

Arginine as a Significant Regulator of Supersaturation in Calcium Oxalate Lithiasis: the Physiological Evidence

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Abstract

Background: At present, the possible effect of arginine as a natural regulator of calcium oxalate (CaOX) supersaturation and crystallization in human urine has been analyzed.

Methods: Two types of experiments have been discussed: clinical laboratory analysis on the urine excretion of arginine (Arg) in patients with CaOX lithiasis and detailed measurements of the kinetics of the dissolution of CaOX calculi in artificial urine, containing various concentrations of Arg.

Results: A detailed analysis showed that 80% of stone formers (SFs) eliminated pathological values: 30% of patients had lower plasma levels compared to controls and about 50% of SFs showed higher concentration. Urine concentrations in these two groups were not reported.

Conclusions: The *in vitro* analytical measurements demonstrate even a possibility to dissolve CaOX stones in human urine, in which increased concentration of Arg has been established. Discussions have arisen to use increased concentration of Arg in urine both as a solubilizer of CaOX stones in humans and on the purpose of a prolonged metaphylactic treatment.

Keywords: Calcium oxalate calculi; Arginine; Dissolution of crystalline; Urine supersaturation

Introduction

The interest towards the formations of renal calculi can be explained by the wide spread of nephrolithiasis [1, 2] and that kind of stones is caused by some gastrointestinal diseases [3, 4]. Despite a number of promising hypotheses, the pathogenetic

mechanism of the intrarenal calcium oxalate (CaOX) stone formation remains obscure; thus the clarification of the main physico-chemical moments of the processes of phase formation in human urine is of significant clinical importance.

In investigating the kinetics of crystal nucleation and growth, and the kinetics of the dissolution of already existing stones, of utmost significance are the issues related to the calculation of the level of supersaturation in biological solutions (such as urine). The urine's saturation is the driving force for the formation of the calculi and still cannot be regulated. In this connection, the results of this study could be put into practice in the clinical treatment of stone formations.

Of all the stone forming substances in the human urine, of most significance is the CaOX precipitation, both, because of the dissemination of the CaOX urolithiasis and because it is the most insoluble stone former (SF). It has to be noted that, in view of the dominant role of hypercalciuria in the pathogenesis of calcium stones [5-7], our therapeutic efforts in respect to those factors are neither clinically, nor scientifically satisfactory.

In recent years, it was shown that the supersaturation in the urine is determined not so much by the concentration of the ions (Ca^{2+} and $\text{C}_2\text{O}_4^{2-}$) constituting the concrements, but by the presence or absence of complex forming agents in the urine. It has been evident since Hammarsten's classic studies [8] that many ions, such as Mg^{2+} , citrate or HPO_4^{2-} , which are normally present in the urine, increase the solubility of CaOX in aqueous solutions by forming complexes with either the Ca^{2+} or the $\text{C}_2\text{O}_4^{2-}$ ions. However, the oral administration of these complexing agents does not lead to encouraging clinical results, as they metabolize quickly in the organism.

There has been an increasing interest, particularly, in Ca-binding amino acids, due to their role in many calcium-dependent physiological processes. The possible inhibiting impact of various amino acids (alanine, ornithine, tryptophane, etc.) on the type of the CaOX formation and on the subsequent CaOX growth in the urine is often discussed in academic literature. The above mentioned amino acids, which are also common in the urine, have an inhibiting effect on the CaOX growth even at minimal concentration [9-11].

According to our *in vitro* data [12], arginine (Arg) is a weak complexing agent with respect to calcium. Arg ($\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$) is present in the human urine at lower concentrations relative to other amino acids; its urine's excretion is 7.0 - 47.0 $\mu\text{mol}/24$

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h [13]. We found that in physiological solutions, resembling the human urine in their composition, the complexing effect of L-Arg with respect to Ca^{2+} ions was surprisingly higher. Those preliminary results and the possible biological significance of Arg provided the impetus for a thorough examination of the kinetics of the CaOX concretions dissolution in physiological solutions containing various concentrations of acid.

Up to now, no analysis has been performed on the possible correlation between the Arg's concentration in the urine and the inclination to develop CaOX calculosis. The results obtained in our *in vitro* experiments [12] regarding the dissolution of CaOX in physiological solutions, containing an increased concentration of Arg, stress the importance of those analyses.

Patients and Methods

Two types of experiments were performed in the framework of this investigation: 1) analysis on the concentration of the Arg in serum and urine in patients with CaOX stones, as well as, in a healthy control group; and 2) an investigation on the kinetics of dissolution of CaOX calculi in physiological solutions, containing various concentrations of Arg.

Patient studies

SFs

Fifty-six patients (30 men and 26 women) were included, whose age ranged from 14 to 65 years, at the beginning of the calculosis, and who had had their CaOX renal calculi removed spontaneously, by surgery or through extracorporeal shock-wave lithotripsy (ESWL). Each patient had a known clinical and family history of this disease, including data for episodes of a renal colic and of concretions elimination, the presence of metabolic disorders and so forth. During the conduction of the study, the patients were on free diet and fully compensated renal function. They did not report any liver disease.

Controls

Fifteen healthy subjects were included who never had any urological and hepatic trouble.

Calculi

The calculi were taken at random from patients who had undergone surgical or ESWL removal of the stones.

Methods

A 24-h urine collection was obtained from each patient. During the period of urine collection, specimens were refrigerated and

aliquots of the 24-h volume and sample were immediately frozen until analyzed. The volume of urine in every sample was recorder on completion of the collection and pH was measured by using a glass electrode pH-meter.

Serum was obtained from the same patients as those whose urine was collected from. Heparinized plasma was separated by centrifugation and was also stored at temperature -25°C .

The amino acid (Arg) contents of the sample were determined using a Hewlett Packard HPLC 1050, coupled to a fluorescence detector. Ethyl alcohol was added to the urine specimens to allow the precipitation of proteins and the extraction of free amino acids. An automated precolumn orthophthalaldehyde derivation procedure was employed. Separations were done using a reversed-phase column (Waters Corp). Amino acid concentration of the samples was determined by comparison with values obtained from a standard curve. Amino acid concentrations are expressed in μM .

The amino acid (Arg) in urinary calculi was evaluated as follows. The calculi were carefully rinsed in saline to remove any contaminations (blood clots, etc). The calculi were dried with filter paper and ground into a powder in an agate mortar. To 0.5 g of the powder, 4.5 mL 90% ethyl alcohol were added, well mixed and left for 10 min to allow the precipitation of proteins and extraction of free amino acids and then centrifuged. The supernatant was evaporated to dryness under vacuum at a temperature not exceeding 55°C . The residue was re-dissolved in 0.2 mL 10% isopropyl alcohol. The qualitative analysis of the calculi was carried out by the HPLC.

Statistical analysis

Statistical analysis of the data obtained from both the SF patients and from the control group was performed using Student's *t*-test to establish the significance of the difference between mean values. All results were expressed as mean \pm SEM and the differences were considered significant if $P < 0.05$.

Dissolution of CaOX concrement: basic theoretical considerations

Human urine is a complicated physiological solution. The physic-chemical formalism of the kinetics of dissolution of kidney stones has been developed, in details, in our papers [14, 15]. One can see that simple formulae can be obtained, describing the effect of complex forming agents (present in the solution at various concentrations) on supersaturation, solubility, and growth velocity of CaOX crystals growing or dissolving in solution, resembling human urine. It can be shown that if we introduce an increasing concentration C_{Arg} (e.g. L-arginine) of a Ca^{2+} -binding complex forming agent, having a solubility constant K_{Arg} into the solution, a linear dependence between the solubility S_{Arg} and C_{Arg} for $\text{Ca}^{2+} \gg \text{C}_2\text{O}_4^{2-}$ can be predicted by:

$$S_{\text{Arg}} = S (1 + K_{\text{Arg}} C_{\text{Arg}} / \alpha') \quad (1)$$

where α' is the α factor in the absence of the complex forming agent "Arg" (here indicating L-arginine).

Thus, the dependence of the supersaturation $\Delta\mu$ on C_{Arg}

Table 1. Serum Levels and Urine Excretions of Amino Acid Arginine in the Patients With a Calcium Oxalate Calculosis and Control Group [13]

	Ref. value [13]	Controls (n = 15)	Stone formers (n = 56)
Arginine, x (μmol/L) (in serum)	49.7 - 132.1 (Male) 46.9 - 139.7 (Female)	94.7 ± 54.5	109.9 ± 39.4
Arginine, x (μmol/24 h) (in urine)	7 - 47	-	-

for the physiologically significant case $\text{Ca}^{2+} \gg \text{C}_2\text{O}_4^{2-}$, determining the supersaturation in urine is:

$$\Delta\mu \cong \Delta\mu_0 - 1/2 \ln (K_{\text{Arg}} C_{\text{Arg}} / \alpha') \quad (2)$$

where $\Delta\mu_0$ is the supersaturation in respect to the CaOX-precipitation without Arg added.

It is also of interest that in the case of the dissolution of CaOX concrements in the presence of a fixed initial concentration of CaOX (or, which in the case of $\text{Ca}^{2+} \gg \text{C}_2\text{O}_4^{2-}$ is the same, in the presence of constant concentration C_o^* of oxalic anions) we have to rewrite (1) as follows:

$$S_{\text{Arg}} = S (1 + K_{\text{Arg}} C_{\text{Arg}} / \alpha') - C_o^* \quad (3)$$

Thus a plot of S_{Arg} versus C_{Arg} should result in a straight line with a slope of $-SK_{\text{Arg}}/\alpha'$, cutting from the ordinate axis a segment $S_{\text{Arg}}(0) = S - C_o^*$. In this way, both S_{Arg} and K_{Arg} can be determined at a known value of α (according to data in Robertson et al [16], α' in human urine is approximately 2).

Thus, depending on the concentration C_{Arg} , that is, on the sign of $\Delta\mu$ (i.e. $\Delta\mu > 0$ during growth, $\Delta\mu < 0$ during dissolution), growth or dissolution of CaOX concrements can be achieved simply by changing the concentration C_{Arg} of the Arg added.

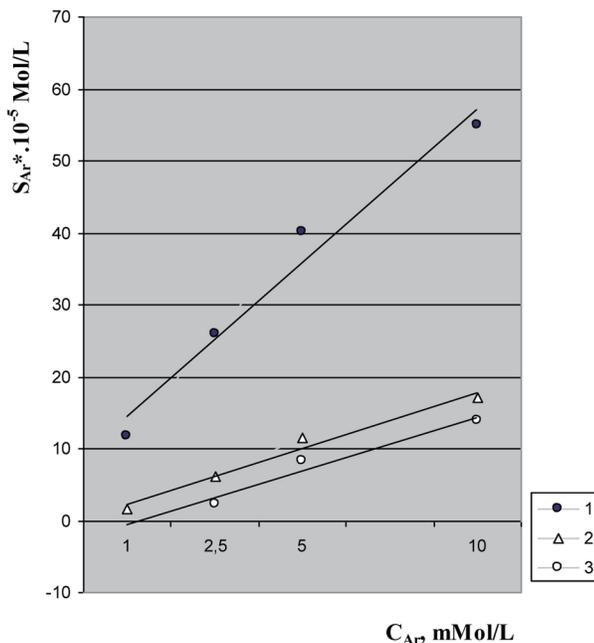


Figure 1. Solubility of calcium oxalate calculi in artificial urine as a function of L-arginine concentration. Solubility in zero saturation urine (curve 1). Solubility in artificial urine with lower saturation (curve 2). Solubility in artificial urine with normal saturation (curve 3).

Instrumental techniques

The experiments on the kinetics of the dissolution of CaOX renal calculi were performed in Jena glass round bottom flasks thermostated at 25 °C. The volume of the studied solution was 1,000 mL and it was stirred (about 200 rpm) by an electromagnetic stirrer. The Archimedean weight G (t) of the samples of CaOX calculi, put in a platinum net basket and suspended to a torsion balance, was continuously measured with a sensitivity of ± 0.5 mg [14, 15].

The CaOX calculi used had been formed in the urinary tract and eliminated spontaneously by the patients. The calculi were selected to have a weight of 100 to 200 mg and to be of identical mineral composition, mainly $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ (weddelite). The composition of the calculi was checked by polarized-light microscopy and thermogravimetry (DTG).

We employed two different types of aqueous solutions, mimicking urine [17, 18], with our solvent (Arg) introduced in several different concentrations.

Results

Clinical laboratory data

Table 1 [13] shows the plasma levels ($\mu\text{mol/L}$) and the urine excretion ($\mu\text{mol/24 h}$) of amino acid Arg in the groups of the patients (SFs) and control subjects. A mathematical estimation of the deviation from the observations on amino acid in patients has been made.

Thus, plasma levels of Arg in the patients were non-significantly higher compared to those of a control group. A detailed analysis shows that 80% of SFs eliminated pathological values: 30% of patients had lower plasma levels compared to controls and about 50% of SFs showed higher concentration.

Urine concentrations in these two groups were not reported.

Data of *in vitro* experiments

The solubility of CaOX in artificial urine with zero supersaturation is considerably increased (8.7×10^{-5} mol/L) compared with its solubility in pure water (5.5×10^{-5} mol/L) due to the presence of complexing ions (Mg^{2+} , citrate ions, etc.) in this solution as predicted by α_i coefficients known from an analytical chemistry [8, 14]. When L-Arg is introduced into the same physiological solution, a dramatic change in solubility (up to

Table 2. L-Arginine as a Solvent of Calcium Oxalate: Comparison With Classical Complex Forming Agents [8, 14, 16]

Ligand	Complex former	Solution	T (°C)	pH	K _i (L/mol)	Reference
C ₂ O ₄ ²⁻	Mg ²⁺	0.3 M NaCl	25	5.0	4.0 × 10 ³	[8]
C ₂ O ₄ ²⁻	Mg ²⁺	0.3 M NaCl	37	-	5.6 × 10 ³	[14]
Na-EDTA	Ca ²⁺	Physiological solution	25	7.0	5.0 × 10 ⁵	[14]
Citric anion	Ca ²⁺	Pure water	25	-	5.0 × 10 ²	[16]
L-arginine	Ca ²⁺	0-artificial urine	25	5.4	5.6 × 10 ³	Fig. 1

55 × 10⁻⁵ mol/L (i.e. about 70 mg/L) is observed, as shown in Fig. 1.

A similar effect of L-Arg is also seen in artificial urine, in which distinct supersaturation (due to the presence of a normal concentration of Ca²⁺ and a medium concentration of C₂O₄²⁻ ions [12]) has been maintained (Fig. 1, curves 2 and 3).

In accordance with Eq (1), a linear dependence of the solubility on C_{Arg} is observed (Fig. 1) for each series of measurements, in which three different supersaturation values (zero, medium and normal) have been established. The effect of the increasing concentration of L-Arg on the solubility of CaOX crystals is clearly evident; the initially supersaturated solutions are transformed into undersaturated systems. The effect of the presence of Ca²⁺ of the course of the straight lines 2 and 3 in Fig. 1 when compared with the no-Ca²⁺ case (line 1 in the same figure) is to be noted. A sharp decrease in the slope of lines 2 and 3 is observed, too. Since the two solutions have the same Ca²⁺ concentration, curves 2 and 3 are parallel, as expected from the formalism, as discussed above [14].

Discussion

This paper examines the role and place of the amino acid Arg in the pathogenesis of CaOX renal calculosis. It has been found that Arg is a very effective solvent of CaOX calculi in solutions characterized by the composition and ionic strength of human urine. We have found that the mechanism of dissolution of CaOX calculi follows the Nernst model of a diffusion-limited process as it is discussed in detail in literature [12, 14, 15]. The dissolution of CaOX with Arg is a relatively slow process, taking approximately 1 month to dissolve about a 70 mg sample at zero supersaturation. Detailed analysis performed in our study indicates that Arg is comparable, in its solubility effect, to the best known classical complex binders of Ca²⁺ or C₂O₄²⁻ ions in urine, that is, Mg²⁺ and citrate anions. This can be seen from Table 2 [8, 14, 16], in which the stability constant K_{Arg} of Arg calculated in our results (Fig. 1, Eq. 1) is compared to the K_i values of Mg²⁺, Na-EDTA, and other known complex-formers of CaOX.

Arg is one of the amino acids constituent of normal human urine. Arg is found in all proteins, which means that it is necessary for life. From bases and arginaza enzyme, which is found in the liver, it breaks down into urea and ornithine (ornithine cycle). The addition of Arg can reduce the binding of free radicals in tubular membranes, respectively to reduce nephrocalcinosis [19, 20].

Insignificantly higher are the serum levels of Arg ((NH)₂C(NH₂)CH₂CH₂CH₂CH(NH₂)COOH) for the patients to a CaOX lithiasis compared to control group. Of all found free amino acids in CaOX stones, Arg is amino acid with highest frequency - 21.6 nmol/mL. But do not expect that fact alone may explain the lack of Arg in urine collections. One possible explanation of this is the disintegration of the acid by the action of the enzyme arginaza to ornithine (H₂NCH₂CH₂CH₂CH(NH₂)COOH), i.e. we cannot consider separately excretion of ornithine from Arg's metabolism. On the other hand, free acid ornithine is not detected into the proteins, i.e. it is the main "body" of the amino acid Arg. Ornithine is the amino acid with a clearly complex action [21] and in this context, lower urinary excretion of ornithine (more than 60% of patients [22]) may explain low inhibitory activity of urine of these patients. From the analytical chemistry, it is known that the ornithine is an agent forming complex compound with metals (for example, calcium) leading to lower levels of urine saturation in unaffected patients.

Arg was not detected in the urine of patients with CaOX stones. The absence of this acid in groups of CaOX-SFs is of great interest, especially in light of non-difference Arg levels in the plasma of SFs and controls. More so than 30% of patients with CaOX stones have significantly lower plasma levels of Arg in relation to control group. The increased concentration of Arg in the urine of these patients would have a positive impact on the prophylaxis and therapeutic effects of our preventive treatment. The latter is of high importance, especially due to the fact that Arg is very similar in structure to proven complexing agents, such as ornithine, Na-EDTA (complexon III), etc.

This paper does not discuss the metabolic pathway of the Arginine.

Conclusion

The results of present study of Arg indicate a level of stone growth dissolution that considerably surpasses the one of any other amino acid. Our analysis proves that the positive impact of Arg on the CaOX dissolution is mainly due to the lower supersaturation of human urine, as a result of the administration of the agent. The "injection" of Arg to the human body through medication or diet could very effectively prevent the formation or further development of CaOX stones. Thus, we have assigned to Arg a promising place among the major solvents of CaOX calculi.

Disclosure

All the authors declared no competing interests.

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