Urol Res (1982) 10:169–172

Urological Research

Retardation of Calcium Oxalate Precipitation by Glutamic-oxalacetic-transaminase Activity

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Accepted: April 16, 1982

Summary. It has been found that calcium oxalate stone formers have low UGOT activity compared to healthy individuals (controls). Urine from stone formers with no GOT activity and no effect on calcium oxalate precipitation was incubated with GOT for various periods. Subsequently calcium oxalate precipitation was decreased and found to be considerably retarded i.e., the pathological urine after the incubation acted in a way similar to that of normal urine. The yield of Glutamic-Oxalacetic Transaminase (GOT) activity is gultamic acid. It was shown that glutamic acid has a significant retardation effect on the precipitation of calcium oxalate stone formation. Therefore it may be suggested that GOT activity involved in glutamic acid creation in situ, has a role in kidney stone formation.

Key words: Calcium oxalate, Kidney Stones, UGOT, Retardation, L-glutamic acid.

Introduction

The formation of stones in the urinary tract is one of the oldest diseases suffered by human beings. The majority of idiopathic renal calculi contain calcium oxalate hydrates [1], since urine is usually super-saturated with respect to this salt [2]. The crystallisation theory of stone formation is based upon a model in which the concentration of precipitating substances is the controlling factor. A mucoid substance containing proteins and carbohydrates is found in most stones [3] and may represent organic material trapped by co-precipitation in the developing stone [4]. The organic component, in terms of this model, might modify the crystallisation process and play an important role in stone formation.

Although the urine is supersaturated with respect to calcium oxalate, its precipitation is inhibited/retarded in the normal urine [5]. There is considerable evidence, from both in vitro und in vivo studies, suggesting that inhibitors of crystallisation of calcium oxalate are involved in the process of normal and pathological calcification of tissue. These inhibitors seem to be partly absent in the stone formers' urine.

Previous results obtained in this laboratory confirm this conclusion and indicate a significant difference between the sum of (UGOT) Urinary Glutamic Oxalacetic Transaminase and (UGPT) Urinary Glutamic Pyruvic Transaminase activity in normal and in stone formers' urine [6]. Moreover, one can see that L-glutamic acid, which is the end product of the UGOT and UGPT activity has an influence on calcium oxalate precipitation and may thus play a role in calcium oxalate stone formation [6].

Sine the presence of several enzymes in the urine has been reported [7] the object of this study was to demonstrate the "in situ creation" by UGOT activity of potent compounds in pathological urine which evidently increase the inhibitory properties of the urine.

Materials and Method

Glutamic-Oxalacetic-Transaminase (GOT) was purchased from Sigma Chemical Company. Fresh mimic urine was prepared, according to the procedure proposed by Gardner et al. [8], except for the calcium and oxalate concentrations.

First urine samples were obtained from patients in the Urology Clinic of the Hebrew University-Hadassah Hospital in Jerusalem, who had undergone surgery for the removal of kidney stones. The extracted stones were analysed, and were found to be calcium oxalate stones.

UGOT activity was measured using Technicon SMAC Analysis. 80 units of GOT were added to 100 cc fo stone formers' urine and stirred with a magnetic stirrer at room temperature.

At various time intervals samples were taken from the incubation vessels and the D.I., which reflects the inhibition power of the urine, was determined according to the method of Sarig et al. [5]. The experimental details concerning D. I. determinations have been described elsewhere [5]. After the termination of the test the crystals were filtered through 0.2 μm millipore filters, coated with gold and viewed under the SEM (Jeol 35). The same procedure was followed using mimic urine.

0300-5623/82/0010/0169/$01.00

Table 1. The variation of D.I. values as a function of incubation time of a pathological urine with GOT

Incubation time [min]	D.I. values
0	1.8[a]
10	0.8
20	0.51
30	1.02
40	0.52
50	0.62
80	1.00
120	0.80
160	0.62
180	0.48

[a] Sedimentation (all other samples were turbid without onset of sedimentation)

Results

UGOT Activity in Stone Formers' Urine

In order to ascertain that UGOT activity is low in stone formers' urine compared to healthy people, a first morning urine sample was tested in the Technicon SMAC System. Only a non-specific activity was detected in the stone formers' urine, which did not increase with increasing substrate or urine concentration.

Since in previous studies it has been reported that amino acids inhibit/retard calcium oxalate crystallisation in vitro, it was of interest of determine whether endogenous enzyme known to be present in human urine [7] is actually connected with calcium oxalate crystallisation. To clarify the question, the urine was incubated with GOT in order to create "in situ" a potential inhibitor to the precipitation process.

This treatment increased considerably the inhibition/retardation effect. One can infer this from the change of D. I. values. Table 1 demonstrates that after 10 min of incubation there is a 50% reduction in the D.I. value. This overall effect diminishes with time, however, and decreasing, fluctuation of the D.I. values are observed. These fluctuations are obviously parallel to the fluctuations of precipitation rate decrease caused by addition of linearly increasing amounts of L-glutamic acid to mimic urine [6]. Actually such fluctuations were observed in earlier studies without being commented upon [9].

Incubation of the stone formers' urine with the GOT resulted in the production of glutamic acid. One can deduce that GOT activity yields an inhibitory substance, most probably glutamic acid. In control incubations of GOT with mimic urine no change in the D.I. value was noted, even after long incubation periods. In our previous studies, it has been observed that precipitation of calcium oxalate forms a visible sediment in the reaction vessel, in the presence of normal and pathological urines as well as in mimic urine. This is regardless of the addition of any inhibitory agent, which when present, subsequently slows down the rate of calcium ion concentration decrease. In the presence of L-glutamic acid the solution turns turbid but no visibile sedimentation can be detected [6]. The same effect of turbidity formation without sedimentation was evident in the stone formers' urine incubated with GOT. The turbid solutions were filtered and viewed under SEM. The photomicrographs (Fig. 1) show calcium oxalate crystals formed in the presence of GOT (80 μ/100 cc) at various times of incubation, as compared to crystals fromed in the same conditions in stone former urine without addition of GOT and incubation treatment. These will serve as controls (Fig. 1a, 1b).

The crystals formed after some time seem to grow thinner (compare 1b, the control, with 1c, after 40 min of incubation). The crystals formed after prolonged times of incubation with GOT appear to be less well developed with frayed edges (compare 1d, after 80 min with 1a and 1b). After 3 h they become small and markedly irregular (compare le with controls).

Previous studies with mimic urine showed a similar trend when the variable was L-glutamic acid concentration [6].

Discussion

In vitro studies demonstrated retardation of calcium oxalate crystallisation in mimic urine in the presence of several substances including amino acids [10]. In vivo studies with rats confirm this result [11, 12]. These studies have shown that the organic molecules in the stone formers' urine are inactive and do not affect the crystallisation process. The urine of healthy individuals possesses active modifiers which constitute the inhibitory potential of the urine.

The modifiers retard the rate of calcium oxalate formation. Moreover, when crystals are finally formed they are of poor crystallographic quality; thin, not well developed, irregular and small with particularly high surface to mass ratio, and thus can be easily washed out from the body. An analogous mechanism of scale control in sea water desalination plants was described [13].

In a previous study we showed that aspartic acid is an indifferent molecule whereas glutamic acid is a molecule capable of modifying activity. On adding GOT to stone formers' urine the precipitation progress was prevented. In our previous report it was suggested that the lack of UGOT and UGPT activity in stone formers' urine allows unhindered precipitation of calcium oxalate crystals in the stone formers' urine. The present report confirms this conclusion. It demonstrates that the crystallisation process of calcium oxalate in the urine can be regulated and controlled by the presence of appropriate concentrations of endogenous substances produced by urinary enzyme. The lack of enzyme activity may be one of the causes of the low inhibition/retardation effect at the stone formers' urine and consequent calcium oxalate stone formation.

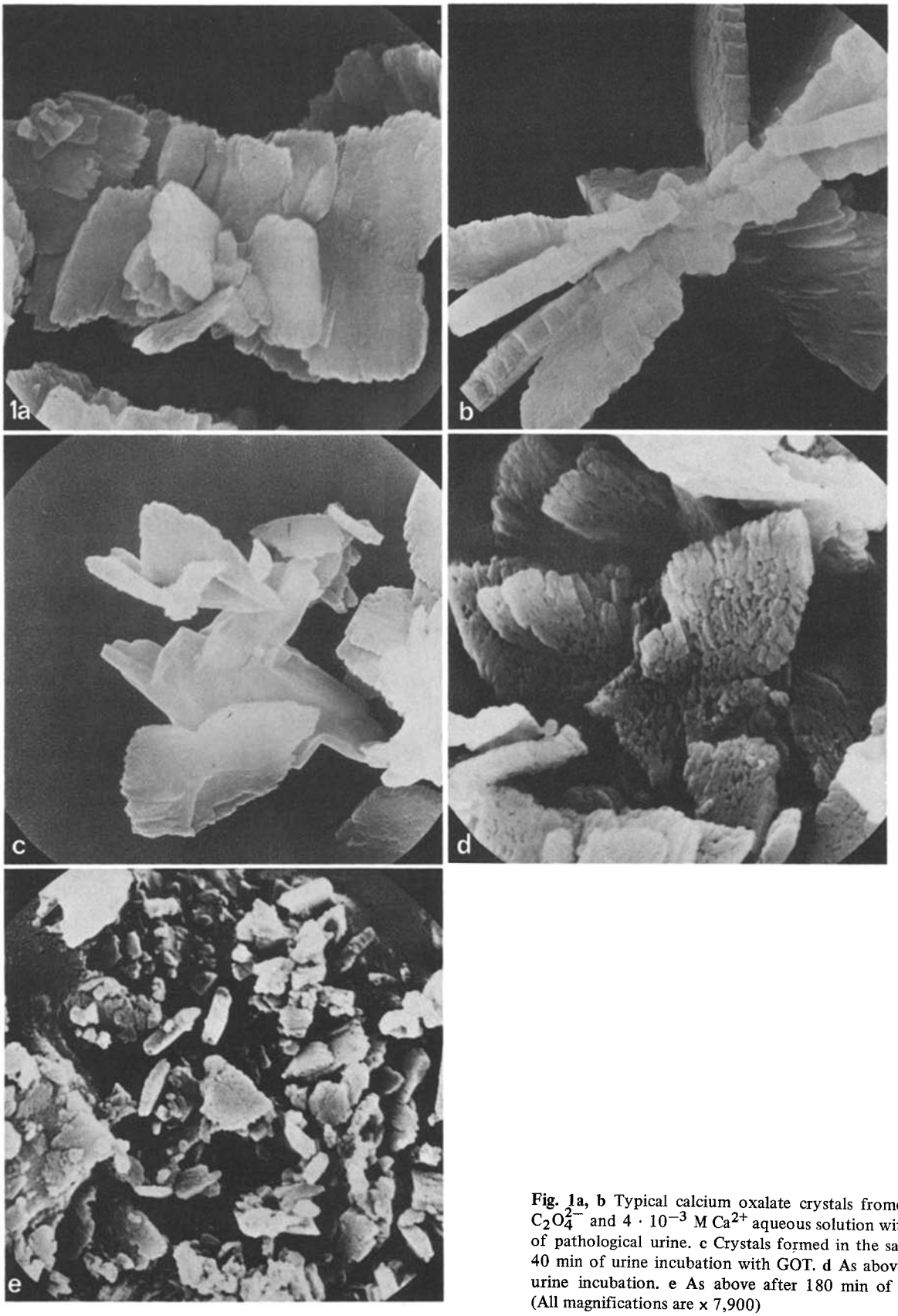

Fig. 1a, b Typical calcium oxalate crystals fromed in $6 \cdot 10^{-3}$ M $C_2O_4^{2-}$ and $4 \cdot 10^{-3}$ M Ca^{2+} aqueous solution with 10% admixture of pathological urine. **c** Crystals formed in the same solution after 40 min of urine incubation with GOT. **d** As above, after 80 min of urine incubation. **e** As above after 180 min of urine incubation. (All magnifications are x 7,900)

Acknowledgments. Thanks are due to: Miss Karpuj Idit, for the asssistance in performing the experiments; Mrs. Kupfer Gila, for the assistance in the enzymes assays; Mrs. Mila Folhan, for the SEM microphotograph.

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