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Solubility and dissolution kinetics of calcium oxalate renal calculi in solutions containing L-arginine: In-vitro experiments

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ABSTRACT

The kinetics of dissolution of calcium oxalate (CaOX) calculi in physiological solutions containing L-arginine at different concentrations were studied using the change in the Archimedean weight of samples immersed in the solution. It was faound that arginine, which is a normal constituent of human urine, acts at increased concentrations as a dissolving agent with respect to CaOX calculi. The possible effect of L-arginine as a natural regulator of CaOX supersaturation and crystalization in human urine is also disscused.

1. Introduction

Interest in the formation of renal calculi can be explained by widespread nephrolithiasis and by the clinical importance of main physiological problems these processes can have [1,2]. Of even greater medical consequences could be the study connected with the solubility of urinary stone-forming substances and with the possibilities of dissolving calculi already formed in the urinary tract [3,4].

The solubility of the most widespread of renal calculi – those formed from the various hydrates of calcium oxalate (CaOX) – has been the subject of many investigations. Two main factors still characterize the CaOX calculus as the problem stone of our age: on one hand its frequency and high rate of recurrence, and the other, the lack of much therapeutic effect from dietary or pharmacological attact.

Unfortunately, the alteration of pH within physiologically tolerable limits changes the solubility of CaOX only slightly [5]. It has to be said that, in view of the dominant role of hypercalciuria in the pathogenesis of calcium stones [6], our therapeutic efforts with respect to these factors are neither clinically nor scientifically satisfactory.

In recent years, there has been increasing interest in a particular Ca-binding amino acid, with regard to its role in many calcium-dependent physiological processes. Research concerning its role in urinary calcium stone formation has been going into further details. The influence of amino acids such as aspartic, ornithine, tryptophan, etc. [7,8] on the CaOX growth process in urine and the type of formed Ca-oxalate phase is often discussed in the literature. In Refs. [9–11], we observe the influence of some amino acids crucial for the human organism, such as lysin, α-ketoglutaric acid, hippuric acid and other similar compounds, which have been proven to be Ca-binding.

In the present in-vitro investigation we indicate that there is another normally present component of urine, L-arginine ($C_6H_{14}N_4O_2$), which increases the solubility of calcium oxalate in various physiological solutions. Arginine is present in the human urine in considerable concentrations (according to Ref. [12] the excretion is about 7–47 μmol/day), surpassing the urinary concentration of all other amino acids.

We found that in physiological solutions resembling artificial human urine in their composition, the complexing effect of arginine with respect to $[Ca^{2+}]$ is unexpectedly increased due to its complexing ability in pure water. These preliminary results and the possible biological significance of arginine provided the impetus for a thorough examination of the kinetics of CaOX concrement dissolution in physiological solutions containing various concertrations of arginine.

2. Material and methods

2.1. Instrumental techniques

Experiments on the kinetics of dissolution of CaOX renal calculi were performed in Jena glass round botton flasks termostated at 37 °C. The volume of the studied solution was 1000 ml and it was stirred (~200 rpm) by an electromagnetic stirrer. The Archimedean weight $G(t)$ of the samples of CaOX

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calculi put in a platinum net basket was measured with a torsion balance, with a sensitivity of ± 0.5 mg. (see Ref. [9]).

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 200–400 mg and to be of identical mineral composition, mainly $CaC_2O_4 \cdot 2H_2O$ (weddellite). Polarized-light microscopy and thermogravimetry (DTG) were used to check the composition of the calculi.

We employed two different types of aqueous solutions with varying concentrations of the solvent–L-arginine.

- "Artificial urine", i.e. a solution with the mean ionic composition of human urine and ionic strength $\psi = 0.3$. In the human urine, the concentration of oxalic acid varies widely. The percentage of patients with hyperoxaluria is relatively higher than those of hypercalciuria. Therefore, in the current in-vitro experiments, we decided to reproduce the urinal saturation in normal physiological boundaries through the variation of $[C_2O_4^{2-}]$ ions. We employed a composition recommended by several authors [13,14] but varied the concentration of oxalate ions in two different series of experiments by introducing different concentrations of oxalate ions $[C_2O_4^{2-}]$ into it: the lower supersaturation urine with 2.4 mmol/l $[Ca^{2+}]$ and 0.024 mmol/l $[C_2O_4^{2-}]$, and medium saturation system with 2.4 mmol/l $[Ca^{2+}]$ and 0.24 mmol/l $[C_2O_4^{2-}]$.
- "O-artificial urine", of the above composition, in which, however, both $[Ca^{2+}]$ and $[C_2O_4^{2-}]$ were absent, i.e. this is a solution with zero initial supersaturation.

L-arginine was added to all solutions in varying concentrations above the physiological norm (7–47 μmol/day) for this substance in human urine. Because of the buffering action of the complex composition of artificial urine its pH value was 5.5–5.7 in all cases.

Each experiment was repeated under identical conditions three times, a new calculus being employed each time. The shape of the dissolution curves, and especially the data on the change in sample weight, did not vary by more than 15% in the repeat experiments.

2.2. Basic theoretical considerations

Human urine is a complicated physiological solution. Taking into account specific features of CaOX as a solute and of urine as a solvent, the solubility S of CaOX at temperature T can be calculated according to procedures normally used in analytical chemistry in the following way as discussed in more retail in Ref. [9]:

$$S = f(1/\gamma) Lp_o \alpha_a \alpha_b \quad (1)$$

where Lp_o is the solubility product of CaOX in pure water (at the same temperature T) and the α factors are determined from the concentrations C_L, C_J and solubility constants K_L, K_J of Ca^{2+} or $[C_2O_4^{2-}]$ binding ions in the investigated biological solution.

For $[Ca^{2+}]$-binding complexing cations

$$\alpha_a = 1 + \sum C_L K_L \quad (2a)$$

and for $[C_2O_4^{2-}]$-binding complexing anions

$$A_b = 1 + \sum C_J K_J \quad (2b)$$

It can be shown that if we introduce an icreasing concentration C_{Ar} (e.g. L-arginine) of a $[Ca^{2+}]$-binding complexing agent having a solubility constant K_{Ar} to the solution, the linear dependence between the solubility S_{Ar} and C_{Ar} for human (e.g. $[Ca^{2+}] \gg [C_2O_4^{2-}]$) system will be predicted by

$$S_{Ar} = S(1 + K_{Ar} C_{Ar} / \alpha'_{Ar}) \quad (3)$$

where α'_{ar} is the α factor in the absence of the complexing agent Ar (L-arginine).

It is also of interest that in the case of dissolution of CaOX concrements in the presence of a fixed initial concentration of CaOX (or – which in the case $[Ca^{2+}] \gg [C_2O_4^{2-}]$ is the same – in the presence of constant concentration C^*_o of oxalic anions) we have to rewrite Eq. (3) as follows:

$$S_{Ar} = S(1 + K_{Ar} C_{Ar} / \alpha'_{Ar}) - C^*_o \quad (4)$$

Thus a plot of S_{Ar} versus C_{Ar} should result in a straignt line of slope SK_{Ar}/α'_{Ar} cutting the ordinate axis at $S_{Ar}(0) = S - C^*_o$ In this way, both S and K_{Ar} can be determined at a known value of α. According to data in Robertson et al. [15], α' for human urine is approximately 2.

3. Results

The solubility of CaOX in pure water at 25 °C is about 4 mg/l [9]. The solubility of CaOX in artificial urine with zero supersaturation (Fig. 1) is considerably increased compared with its solubility in pure water (5.7×10^{-5} mol/l) due to the presence of complexing ions ($[Mg^{2+}]$, citrate ions, etc.) in this solution as predicted by the α_a, α_b coefficients in analytical chemistry [9]. When L-arginine is introduced into the same physiological solution a dramatic change in solubility (up to 74 mg/l or 42.5×10^{-5} mol/l) is observed, as shown in Fig. 1. These solutions, as already mentioned, differ from normal urine because of lack of $[Ca^{2+}]$ and $[C_2O_4^{2-}]$ ions in them.

A similar effect of L-arginine is also seen in artificial urine in which a distinct supersaturation (due to the presence of a normal concentration of $[Ca^{2+}]$ and a medium (or lower) concentration of $[C_2O_4^{2-}]$ ions) has been maintained (see Figs. 2 and 3). In accordance with Eq. (3) a linear dependence of solubility on C_{Ar} is observed (Fig. 4) for each series of measurements, in which three

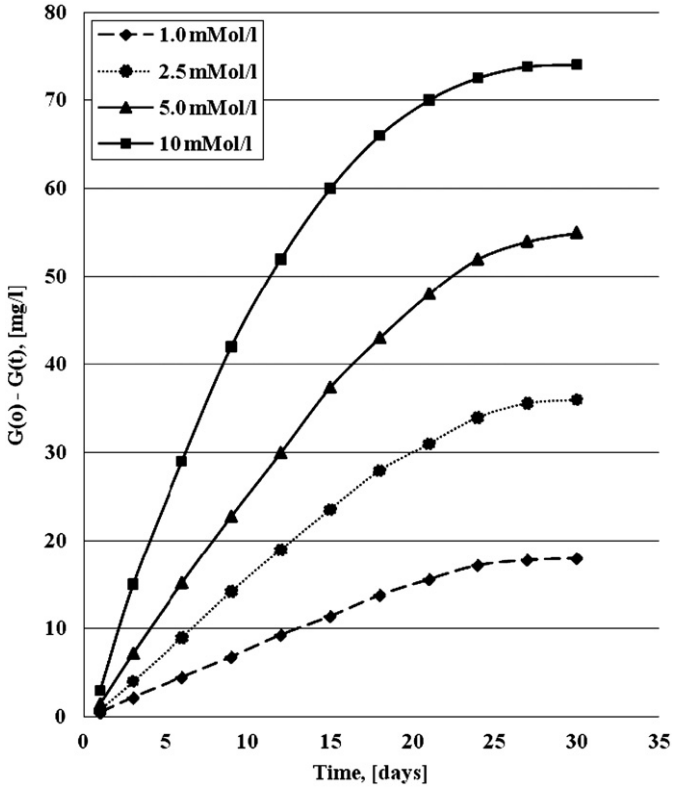

Fig. 1. Weight change in the dissolution of calcium oxalate calculi in artificial urine with zero supersaturation.