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Determination of GOT Activity on Nucleation and Crystal Growth of Calcium Oxalate

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Summary. Crystal Size Distribution (CSD) and the yield of Calcium Oxalate Crystals in solutions with an admixture of 5 normal and 3 stone forming urines, were determined. A positive correlation was found between the median size, the number of particles and the overall inhibitory potentials of the urines toward calcium oxalate precipitation in vitro as reflected by Discriminating Index (DI) measurements. Incubation of two samples of stone formers' (SF) urines with glutamic-oxalacetic-transaminase (GOT) caused a reduction of aspartic acid concentration, an increase in glutamic acid concentration and a parallel decrease in the DI values. After 90 min of SF urine incubation with GOT the DI in three samples was improved and both the median size and number of particles reduced, by 28% and 45% respectively. These results could indicate that GOT activity changes the inhibitory power of the SF urine by transforming aspartic acid into glutamic acid, having thus most probably a part in the inhibition of CaOx stone fromation.

Key words: Calcium oxalate crystal growth, Glutamic – oxalacetic – transaminase.

Introduction

The processes of nucleation, crystal growth and aggregation of calcium salt crystals subsequently lead to stone formation. These factors depend not only on the excessive amounts of calcium and oxalate present in urine but also on a deficiency of natural inhibitors [1–4]. Agents such as pyrophosphate, citrate, magnesium and others seem to have an inhibitory effect when added to in vitro systems [2–5]. However, there is no significant difference between their concentration in the urine of stone formers (SF) and healthy persons.

In previous studies it has been shown that glutamic acid might have a unique role in the crystallization process of CaOx in urine-like solutions [7]. Glutamic acid is present in the urine of normals and stone formers. However, the groups differ in the activity of trasaminase enzymes (GOT and GPT) which form glutamic acid. This activity was found to be about 12 IU in the urines of stone formers, while it was about 37 IU in the urines of healthy controls [7]. Also it has been demonstrated that incubation of SF urine with GOT increases the inhibitory potential of the urine toward calcium oxalate precipitation in vitro by 60% or more as expressed by the DI values [8].

In the present research, the effect of GOT activity in SF urines on amino acid composition and on the nucleation and crystal growth of CaOx was evaluated.

Experimental Method

a. Healthy and SF Urines Characteristics

First urine samples were obtained from three stone forming patients in the Urological Clinic of the Arizona Health Science Center in Tucson, Arizona. The urines of five healthy donors were examined as controls.

The urines were centrifuged and filtered on 0.8 μm millipore to remove suspended matter, and subsequently subjected to the DI test of Sarig et al. This test, which is described elsewhere [9], reflects the overall inhibitory potential of the tested urine with respect to CaOx crystallization in vitro under standardized conditions. In each case the suspension of crystals formed during the test was well mixed and an aliquot of 10 ml was taken for crystal size distribution (CSD) determination.

This sample was diluted with 90 ml conductivity solution (1% LiCl in Methanol). The number and median size of the crystals in the precipitate were determined by a PDI Analyzer (Particle Data Inc.) using a 150 μ orifice tube. The whole procedure was carried out three times for each SF urine. Data from the Counter were analyzed by a PDP8 minicomputer program [10].

b. Incubation of SF Urines with GOT

Samples of 200 ml of stone formers' urines were incubated with glutamic-oxalacetic-transaminase (GOT) (purchased from Sigma Chemical Co.), according to the conditions which are described

Table 1. The relationship between Discriminating Index (DI) and Crystal Size Distribution (CSD) in calcium oxalate precipitation in the presence of urine

Contributor	Definition of State	DI	Median Size Lm (μm)	Number/ml
1	Healthy	0.25	5.84 ± 1.20	49,991
2	Healthy	0.19	6.25 ± 1.60	44,580
3	Healthy	0.29	8.31 ± 1.95	52,660
4	Healthy	0.86	6.94 ± 1.41	77,247
5	Healthy	0.99	9.26 ± 2.46	57,526
I	SF	1.65	8.81 ± 2.57	74,539
II	SF	1.85	8.77 ± 1.90	81,847
III	SF	2.20	9.01 ± 2.10	89,050

Table 2. The effect of incubation of SF urines with GOT on CSD and DI values

Duration of incubation	Patient No.	DI	Lm (μm)	#/ml
0	I	1.65	8.805 ± 2.57	74,539
	II	1.85	8.72 ± 1.90	81,847
	III	2.20	9.01 ± 2.1	89,050
10	I	0.96	7.87 ± 1.77	54,280
	II	1.12	8.75 ± 2.2	67,180
	III	1.89	8.80 ± 2.02	58,566
30–35	I	1.2	7.64 ± 1.59	65,403
	II	0.95	8.25 ± 2.02	60,253
	III	1.25	7.95 ± 2.20	58,497
60–65	I	0.80	8.06 ± 1.81	64,291
	II	0.70	5.95 ± 1.12	58,976
	III	0.72	7.38 ± 1.82	59,564
90–105	I	0.44	7.42 ± 1.59	58,515
	II	0.44	5.85 ± 1.44	36,618
	III	0.55	6.1 ± 1.95	42,440
170–180	I	0.39	6.98 ± 1.74	47,850
	II	0.45	6.44 ± 1.26	42,631
	III	0.51	6.6 ± 2.2	44,590

elsewhere [8]. Urinary-GOT activity was measured in the University Hospital official lab by Gilford System 5. The concentrations of aspartic and glutamic acids were determined by Amino Acid Analyzer, model LKB 4400, equipped with LKB gold column.

Results and Discussion

Determination of the state of the contributors' urines as stated by them with respect to DI tests is satisfactory. Table 1 shows that for the SF donors the values of DI are above 1.65 while for the controls the values are below 1.00. It was also shown that among the normals there is a subdivision: contributors 1–3 had DI values below 0.3 while contributors 4 and 5 had values of 0.86 and 0.99. Actually the latter values are in the region referred to as the "in between" population [9]. This region is the overlap between the extremes of SF and control distributions in the DI scale [9]. The crystal size distribution (CSD) analysis of the crystals formed during the DI determination test provides data characterizing the crystals, i.e. the median size and the number of particles. Thus additional important information is added to characterize the state of the urine.

The number of particles as determined for the 1–3 controls ranged between 44,580 and 52,660 per ml, while the lowest number of particles for the SF's was 74,539 per ml and the highest was 89,050 per ml. This was a clear-cut difference which was in agreement with the DI determinations and agreed with the high SF nucleation rates observed by Drach et al. [11]. The median size data, though furnishing less sharp discrimination because of the large standard deviations, also showed that on the whole the particles formed in solution with addition of SF urine were relatively large.

Low DI values resulted from retardation of CaOx crystal formation in the test solution, i.e. sparse precipitate: they correlated well with low numbers and small sizes of crystals. The almost undisturbed fast precipitation in the presence of SF urine resulted in larger numbers and larger particles. These measurements showed good agreement between the two experimental methods.

The "in-between" values should coincide with a medium quantity of precipitate. This should show up either in an increase of numbers as compared to the 1–3 controls while the size remains small, or in an increase of size while the number remains small. Accidentally, and even surprisingly, both possibilities were realized in the analyses of contributors 4 and 5 respectively.

The significance of the correlation between DI and the crystal size and numbers was assessed by Kendall Rank Correlation Coefficient [12]. The values of DI were positively correlated with the size ($\tau = 0.64$, $P < 0.002$) and with the number of crystals per ml ($\tau = 0{,}82$, $P < 0.001$).

The additional information sought after and gained in the present study concerned the characteristics of the calcium oxalate precipitate in correlation with the decrease of DI in SF urines due to incubation with GOT. The values of DI and CSD in the pathological urines incubated during periods from 10 to 180 min are listed in Table 2. The relative decrease in both parameters as a function of time is shown in Fig. 1.

It can be seen that after 180 min of incubation with GOT the DI values were transferred from the range of SF to the range of normals. The values of the median size, number per ml and DI were similar to those of the healthy controls. In fact they were lower (i.e. better) than those of the "in between" population. The decrease in the median size and number of crystals as well as in the value of DI was gradual. It is interesting to note that the values after 60–65 min of incubation corresponded well with the values of the "in between" population (Table 1 and 2).

Thus, Robertson's results which showed that SF urine contains more and larger crystals than normal urine [13] are in excellent agreement with the present study.

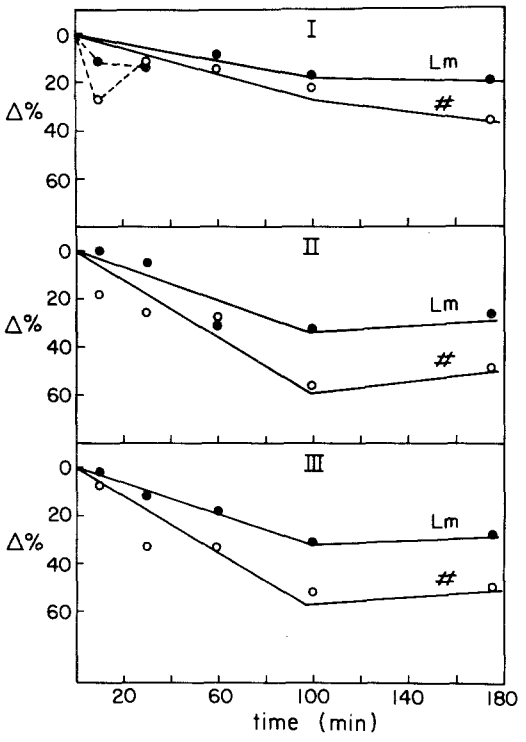

Fig. 1. The decrease of median size and numbers of CaOx crystals as a function of incubation periods with GOT

Table 3. Aspartic and glutamic acids concentrations (nM ± 3%) in two SF urines during incubation with GOT

Incubation time (min)	Patient I DI	Asp. acid (nM)	Glu. acid (nM)	Patient II DI	Asp. acid (nM)	Glu. acid (nM)
0	1.20	0.546	0	1.44	0.631	0.012
10	0.38	0.549	0.046	0.43	0.604	0.055
25	0.19	0.451	0.057	0.35	0.489	0.071
40	0.24	0.463	0.062	0.20	0.492	0.100
55	0.53	–	0.147	0.41	0.366	0.192
85	0.27	0.362	0.170	0.30	0.358	0.298
125	0.47	0.333	0.271	0.35	0.304	0.285

The DI determination utilizes the spontaneous precipitation of calcium oxalate, which is very susceptible to the presence of active impurities. It has been shown by us that the precipitation process is strongly affected by glutamic acid [8, 14]. It could have been inferred that GOT transforms glutamic acid from aspartic acid in situ, thus inducing the improvement of DI in SF urines following incubation with the enzyme [8]. Direct evidence has been supplied by amino acid analyses of two SF urines incubated for 125 min. The concentrations of both aspartic and glutamic acids and the corresponding DI values determined for samples withdrawn during the incubation, at the indicated times are presented in Table 3; the decrease in aspartic acid and the parallel increase in glutamic acid concentrations is obvious. The DI values of the urines, characteristic of SF (1.20 and 1.44) were transformed into the range of values found in healthy urines [9]. The DI values were shown to be well correlated with the number and size of calcium oxalate crystals formed during standardized enforced precipitation (Table 1) [14]. The DI values are drastically changed during incubations of SF urines with GOT (Tables 2 and 3), [8]. It is well known that GOT transforms aspartic into glutamic acid, therefore it may be concluded that the glutamic acid created in situ reduces the DI value by retarding calcium oxalate formation. As reduction of DI values has been correlated with increased efficacy to prevent recurrence of calcium oxalate kidney stone formation [15] it may be inferred that GOT may beneficially affect the inhibition of calcium oxalate stone formation via formation of glutamic acid. This hypothesis is strengthened by the findings that the mean UGOT and UGPT (which produces glutamic acid from aspartic acid and from alanine) is 37.4 ± 14.8 in normals and 12.1 ± 4.85 units/liter in stone formers [7].

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