composition was unaffected by the treatment. Similar diurnal excretion patterns were also observed.

Discussion

Calcium oxalate crystals were observed in most samples with a pH below 6.25. It needs to be emphasized that this occurred despite an *AP(CaOx)-index* below 2.8, which approximately corresponds to the formation product of calcium oxalate [15]. These results therefore appear to support the hypothesis that heterogeneous nucleation is the first step in calcium oxalate stone formation [4]. All urine samples had an *AP(CaOx)-index* above 0.2, and were thus metastably supersaturated with respect to calcium oxalate [12].

Another possible explanation is that these crystals were formed during a shorter period of supersaturation [2], or in some part of the kidney where supersaturation differs from what is found in whole urine. Such a mechanism is supported by the observation by Hautmann and co-workers [7] of different concentrations of calcium and oxalate in different parts of the kidney. Calcium oxalate crystals formed in this way should probably remain unaffected despite subsequent reduction of the level of saturation [19].

Like Brandes and co-workers [3], but in contrast to other observations [17, 19], we were unable to demonstrate a positive correlation between calcium oxalate supersaturation and calcium oxalate crystal volume. This is probably attributable to the narrow concentration range of calcium and oxalate in our urine samples. Different concentrations

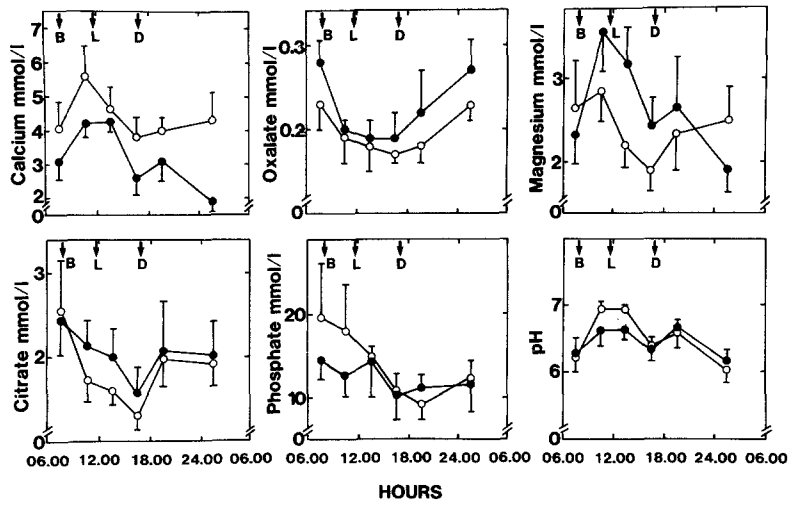

Fig. 5. Diurnal variation of concentrations of different urine variables (mean ± SEM), before (○) and during (●) treatment with bendroflumethiazide

of crystallization inhibitors might also influence the results, but information on the effect of inhibitors in whole urine is too scanty to enable valid conclusions in this respect.

Crystals of calcium phosphate were apparently more unstable than crystals of calcium oxalate, and it is likely that the rapid variations in particulate activity in fresh urine observed by Adamthwaite [1] merely reflects changes in calcium phosphate crystalluria. A low pH appears to increase the risk of calcium oxalate crystal formation [24], whereas a high pH is a most important determinant for calcium phosphate precipitation [14, 23]. In accordance with results from other investigations [5, 16], urine pH appeared to be a good discriminator between calcium oxalate and calcium phosphate crystalluria. A urine pH around 6.25 thus appeared to be optimal in reducing crystal content. In many samples with a pH above 6.50, where crystal growth continued in the period immediately after voiding, high values of the *AP(CaP)-index* were recorded. There also was a correlation between calcium concentration and calcium phosphate crystal volume.

Whereas calcium oxalate crystalluria remained unaffected by treatment with bendroflumethiazide, the volume of calcium phosphate crystals decreased. This is in accordance with the results presented by Hallson and Rose [5], but in contrast to a report by Brandes and coworkers [3]. However, the latter authors did not discriminate between crystals of calcium oxalate and calcium phosphate. Because the samples from our patients, according to the experimental situation, were more diluted than would be anticipated under normal physiological conditions, it is still possible that thiazides might affect calcium oxalate crystallization in more concentrated urine. However, Robertson and coworkers [19] showed that changes in calcium concentration had a very limited influence on calcium oxalate crystalluria.

The effect of bendroflumethiazide on calcium phosphate crystalluria is noteworthy. It is possible that bendroflumethiazide to some extent exerts its clinical effect either by reducing the amount of calcium phosphate available for heterogenous calcium oxalate crystallization [10, 11], or by decreasing the rate of calcium phosphate deposition on calcium oxalate.

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