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Urothelial injury to the rabbit bladder caused by calcium dissolving agents including two new citrate solutions

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Abstract We compared the urothelial injury to the bladder caused by four agents capable of dissolving calcium salts. The solutions were administrated in an antegrade way through left ureterostomies in 54 rabbits for periods of 24, 48 and 72 h. The bladders were then removed and three routine histological sections were made for each. The following six solutions were used: physiological sodium chloride solution (Phys), artificial urine (Art), 0.03 M disodium EDTA buffered to pH 8.5 with triethanolamine (EDTA), 10% Renacidin (R), test solution 2 (S2, using D-gluconic acid-lactone and other compounds that differ from R in terms of ingredients or quantity), and test solution 1 (S1, using D-gluconic-acid instead of D-gluconic acid-lactone in S2 but keeping the other ingredients the same) for irrigation. At 24 h there was no observable urothelial damage caused by perfusion with Phys or Art; solutions R, S1 and S2 caused approximately the same level of injury to the rabbit bladder mucosa; however, irrigation with disodium-EDTA caused more serious urothelial injury than R, S1 and S2 ($P < 0.05$, χ^2-test) and may be unacceptable. The damage to bladder tissues treated with S1 and S2 was less than that caused by R, but this was not significant ($P > 0.05$, χ^2-test). Following a prolonged irrigation time, all of these solutions cause further urothelial damage, but EDTA caused the most, followed by R, S1, S2, Phys or Art, respectively, at 48 and 72 h. In view of the better solubility effect of solutions S1 and S2 compared with R, it might be justified in accepting the more pronounced urothelial irritation caused these solutions, but in order to enhance their effectiveness and reduce urothelial injury further study will be needed.

Keywords D-gluconic acid-lactone · D-gluconic-acid · Solution · Bladder infusion · Mucosal injury

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Introduction

The best means of treating stone disease is still definitive calculus removal. The advances in technology and the development of new percutaneous techniques enable us to remove most urinary stones effectively, and have left very few indications for open surgical extraction. These advances notwithstanding, the search continues for a medicinal approach to stone management, not as an alternative to today's highly successful treatment modalities, but as a complement to these effective techniques. Though the chemolysis of urinary stones is seldom used clinically, we endeavor to discover an agent which dissolves phosphate urinary stones effectively and with limited urothelial injury.

Chemical dissolution of urinary calculi has been investigated for many years. In the 1940s, Suby and co-workers [1, 2] reported rabbit bladder mucosa injury from hemiacidrin (R) [3–5]. Hitherto, many solutions have been tested in the quest for a high dissolution efficiency and limited urinary tract injury formula. We know that slightly alkaline solutions [6] infused through ureteral or percutaneous catheters may dissolve acid stones; the addition of acetylcysteine to such solutions may bring cystine stones into solution [7]. Slightly acidic solutions may cause alkaline stones to dissolve. The use of a chelating agent can bring even the insoluble calcium oxalate compounds into solution [8]. Unfortunately, in most solutions either pH dependent or chelating agents produce unacceptable damage to the urothelial tissue. In this study, we performed an experiment with rabbits to detect the injury to urinary

mucosa caused by two new complex solutions which are tested for their ability to dissolve phosphate calculi effectively.

Materials and methods

The experiments were approved by the local animal ethics committee. The following six solutions were selected for study:

1. Physiologic sodium chloride solution at pH 7.0 (Phys).
2. Artificial urine (Art, pH 5.7, according to Griffith et al. [9]) consisting of urea (25 g/l), sodium chloride (4.6 g/l), potassium dihydrogen phosphate (2.8 g/l), sodium sulfate (2.3 g/l), potassium chloride (1.6 g/l), ammonium chloride (1.0 g/l), and minor quantities of calcium chloride dihydrate, sodium oxalate, and sodium citrate.
3. 0.03 M Disodium ethylenediaminetetraacetic acid buffered to pH 8.5 with triethanolamine (EDTA, pH 8.5).
4. 10% Renacidin solution (R, pH 3.9), consisting of citric acid (28.2 g), gluconic acid (5.0 g), calcium carbonate (1.0 g), magnesium bicarbonate (14.5 g), citrate magnesium (2.5 g), dissolved in 1,000 ml distilled water.
5. Test solution 2 (S2), prepared using citric acid (18 g), citrate magnesium (1.0 g), calcium carbonate (0.5 g), magnesium carbonate (7.5 g), D-gluconic acid-lactone (3.0 g), altogether 30.0 g in 100 ml distilled water. These solvents were kept at 37°C for 3 days, then the clarified solution was removed, diluted with 140 ml distilled water, and the test solution is prepared (pH 4.0, these ingredients can be completely dissolved by heating).
6. Test solution 1 (S1), prepared using citric acid (18.0 g), citrate magnesium (1.0 g), calcium carbonate (0.5 g), magnesium carbonate (7.5 g), D-gluconic-acid (3.0 g), dissolved in 100 ml distilled water, and the same process as S2 was carried out (pH 3.9).

All chemicals were bought from Sigma-Aldrich China.

A total of 54 New Zealand rabbits were used in our experiment, with each group containing nine animals. These rabbits were anesthetized with pentobarbital and ketamine hydrochloride and the incision area shaved and prepared with betadine. Via a midline abdominal incision, the left ureter was exposed and ligated close to the renal pelvis. Just distal to this ligation a ureterotomy was carried out and a piece of medical grade Teflon tubing (internal diameter 0.012 inches and external diameter 0.03 inches) was introduced such that its distal end was approximately 1.5 cm above the uretero-vesical junction. The proximal end of the tubing was brought out through a subcutaneous tunnel in the animal's back and connected to a single fluid channel standard swivel (Spalding Medical Products, Arroye Grande, Calif.) which was in turn connected to infusion equipment. Solutions were infused at a rate of between 15 and 20 cc/h, which is approximately the urine flow rate in rabbits. After irrigation, three animals were sacrificed from each group at 24, 48 and 72 h, respectively. At that time, verification of the appropriate catheter position was confirmed and the bladder was removed and fixed in Bouin's solution for later histological examination. Three different areas were selected according to their injury level and designated as slight, middle and serious, with one slid made from each area.

Damage to random bladder sections was scored by two pathologists according to the code in Table 1.

Histological changes included edema, urothelial cell damage and neutrophil infiltration. Endothelial damage ranged from cytoplasmic vacuolization, shrinkage and desquamation, to diffuse necrosis with the formation of shallow ulcerations.

Results

There was no observable damage to urothelial tissue produced by irrigation with Phys or Art (Fig. 1), and all minimal alterations were associated with the high flow rates used in the experiments or the tissue preparation procedure. On the other hand, irrigation with disodium-EDTA caused more serious urothelial injury (Fig. 2) than R (Fig. 3), S1 (Fig 4) or S2 (Fig 5) at 24 h ($P < 0.05$, χ^2-test). R caused a little more injury than S1 or S2 but this was not significant ($P > 0.05$, χ^2-test). There was a diffuse area of edema, neutrophil infiltration, and endothelial necrosis in every bladder from the S1 and S2 groups, and each was given a rating of 2 or 3 (Table 1). In many instances, the damage caused by EDTA were subjectively worse than in the other groups. All nine EDTA treated bladders were scored 3 or 4. The nine bladders from the Phys or Art, all were scored 0 or trace score (Tr) (Table 2).

Following prolonged irrigation time, all of these solutions caused further urothelial damage (EDTA caused the most, followed by R, S1, S2, Phys or Art, respectively, at 48 and 72 h; Table 3).

The gross appearance of the bladders irrigated by EDTA became black, congested with diffuse necrosis

Table 1 Scores of bladder injury

Scores	Injury
0	Normal
Tr	Could not be definitely detected
1	<20%
2	20%–50%
3	50%–80%
4	>80%

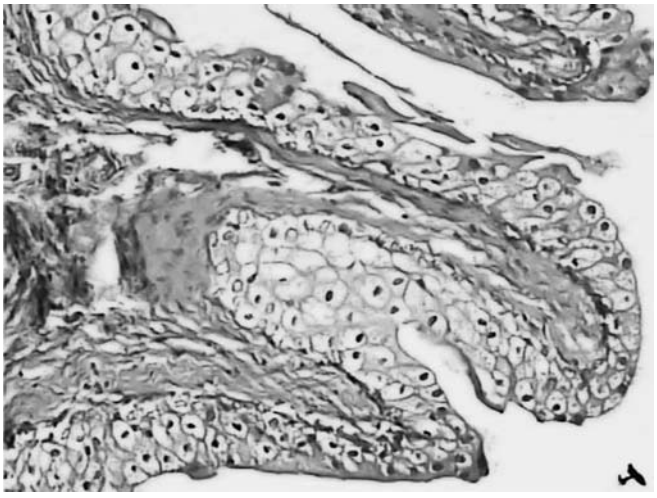

Fig. 1 Phy 24 h, normal mucosa, 4×

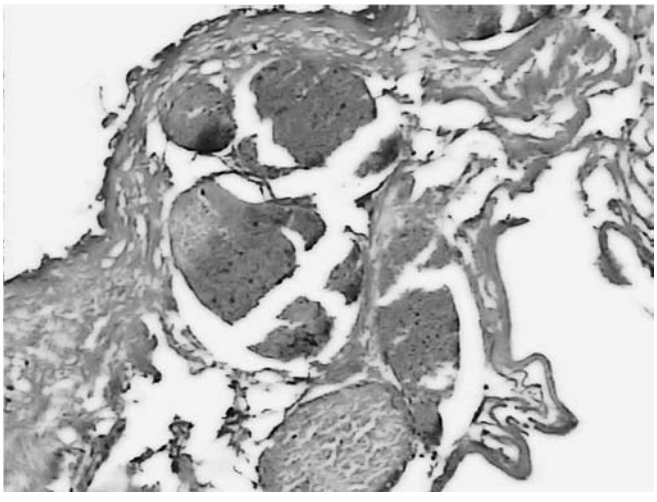

Fig. 2 EDTA 24 h, vesicular inflammatory dilatation, interstitial swelling and collagen degeneration, 10×

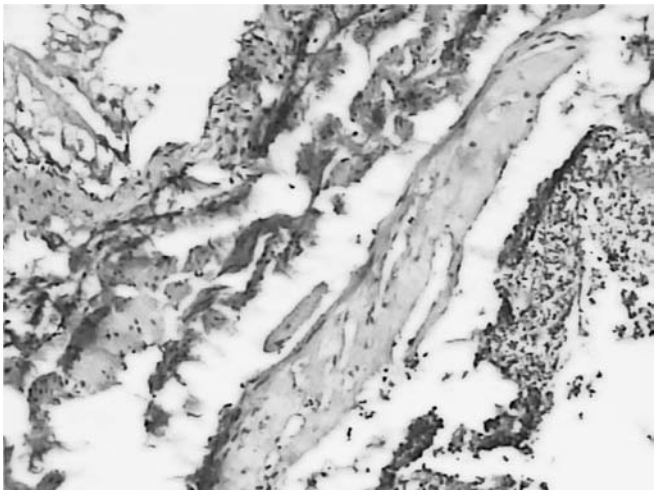

Fig. 3 Renacidin 24 h, vesicular hyperplasia, dilatation and hypereima, matrix swelling, neutrophil infiltration 10×

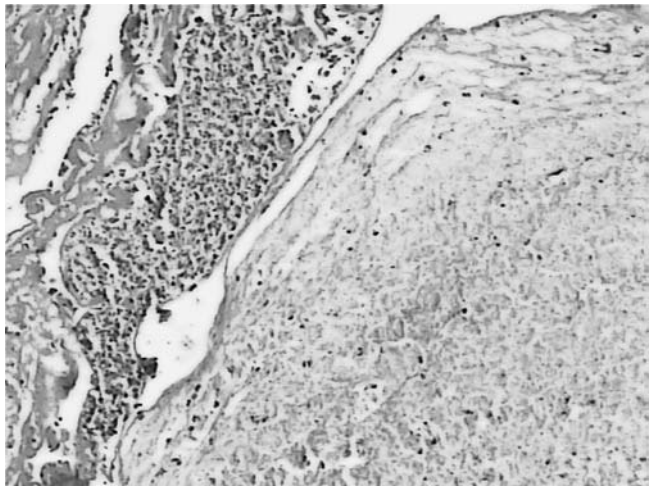

Fig. 4 S1 24 h, vesicular inflammatory dilatation and interstitial swelling infiltrated by leukocytes, 10×

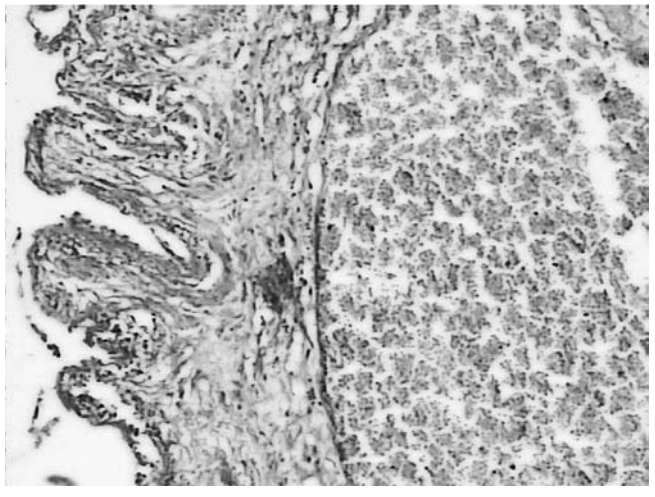

Fig. 5 S2 24 h, mucosal swelling, interstitium infiltrated by inflammatory cells, 10×

and formation of shallow ulcerations. The bladders irrigated by R, S1 and S2 became red accompanied by edema and congestion.

Discussion

The chemical dissolution of urinary calculi has been used in therapy for many years, mostly for dissolving struvite, uric acid and cystine stones [6, 7]. There are several effective solutions, most of which are composed of chelating agents. These are a compounds that can form soluble non-ionic complexes with polyvalent metal ions, having tolerable toxicity. This means that chelating agents have the advantage of improved mineral solubility at different pHs. By altering urinary pH with these solutions, struvite, uric acid and cystine stones can often be brought into solution.

Table 2 Score of bladder lesions after perfusion for 24 h with different solutions

Solution		Score of bladder lesions					Total
		0	Tr	2	3	4	*n*
Art	pH 7.0	24	3	0	0	0	27
Phys	pH 7.0	25	2	0	0	0	27
EDTA	pH 8.5	0	0	0	2	25[a]	27
R	pH 4.0	0	0	18[ab]	9[ab]	0	27
S1	pH 4.0	0	0	14[abc]	13[abc]	0	27
S2	pH 3.9	0	0	12[abc]	15[abc]	0	27

[a] $P < 0.01$ vs Art, Phys; [b] $P < 0.05$ vs EDTA; [c] $P > 0.05$ vs R

Table 3 Score of bladder lesions after perfusion for 72 h with different solutions

Solution		Score of bladder lesions					Total
		0	Tr	2	3	4	*n*
Art	pH 7.0	20	7	0	0	0	27
Phys	pH 7.0	21	6	0	0	0	27
EDTA	pH 8.5	0	0	0	0	27[a]	27
R	pH 4.0	0	0	6[ab]	21[ab]		27
S1	pH 4.0	0	0	6[abc]	21[abc]		27
S2	pH 3.9	0	0	7[abc]	20[abc]		27

[a] $P < 0.01$ vs Art, Phys, [b] $P < 0.05$ vs EDTA, [c] $P > 0.05$ vs R

Disodium-EDTA binds calcium in a water-soluble complex, and even the most insoluble calcium calculi can be brought into solution. However, it produces unacceptable tissue damage when used as an urothelial irrigant. The most useful solution used to dissolve phosphate calculi is Renacidin, a buffer solution of citrate-citric acid complex which creates an acidic environment essential for dissolving. Reckler et al. [6] reported that all of the pH 4.0 solutions which they tested produced significant injury to the bladder mucosa of the rabbit. However, Kane et al. reported that it is the chelating property and not the inherent structure of the chemical or pH of the solution which is injurious to the mucosa [8]. It is possible that any solution which can effectively extract calcium from the tight crystal lattice of a calcium oxalate stone will also remove calcium or other important cations from the cell membrane, causing urothelial disruption. The addition of magnesium to the R irrigating solution reduces the damage to rabbit bladder mucosa, but this will obviously reduce the speed of dissolution of struvite stones [1, 2]. However, a contradictory conclusion was made by a Dutch study [10] which showed that the magnesium in R may promote stone dissolution by cation exchange with calcium in apatite [11].

Our results show that irrigation with 0.03 M disodium-EDTA at pH 8.5 is severely toxic to rabbit bladder mucosa, as reported by Kane et al. [8]. R, S1 and S2 are also chelating compounds which dissolve phosphate calculi effectively [72 h mean dissolution rate: 5.75 ± 0.44 mg/h (S1), 5.2 ± 0.63 mg/h (S2), 4.55 ± 0.46 mg/h (R)]. They cause less toxicity than EDTA to rabbit bladder mucosa. The injury caused by R is slightly more than S1 or S2, but this is not significant. Following prolonged irrigation, all of these solutions caused further urothelial damage, but EDTA caused the most, followed by R, S1, S2, Phys or Art, respectively, at 48 and 72 h. There was no observable damage to urothelial tissue produced by irrigation with Phys or Art.

Although S1 and S2 cause less damage than R, we were not satisfied with our result but could not reduce the damage further by pH regulation or adding cations to these solutions. It is possible that an alteration in our ingredients will have an effect, but this needs further study. Our animals were irrigated antegrade via an ureterostomy and allowed to void spontaneously. The irrigating solution was therefore in contact with the bladder mucosa more than in other models [12, 13], in which the bladder is irrigated and drained via a two-way urethral catheter. We also irrigated for more than 24 h, while other studies only covered 6 h. Part of the reason why our animals showed more severe mucosal injury could, therefore, be due to differences in contact time or continuity, or to a higher concentration of R. However, we believe that our model is more accurate because it eliminates mechanical artifacts in the bladder from either the catheter tip or the irrigation stream [14].

We can try to minimize the mucosal injury produced by S1and S2 by other approaches such as supplementing R with magnesium. Until some way of protecting the mucosa of the urinary tract is found, our studies suggest that S1and S2 would cause urothelial injury to the same extent as R if used clinically. However, in view of the better solubility effect of solutions S1 and S2 compared with R, it might be justified in accepting the more pronounced urothelial irritation caused by these solutions.

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