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The Effect of Crystalline Monosodium Urate on the Crystallisation of Calcium Oxalate in Whole Human Urine

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Summary. (1) Samples of undiluted urine from normal men were preincubated with crystalline monosodium urate and their metastable limits and responses to a standard oxalate challenge were compared with results obtained from control samples preincubated without urate. (2) Preincubation with urate had no significant effect on the metastable limits of the urines, the morphology, size, or growth rates of calcium oxalate crystals precipitated from the urines, or on the total amount of calcium oxalate deposited in a given time. (3) It was concluded that particulate monosodium urate is unlikely to influence calcium oxalate stone formation by binding to and attenuating the potency of urinary inhibitors.

Key words: Calcium oxalate urolithiasis, Calcium oxalate crystallisation, Monosodium urate, Glycosaminoglycans, Whole urine.

Introduction

An apparent beneficial effect of the drug allopurinol in reducing calcium oxalate stone recurrences in individuals exhibiting derangements of uric acid metabolism [1, 2] has formed the basis for a suggested causal role for uric acid in calcium oxalate urolithiasis. The recognition of an association between uric acid and this type of renal stone has become so firmly established that "hyperuricosuric calcium oxalate nephrolithiasis" [3] has come to be regarded as a distinct clinical entity. However, a plausible rationale for the existence of such a sub-group of calcium oxalate stone disease is still without sound scientific foundation.

One theory which has been advanced to explain the purported relationship between uric acid and calcium oxalate stones proposes that colloidal urate in urine binds to the urinary glycosaminoglycans, thereby destroying or reducing their potency as inhibitors of calcium oxalate crystallisation [4]. Although a reduction in the inhibitory activity of the glycosaminoglycan heparin [5, 6], diluted macromolecular urine fractions [7, 8] and dilute, unfractionated urine [6] have been reported following preincubation with crystalline monosodium urate or uric acid, no previous study has addressed the question in whole human urine.

In this study we investigate the effect of pretreating undiluted human urine with crystalline monosodium urate on the capacity of the urine to resist spontaneous nucleation of calcium oxalate and on its response to a standard oxalate challenge above its measured metastable limit.

Materials and Methods

The subjects consisted of 10 healthy men with no history of kidney stone disease. Twenty-four h urine specimens were collected and processed as previously described [9].

Crystalline monosodium urate (Sigma) was added to the urine samples in the ratio of 0.5 mg per ml of urine [5]. The resulting suspensions were then preincubated for 2 h at 37 °C in a shaking water bath. Controls consisted of urine samples incubated in the same way, but with no added urate. At the end of the preincubation period the urine samples were filtered. The practical metastable limits of the urine samples and their responses to a fixed oxalate load were then determined as previously described [9]. The practical metastable limit was defined as the minimum quantity of oxalate necessary to induce detectable crystal nucleation in 20 ml of urine, and the response of the urines to the oxalate load, as the slope of the linear portion of the curve relating increasing crystal volume to incubation time (growth rate), the total volume of crystals precipitated during the incubation period and the position of the crystal size distribution peak.

Urinary urate concentration was measured using a standard Technicon SMA autoanalyser system. Data were compared using the Wilcoxon signed rank sum test.

Results

Most urines precipitated envelope calcium oxalate dihydrate crystals, and the morphology was not altered by the urate treatment.

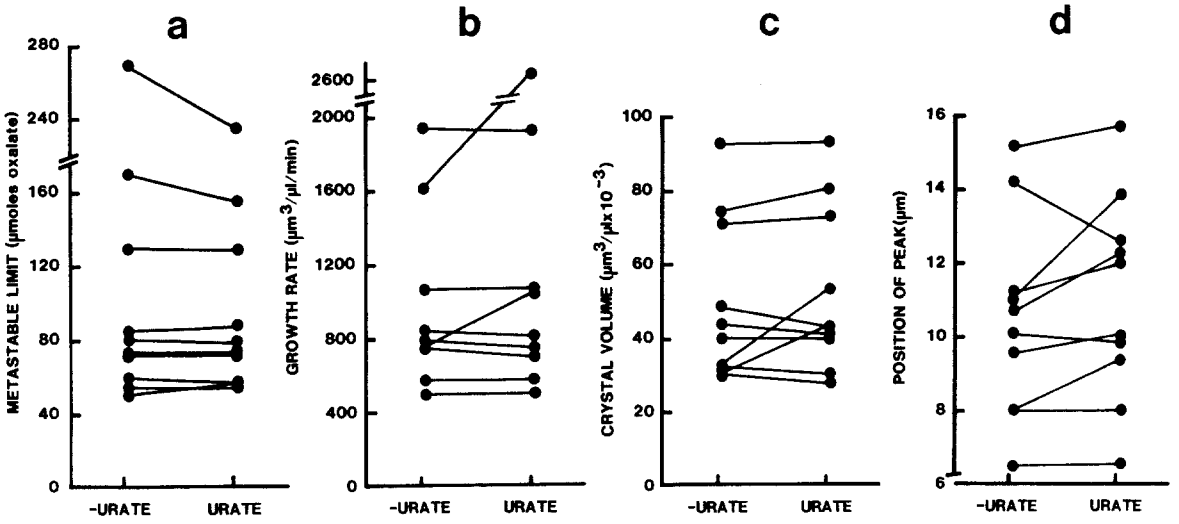

Fig. 1a–d. a The practical metastable limits, expressed as the minimum amounts of oxalate necessary to induce detectable nucleation, in the control urine (-urate) and after preincubation with urate (urate). b The growth rates of the crystals precipitated from the urine samples in response to the oxalate load, after preincubation in the absence (-urate) and presence (urate) of sodium urate. c The volume of calcium oxalate crystals deposited during the 90 min incubation period following addition of the oxalate load, in the control (-urate) and after pretreatment with urate. d The size of the precipitated crystals, expressed as the position of the mode of the volume versus diameter size distribution curve after preincubation with and without urate

The initial pH values of all the urines were in the range 5.6–6.9. Monosodium urate is virtually insoluble in this range [10]. In accord with this, the concentration of dissolved urate in the first three urines examined was not increased by preincubation with sodium urate, and so further urate determinations were not carried out. As would be expected, the pH values of the urines were also unaffected by preincubation with urate.

Fig. 1a shows the metastable limits of the urine specimens after preincubation in the absence and in the presence of monosodium urate. A reduction in the metastable limit of two specimens occurred following pretreatment with urate, but overall, there was no discernible difference between the pairs of values.

The growth rates of the precipitated crystals are shown in Fig. 1b. Two urine specimens exhibited a marked increase in crystal growth rate following preincubation with sodium urate, but when considered in toto, there was no significant difference between the groups of values. The two specimens showing an increased growth rate were different from the two which had reduced metastable limits after the preincubation step. As would be expected from this finding, preincubation with urate had no reproducible effect on the total amount of calcium oxalate precipitated during the 90 min incubation period (Fig. 1c). Neither (Fig. 1d) did it exert a consistent influence on the size of the crystals, expressed as the position of the mode of the crystal volume size distribution curve.

Discussion

In this study the proposal [4] that particulate urate reduces the inhibitory potency of urine by interacting with glycosaminoglycans was tested by preincubating samples of normal human urine with crystalline monosodium urate. If significant binding had occurred, then the glycosaminoglycans would have been removed from the urine when it was filtered after the preincubation period, thus reducing the inhibitory activity of the urine. This might therefore have been expected to lower the practical metastable limits of the urine specimens, to increase the growth rate of the crystals precipitated in response to the oxalate load (and therefore the total amount of calcium oxalate desposited during the 90 min incubation period), or to alter the degree of aggregation of the crystals. In the absence of a difference between the total mass of calcium oxalate deposited, a discrepancy in crystal size would indicate a corresponding difference in the state of crystal aggregation. However, pretreatment with monosodium urate had no consistent effect on any of these indicators of inhibitory activity. These results do not discount the possibility that the urate may have bound to urinary glycosaminoglycans, but they do suggest that even if such binding were to occur in vivo, it would be unlikely to predispose a person towards calcium oxalate stone formation.

Although it has previously been shown that similar preincubation with monosodium urate or uric acid can lower the inhibitory activity of macromolecular urine fractions [7, 8] or unfractionated urine [6], it must be borne in mind that the urine specimens or fractions used in these studies had been diluted. The problems of using results obtained with dilute urine to predict likely effects of inhibitors in whole urine have been documented [11] and it is possible that the effects reported by Fellstrom et al. [7] and Ryall et al. [6] have no relevance in vivo.

Crystalline sodium urate is rarely found in urine or stones [13] and the results presented here suggest that even

were it present in urine, it would be unlikely to contribute to calcium oxalate stone disease by interfering with the inhibitory action of urinary glycosaminoglycans. Furthermore, since the studies were performed in whole urine, this conclusion can be extended to include any macromolecular urinary inhibitors. If indeed allopurinol does reduce calcium oxalate stone recurrences in individuals with hyperuricosuria or hyperuricaemia, then its advantageous effect cannot be ascribed to its indirect effect of preserving the integrity of macromolecular inhibitors. However, its effect might be due to a reduction in the concentration of dissolved urate. It has been reported that increasing the concentration of urate in urine causes precipitation of calcium oxalate [12], presumably by mechanisms analogous to the classic "salting out" of non-electrolytes by electrolytes. This possibility was recently questioned by Hallson et al. [13] who presented data which indicate that high levels of dissolved urate actually appear to decrease calcium oxalate crystal formation in concentrated human urine. Perhaps future studies should therefore be directed at elucidating the effect of dissolved urate on calcium oxalate crystallisation in whole urine.

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