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Respective influence of calcium and oxalate urine concentration on the formation of calcium oxalate monohydrate or dihydrate crystals

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ABSTRACT

Clinical interest for the identification of the crystalline phase of CaOx, either monohydrate (COM) or dihydrate (COD), in crystalluria analysis is debated. In the present study, we simultaneously determined calcium and oxalate concentration and identified the two crystalline phases of CaOx crystals in 1288 first morning urine samples from 407 CaOx stone formers with crystalluria.

The mean concentration of oxalate ions was higher in urine samples containing COM than in those containing COD crystals, and conversely the mean concentration of calcium ions was markedly higher in urine samples containing COD than in those containing COM crystals. COM crystals were predominant (93%) in urine samples with a Ca/Ox molar ratio <5 (i.e. with relative hyperoxaluria) whereas COD crystals were largely predominant (98%) in urine samples with a Ca/Ox ratio >14 (i.e. markedly hypercalciuric). In the intermediate Ca/Ox values, mixed COD and COM crystals were present. Logistic regression analysis showed that increasing the concentration of calcium resulted in a higher risk of COD than of COM crystal formation, whereas increasing oxalate concentration was associated with a greater COM than COD crystal formation. In conclusion, this study demonstrates the calcium-dependence of COD and the oxalate-dependence of COM crystal formation. Thus, a crystalluria mainly composed of COD suggests a search for conditions associated with hypercalciuria whereas an abundant crystalluria made of COM crystals should prompt a search for heavy hyperoxaluria and noteworthy primary hyperoxaluria.

1. Introduction

Calcium oxalate (CaOx) calculi are presently the predominant type of urinary calculi throughout the world. In France, they account for 75% of all calculi in men and 60% in women. CaOx stones may contain one or several crystalline forms among the three crystalline species of CaOx identified in the urine of humans, namely calcium oxalate

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monohydrate (COM) or whewellite, calcium oxalate dihydrate (COD) or weddellite and, more scarcely, calcium oxalate trihydrate (COT) or caoxite. Although the supersaturation level of CaOx is the well established major factor that determines the propensity to form urinary CaOx crystals [1], the respective influence of the urinary concentration of calcium (Ca) and oxalate (Ox) ions on the crystalline phase of CaOx, either monohydrate or dihydrate, is controversial [2–6].

In experimental models, the molar concentration of oxalate was shown to have a greater influence on CaOx crystallization than an equimolar calcium concentration [7]. In our experience [8], the formation product of calcium oxalate (FP_{CaOx}) was decreasing with the CaOx molar product (p_{CaOx}) while oxalate concentration was increasing and calcium concentration was decreasing, thus providing evidence that the molar concentration of oxalate has a greater effect than that of calcium on the CaOx crystallization. However, given the solubility product (Ksp) of CaOx in water at 37 °C, in the range of 2.2 to 3.6×10^{-9} mol/L [9,10], all urine samples appear dramatically supersaturated and therefore should contain numerous CaOx crystals, which is not the case. Indeed, in normal urine, the average p_{CaOx} value is around 6.10^{-7} $(mmol/l)^2$ and no CaOx crystal is found in most cases. Indeed, urine is a complex medium containing numerous electrolytes including ions able to interfere specifically with calcium or oxalate ions, thus reducing the actual CaOx molar product and the CaOx relative supersaturation. These ions are Mg^{2+}, $citrate^{3-}$, HPO_4^{2-}, $H_2PO_4^{-}$, SO_4^{2-}, and $P_2O_7^{2-}$, in various combinations. In addition, according to the Debye–Huckel equation, the ionic strength of the medium, mainly related to the content of Na^+, K^+ and Cl^- also contributes significantly to reduce CaOx supersaturation and thus to increase FP_{CaOx}.

In healthy subjects, the average molar concentration of urine calcium (range of 3 mmol/l) is about ten times greater than that of oxalate (range of 0.3 mmol/l) so that a proportional increase of molar concentration for either oxalate or calcium has a similar effect on calcium oxalate supersaturation [11]. Moreover, in humans, a marked increase in urinary calcium excretion without a concomitant increase in oxalate excretion is frequent in stone formers with idiopathic hypercalciuria, thus considerably increasing the molar Ca/Ox ratio, whereas reciprocally a marked increase in urinary oxalate concentration without a concomitant increase in calcium concentration is only observed in some pathological conditions, such as primary hyperoxaluria.

Thus, different values of the Ca/Ox ratio may be observed within the same CaOx molar product (p_{CaOx}), resulting in the formation of distinct crystalline phases of CaOx crystals [8]. Indeed, correlations between stone composition and urinary parameters have shown that patients with hyperoxaluria preferentially form calculi made of COM whereas those with hypercalciuria form calculi mainly made of COD [12–14] and such was our experience [8,15–17]. However, COM is the thermodynamically stable form of CaOx, and so all other crystalline forms may convert into COM with time. This phenomenon was reported to be fast for calcium oxalate trihydrate (COT), which is able to convert into COM within a few minutes in vitro at 37 °C [18]. At variance with such experimental models, we occasionally found calculi containing COT that had stayed several months or years in the urinary tract. Regarding the crystalline conversion from COD to COM in vivo, a number of reports underline the frequent occurrence of crystalline structures at the surface or within stones suggestive of initial COD formation while polarized light microscopy, infrared spectroscopy or X-ray analysis provide evidence of a high COM content [9–22]. However, the kinetics of such a crystalline conversion is poorly understood [23]. We have incubated a pure COD stone within human urine at 37 °C for several months and failed to observe any transformation of COD to COM (unpublished data). Therefore, what are the biochemical conditions that favor in vivo the primary formation of the various crystalline species of CaOx in urine? It is a very important point from a clinical point of view if we consider that around 60% of stones are predominantly made of COM while 20–25% contain COD as the main component. Because COT crystals are infrequent in urine and calculi, we restricted our investigation to the more common crystalline forms of CaOx, namely COM and COD.

Therefore, we examined the relationship between urinary concentrations of Ca and Ox and the type of CaOx crystals, either COM or COD, in the same urine samples and determined the effects of gradual increments in Ca and Ox molar concentration on the respective risk of formation of COM or COD crystals.

2. Materials and methods

We examined 1288 first morning urine samples from 407 idiopathic calcium oxalate stone formers who had CaOx crystalluria made up of either COM or COD. In every sample, we determined the concentration of calcium and oxalate. Morphologic identification of urinary crystals was made by polarization microscopy, as described elsewhere [24].

In the first part of the study, we determined in stone formers the mean values of urinary Ca and Ox concentrations in urine samples containing only either COM or COD crystals, and reciprocally, we examined the type of crystals with respect to the ratio of Ca to Ox concentration.

In the second part of the study, we examined by logistic regression analysis the effects of increasing concentrations of Ca or Ox above the average values observed in first morning urine samples from 227 healthy subjects. Among 267 healthy controls in our database, 40 (15%) exhibited calcium oxalate crystals in their urine. COD crystals accounted for 92.5% of cases and COM crystals were found in only 2.6% of urine samples (7.5% of samples containing calcium oxalate crystals). Mean calcium and oxalate values found in these samples were very similar to the respective values observed in urine samples of stone formers with calcium oxalate crystals (data no shown). So, we retained 227 urine samples from healthy controls without calcium oxalate crystals. The mean value for calcium and oxalate concentration was 3.31 ± 2.14 mmol/l and 0.35 ± 0.17 mmol/l, respectively, taken as reference values (relative risk 1) in the absence of COM or COD crystalluria.

In the last part of the study, we examined the presence of calcium oxalate crystalluria in relation with p_{CaOx} and the