Urol Res (2012) 40:205–209
DOI 10.1007/s00240-011-0429-z

ORIGINAL PAPER

Chemolysis of calcium oxalate stones: study in vitro and possible clinical application

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Received: 27 May 2011 / Accepted: 30 September 2011 / Published online: 17 November 2011

Abstract The flow cell modeling clinical conditions have been used to study the interaction between dilute chemolytic solutions and large calcium oxalate renal stones. The stone treatment with 5% disodium ethylenediaminetetraacetate aqueous solutions or citrate buffer are found not to provide notable disruption of the samples studied. The significant improvement is reached with the mixed compositions containing both natural and synthetic chelating reagents:citrate and ethylenediaminetetraacetate ions as well as an antibiotic. Description of the chemolytic irrigation, numerical results and their possible clinical application are the main topic of the present research.

Keywords Calcium oxalate stones · Chemolytic irrigation

Introduction

Abnormal biomineralization in the urinary tract afflicts patients worldwide [1–8]. From 3 to 14% of the population in Europe and the United States suffer from renal stones formation, most of which are composed of calcium oxalate hydrates [1–3]. Being attached to the epithelial tissue of a human kidney, such aggregates often pose significant problems for modern little invasive removal techniques [3, 5–7]. Moreover, calcium oxalate renal stones are almost insoluble in well-established chemolytic solutions such as hemiacidrin or Suby G [6–8].

Recently [9, 10] we have shown that aqueous solutions containing citrate or ethylenediaminetetraacetate ions provide rather rapid destruction of oxalate-phosphate, and especially phosphate renal stones. However, there are some problems to be solved to approach the clinical utilization of the results obtained. First, we have found [9] that as phosphate content in the sample decreases, solubility becomes much smaller, indicating that the destruction of pure oxalate samples will be insufficient for clinical purposes. Second, the pH values of Na_3Cit and Na_2H_2EDTA solutions are of 8.6 and 4.6, respectively. It is obvious that these values are far from those usually observed for the physiological pH range of human urine [11]. Third, it is not quite clear how chelating reagents affect renal tissue. Despite the fact that the authors [12–14] confirm low invasive action of dilute complexon solutions on the human urinary tract, some recent studies have indicated that long-time chemolysis with sodium or potassium ethylenediaminetetraacetate solutions with the pH value of 8.5 may cause urithelial injury (see [15] and references therein). Thus, this problem requires additional studies.

The present research focuses on the interaction of synthetic and natural chelating reagents with urinary tract concrements to find more effective solutions for percutaneous chemolysis of calcium oxalate stones.

Materials and methods

Five large kidney oxalate stones were removed by surgical operations in the urological clinic of Ivanovo regional

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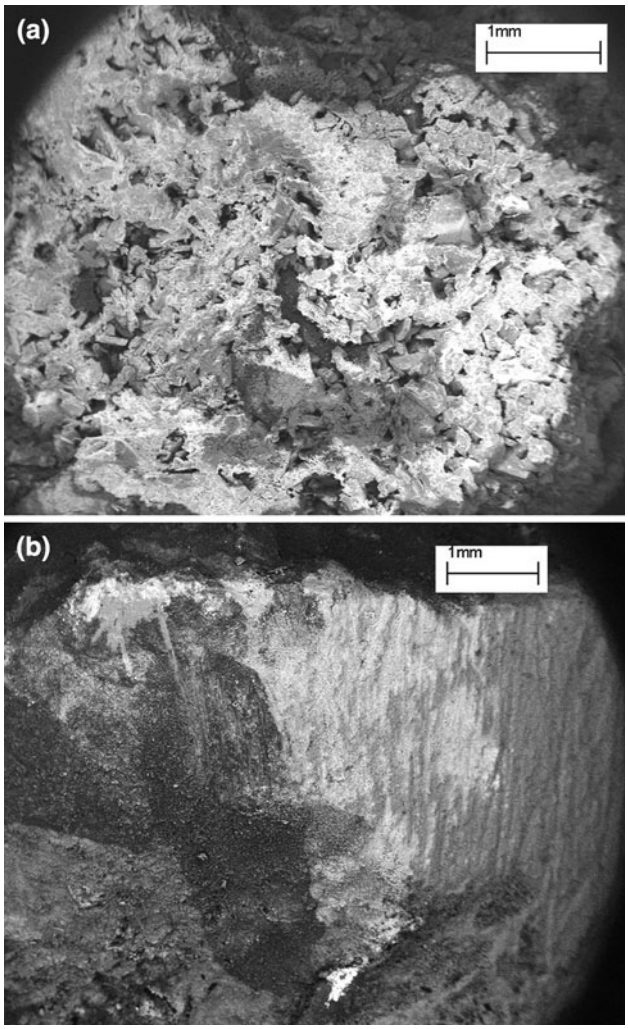

Fig. 1 Images of the calcium oxalate renal stones studied: sample A (**a**) is primarily composed of COD, whereas sample E (**b**) contains almost pure COM

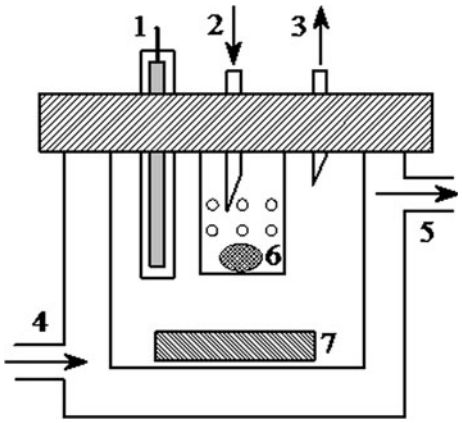

Fig. 2 The flow cell for treating renal stones. *1* a platinum thermometer connected with the standard temperature measuring instrument, *2, 3* steel pipes for input/output of thermostated chemolytic solutions, *4, 5* holes for water input/output for the cell to be thermostated, *6* a renal stone in a glass capsule, *7* a magnetic stirrer

hospital. Two typical samples primarily composed of calcium oxalate dihydrate (COD, $CaC_2O_4{\cdot}2H_2O$) and calcium oxalate monohydrate (COM, $CaC_2O_4{\cdot}H_2O$) are shown in Fig. 1. The qualitative chemical analysis of the samples studied was performed in the clinical biochemical laboratory. The X-ray diffraction studies with the "TOPAS" software (Bruker) were used to obtain detailed information about mineralogical composition of kidney stones.

The flow reaction cell for simulating renal stones chemolysis is described in detail elsewhere [9, 10]. Before the experiment, all the stones were washed by distilled water and dried at first in forced air circulating at 318 K and then at room temperature. For the evaluation of the chemolytic solutions influence on renal stones, we have chosen a simple but informative method based on the analysis of the sample mass loss during the treatment. The stone was put into the glass cylindrical capsule equipped with holes for input and output of a complexon solution, and weighed with the "OHAUS" analytical balances with the sensitivity of 0.01 mg. The capsule was fastened in the 50 ml glass thermostated cell with distilled water (see Fig. 2), and then the system was stirred until thermal equilibrium was reached. The flow rate was adjusted to ensure a constant chemolytic solution concentration in the reaction cell. The experiment duration was 2 h, and the feed rate of a thermostated solution provided by the "Zalimp" peristaltic pump (Poland) was chosen to be equal to 2.5 ml/min. The temperature of the cell measured by the standard temperature measuring instrument was 310 ± 0.1 K during the experiment. Within 2 h, the peristaltic pump and the stirrer were cut off, the capsule with the stone was washed three times by distilled water, dried in forced air at 323 K and then at a room temperature up to a constant weight. The stone mass loss (%) which equals its solubility in this study can be easily estimated from the difference between the capsule mass before and after each experiment and the empty capsule mass.

Electronic microscopy study was performed with the LEO1455VP microscope (Carl Zeiss) equipped with the SiLi semiconductor detector (Röntec) for X-ray spectral microanalysis. The quantitative determination of a chemical element content in a surface layer was performed with the program package enclosed by means of the analysis of the braking radiation level and the comparison of the radiation intensity observed with the etalons provided.

KOH (Aldrich, >95%), H_3Cit (Panreac, >99%), Na_2H_2EDTA (>99%, Chemapol) and ampicillin sodium salt (Belmedpreparaty) were used as-supplied. Aqueous solutions of Na_2H_2EDTA were prepared by weight from the required amount of solid salt and freshly bi-distilled

Table 1 The chemical element content (at.%) on the surface of the samples studied

Element	Sample					
	A R[a]	B E	C E	D R	E	
					R	E
C	37 ± 16[b]	41 ± 15[b]	44 ± 7	35.7 ± 7	22 ± 6	49 ± 18
O	56 ± 20	53 ± 16	46 ± 8	54 ± 11	62 ± 12	41 ± 16
N	–	–	–	–	–	5 ± 6
Ca	7 ± 1	5 ± 1	5.1 ± 0.6	10 ± 2	15.8 ± 2	4.3 ± 0.8
P		1 ± 0.4	0.5 ± 0.1	–	0.2 ± 0.1	0.1 ± 0.1
Na		–	4.1 ± 0.6	–	–	0.1 ± 0.2
K	–		–	–	–	0.1 ± 0.1
S	–		0.3 ± 0.1	0.3 ± 0.2	–	0.4 ± 0.2

Stone composition (mass%): sample A:COM (52%) + COD (48%); sample B:COM (11%) + COD (87%) + hydroxyl apatite (2%); sample C:COM (78%) + COD (15%) + hydroxyl apatite (7%); sample D:COM (100%); sample E:COM (99%) + COD (1%)

[a] Symbols E and R denote the etched and raw surface, respectively

[b] Uncertainties from here on represent the twice standard deviation of the mean

water. Potassium citrate solution (citrate buffer) and the mixed citrate-ethylenediaminetetraacetate compositions were prepared as follows: 19 g of pure H_3Cit or citric acid plus additives was dissolved in 900 ml of freshly bi-distilled water, and then the required amount of pure KOH were added to obtain the pH value of 6.4. Then some amount of water was added to reach the mass of the solution equal to 1 kg.

Results and discussion

Since chelating reagents contact primarily with the kidney stones surface, it is important to reveal changes in its texture and chemical composition before and after the chemolytic irrigation. The morphological analysis of the COM stone (see Fig. 1b) reveals a concentric and radial structure which is nearly identical to the Id type of COM stones described by Daudon et al. [3]. Formation of such aggregates is readily associated with hyperoxaluria [3], most of these stones being rather resistant to extracorporeal shock wave lithotripsy (ESWL).

X-ray microanalysis shows that there are mainly oxygen, carbon and calcium atoms on the stone surface (see Table 1), which is in good agreement with the X-ray diffraction studies. Table 1 shows that the relative content of oxygen and carbon atoms is nearly identical for raw and etched surfaces, whereas the content of calcium atoms is smaller for the etched samples. This observation is not too surprising in light of our [9, 10] and literature [16–18] findings, since chelating reagents containing carboxyl groups reveal a strong tendency for binding with Ca ions both in solutions and in an acute single {101} step on the (−101) face of the COM crystal surface. Figure 3 demonstrates microstructure of sample E before and after the treatment. Although significant changes are easily viewed, appreciable cracks on the COM surface are not detected. The results of X-ray microanalysis given in Table 1 show that even after the 10 h chemolysis, complete demineralization of the COM stone surface does not appear to be reached.

Solubility values given in Table 2 reveal additional important features which are worth noting. First, water does not provide any destruction of the samples studied, which is consistent with our previous results for oxalate–phosphate renal stones [9]. Nevertheless, as the COD content increases, the stone mass loss appears to be slightly larger. The same conclusion can be drawn for Na_2H_2EDTA and potassium citrate aqueous solutions. Thus, renal stones composed primarily of COD should be more easily expelled from a kidney than the COM ones. Second, although the chemolytic solutions containing potassium citrate or Na_2H_2EDTA affect all the samples studied rather identically, the stone mass loss observed is still small. Third and general, mixed compositions with the physiological pH value of 6.4 containing chelating reagents, both natural and synthetic, turn out to be much more effective for treating calcium oxalate stones in comparison with Na_2H_2EDTA or potassium citrate solutions. Table 2 does indicate that for COD + COM mixed stones solubility increases greatly, reaching 3–5 mass% for 2 h, which is, in our opinion, of evident clinical importance. For the samples composed of pure calcium oxalate monohydrate, solubility does not exceed 1.5 mass%. Although this value is much larger than for Na_2H_2EDTA or potassium citrate solutions (see Table 2), it is rather insufficient to destroy such aggregates within reasonable time. Nevertheless, the solubility observed is large enough for removing small residual concrements arising after the shock-wave treatment of pure

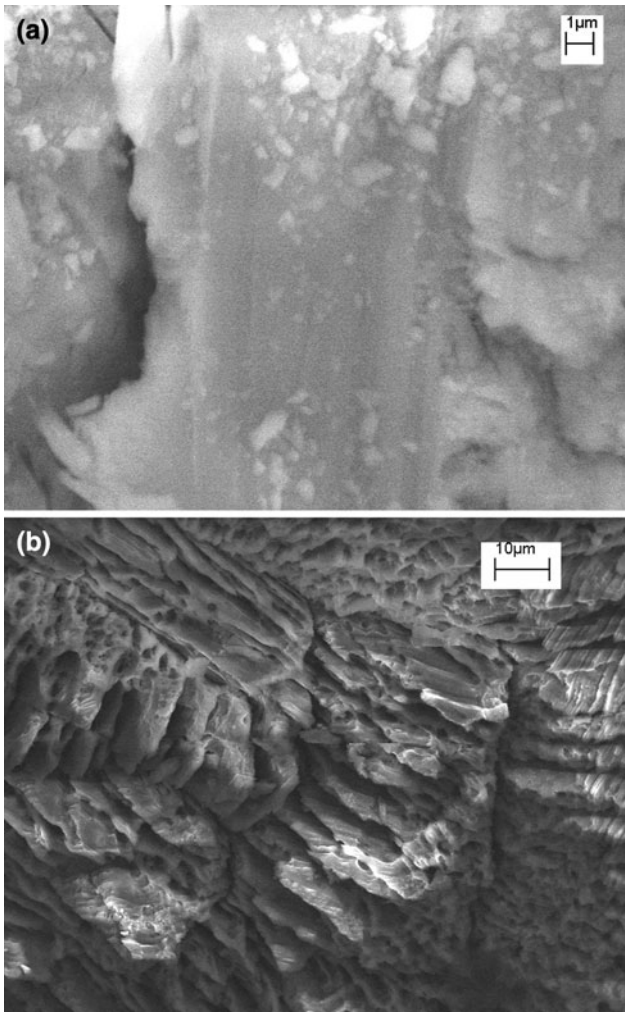

Fig. 3 Images of sample E microstructure: raw surface (**a**), etched surface (**b**). The chemical element content for the regions depicted is given in Table 1

COM stones in the human urinary tract. In fact, our recent estimation does indicate that fragments of COM stones expelled from a urinary tract after ESWL become much better soluble in the mixed chemolytic solution. The stone mass loss for such fragments reaches 8 and more percents for the 2 h treatment. Thus, for such small stones or their fragments (stone paths) arising after the ESWL treatment, the chemolytic irrigation may provide rather rapid stone removal from the kidney. It appears that for 3–5 mm residual fragments, only three or four sessions could be quite enough to expel them from the urinary tract.

Table 2 shows another interesting result. $MgCl_2$ addition to a complexon solution results in decreasing solubility for all the samples studied. This fact, however, appears to be not too surprising from the chemical point of view, since both Ca^{2+} and Mg^{2+} cations reveal a strong tendency to form stable complexes with citrate and ethylenediaminetetraacetate ions in aqueous solutions [18]. We assume that the phenomenon observed simply arises from the competition between cations for polyanionic molecules. It indicates that though potassium–magnesium citrate management is believed to be an effective prophylactic therapeutic drug against recurrent calcium oxalate nephrolithiasis [19], chemolytic solutions containing magnesium citrate or magnesium ethylenediaminetetraacetate become less effective reagents for direct dissolution of calcium oxalate renal stones.

Conclusions

The results obtained do indicate that mixed compositions containing natural and synthetic chelating reagents, citrate and ethylenediaminetetraacetate ions as well as ampicillin sodium salt with the pH value of 6.4, should provide new possibilities for treating calcium oxalate nephrolithiasis. In our opinion, this chemolytic solution should also destroy oxalate–phosphate and, especially, pure phosphate stones including concrements primarily composed of brushite.

Our recent preliminary study of the chemolytic reagents' action on the urinary tract of experimental animals indicates that the chemolytic solutions above are rather slightly invasive for the rat bladder epithelial tissue [20]. Although local changes are detected with light microscopy, they are insignificantly stronger than those appearing in the case of irrigation with the physiological NaCl solution [20]. However, it is obvious that for starting any clinical tests additional comprehensive animal studies are required.

Nevertheless, at present we may propose at least two possible ways for the clinical utilization of the chemolytic solutions above. First, although the widespread use of shockwave lithotripsy as the first-choice treatment is accepted for a variety of urological clinics [3, 8, 12], we believe that joint shock wave action and chemolytic irrigation [4, 6, 7] should provide much more rapid removal of COM and especially, COM + COD stones from the human urinary tract. Second, for some patients, installation of nephrostomic catheters or stents into a kidney is required [6, 12, 14]. It gives additional possibility to destroy calcium oxalate concrements by irrigating the kidney interior with the chemolytic solutions proposed. In this case, however, special attention should be paid to the patient blood and intra-renal pressures at least in the initial period of irrigation. The numerical solubility values listed in Table 2 could give the quantitative physicochemical basis for this treatment. The same procedure can be also used to improve the results of surgical operations by removing small residual COM concrements from the human kidney.

Table 2 Solubility (mass%) of calcium oxalate renal stones in chemolytic solutions at 310 K

Reagent	Sample A $m = 190$ mg $S \sim 130$ mm^2 [a]	Sample B $m = 250$ mg $S \sim 160$ mm^2	Sample C $m = 330$ mg $S \sim 180$ mm^2	Sample D $m = 440$ mg $S \sim 270$ mm^2	Sample E $m = 1250$ mg $S \sim 390$ mm^2
Bi-distilled water	–	0.3[b]	0.2	0.05	0.05
Na_2H_2EDTA, 5 mass% ($m = 0.16$ mol/kg, pH $\sim$ 4.6)	1.1	3.2	1.5 ± 0.1[c]	0.7 ± 0.1	0.6 ± 0.1
Potassium citrate ($m = 0.101$ mol/kg, pH $\sim$ 6.4)[c]	1.4	3.5	1.8	0.9	0.8 ± 0.1
Potassium citrate ($m = 0.101$ mol/kg) + 1 mass% of Na_2H_2EDTA, (pH $\sim$ 6.4)	3.1 ± 0.2	5.3 ± 0.6	5.0 ± 0.5	1.7 ± 0.2	1.5 ± 0.1
Potassium citrate ($m = 0.101$ mol/kg) + 1 mass% of Na_2H_2EDTA + 0.2 mass% of ampicillin sodium salt (pH $\sim$ 6.4)	3.3 ± 0.3	5.4 ± 0.4	5.2 ± 0.7	1.6 ± 0.2	1.4 ± 0.15
Potassium citrate ($m = 0.101$ mol/kg) + 1 mass% of Na_2H_2EDTA + 0.5 mass% of $MgCl_2$ (pH $\sim$ 6.4)	2	3.6	2.6	–	–

[a] For calculating the stone surface area concrements have been approximated with spheroids

[b] The result of the single experiment

[c] Molality represents mol of citrate or ethylenediaminetetraacetate ions per 1 kg of the solvent containing water and additives

Acknowledgments We thank Dr. O. V. Gusakova for providing X-ray spectral microanalysis studies. The authors gratefully acknowledge partial support of this work by the Russian Foundation for Basic Researches (grant N 08-03-12053-ofr) and Carl Zeiss company through the grant for young scientists.

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