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Toxicity of litholytic ethylenediaminetetraacetic acid solutions to the urothelium of the rat and dog*

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Summary. The toxicity to the urothelium of bipotassium ethylene-diaminetetraacetic acid (K_2-EDTA) buffered with 0.2 *M* triethanolamine (TEA) at pH 8 and 8.5 was tested in rats and dogs. Even at a low concentration of 3.125 m*M*, K_2-EDTA is very noxious to the bladder mucosa. This toxicity is not due to the buffer TEA, which is well tolerated. Although buffered K_2-EDTA, at pH 8.5 is an excellent chemolytic agent for calcium-containing stones, its clinical use is limited by this toxicity.

Key words: Renal lithiasis – Chemolysis – Ethylenediaminetetraacetic acid (EDTA)

Since the appearance of kidney stone lithotripsy, the idea of chemolysis has received renewed attention. Indeed, the surface of kidney stone increases drastically after stone disintegration, enabling its more effective dissolution by chemolytic solutions. In previous studies, we found buffered ethylenediaminetetraacetic acid solutions (EDTA) to be among the most effective agents in dissolving calcium-containing calculi [5, 6]. The aim of the present study was to evaluate the local toxicity of these solutions to the urothelium of the rat and dog.

Materials and methods

Rat experiments

Female Wistar rats weighing 180–240 g were used. They were anaesthetized by a single intraperitoneal injection (0.5 ml/100 g body weight) of a 30% urethane solution in water, which provokes a rapid and long-lasting anaesthesia for up to 24 h. The bladder was exposed by a short suprapubic midline incision. The dome of the bladder was grasped and opened with a short transverse incision, and a double-loop polyethylene catheter was introduced. The end of the inflow tube has an enlarged brim and is thus easily retained in the bladder by the suture through the bladder dome around the entrance of the tubes. The opening of the outflow catheter was placed 1 cm lower in the bladder and fixed together with the inflow catheter outside the bladder using tape.

A perfusion pump provided a continuous flow of 1.4 ml/h. During perfusion the rats were kept warm on an electric cushion and the abdomen was held closed with a bulldog clamp. At the end of the perfusion an overdose of urethane was injected intraperitoneally and the animal was killed by cutting the aorta. The bladder was completely excised, fixed in 10% formalin and routinely embedded in paraffin. Sections (5 µm) were cut and stained with H&E for light microscopy; at least six sections from each bladder were examined. Damage to the urothelium was classified according to scores from 1 to 5 as follows:

1. Normal urothelium and submucosal layers
2. Absence of, damage to, or erosion of $<20\%$ of the bladder mucosa
3. Absence of, damage to, or erosion of 20%–50%
4. Absence of, damage to, or erosion of 50%–80%
5. Absence of or damage to $>80\%$ of the urothelium

The quantity of epithelial lesions was established at two different levels of transversally cut bladders in two histological sections per bladder. The mean of these four determinations represented the grade of change. The investigator carrying out the histological examination was not informed as to which perfusion solution had been used.

Dog experiments

Five street dogs weighing 22–35 kg that had undergone a unilateral nephrectomy in another experiment were used. The blind-ending ureter was catheterized and connected with a 21 drip infusion of 125 m*M* K_2-EDTA + 0.2 *M* TEA (pH 8.5) for a period of 6 h. The dogs were killed at the end of the experiment with an overdose of $MgSO_4$. The ureter and bladder were excised and further worked up as in the rat experiments.

Solutions

The following solutions were used in all experiments:

1. Physiologic solution of 0.9% NaCl in water.
2. Multi-ionic physiologic solutions consisting of 84.8 m*M* Na^+, 10 m*M* K^+, 30 m*M* CA^{2+}, 1.5 m*M* Mg^{2+} and 157.8 m*M* Cl^-. This solution was prepared using analytical-grade NaCl, KCl, $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$.
3. As a buffer, 0.39 *M* (TEA, pH 8) was prepared with triethenolamine (Merck 8379) by adding HCl to obtain a pH of 8.
4. K_2-EDTA − 0.2 *M* TEA at pH 8.5, with concentrations of K_2-EDTA ranging from 3 to 125 m*M*, was prepared with $K_2 \cdot 2H_2O$ (Fluba 03660). After dissolution of the EDTA salt and addition of 0.2 *M* TEA, the pH was adjusted with HCl.
5. The same as solution 4, but at pH 8.
6. Suby solution, consisting of 32.3 g citric acid, 3.9 g anhydous magnesium oxide, 4.3 g anhydrous sodium carbonate and 1 l H_2O at pH 4.

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Table 1. Score of bladder lesions in rats after bladder perfusion, as a function of the composition of the solution and of perfusion time, and statistical comparison of the different solutions with 9% NaCl and Suby solution

Solution	Perfusion time (h)	Score of bladder lesions					Total number	*P* value when compared with	
		1	2	3	4	5		9% NaCl	Suby
9% NaCl in water	6	2	23	9	2	0	36		
	24	3	7	2	0	1	13		
Multi-ion physiologic solution	9	1	9	5	0	0	15	*	–
Buffer: Triethanolamine 0.39 *M*, pH 8	6	2	14	8	0	0	24	*	–
Suby, pH 4	6	0	8	8	4	0	20	*	–
K_2-EDTA 3.15 m*M* + TEA 0.2 *M*, pH 8.5	6	0	2	14	7	1	24	***	*
K_2-EDTA 6.25 m*M* + TEA 0.2 *M*, pH 8	6	0	1	4	13	6	24	***	***
K_2-EDTA 6.25 m*M* + TEA 0.2 *M*, pH 8.5	6	0	2	6	11	4	23	***	**
K_2-EDTA 12.5 m*M* + TEA 0.2 *M*, pH 8	6	2	5	7	7	1	22	**	*
K_2-EDTA 12.5 m*M* + TEA 0.2 *M*, pH 8.5	6	3	3	8	9	1	24	***	*
K_2-EDTA 12.5 m*M* + TEA 0.2 *M*, pH 8.5	24	0	0	0	2	13	15	***	–
K_2-EDTA 12.5 m*M* + TEA 0.2 *M*, pH 8	24	0	1	0	5	6	12	***	–
K_2-EDTA 25 m*M* + TEA 0.2 *M*, pH 8	6	0	2	6	9	7	24	***	***
K_2-EDTA 25 m*M* + TEA 0.2 *M*, pH 8.5	6	0	1	8	20	4	33	***	***
K_2-EDTA 12.5 m*M* + TEA 0.2 *M*, pH 8.5	1½	4	4	6	2	0	16	–	–
K_2-EDTA 50 m*M* + TEA 0.2 *M*, pH 8.5	6	0	2	7	10	6	25	***	***
K_2-EDTA 100 m*M* + TEA 0.2 *M*, pH 8.5	6	0	1	0	5	6	12[a]	***	***

[a] 12 animals died during the experiment
Statistical comparison: * $P \geq 0.05$; ** $0.01 < P < 0.05$, statistically significant; *** $P < 0.01$, highly significant

Statistical analysis of the results

The effect of the composition of the perfusion solution on the incidence of lesions according to the score of damage was evaluated on the basis of a 2×5 contingency table using the exact Fischer's test [1]. All solutions were individually compared with physiologic solution and Suby solution.

Results

The number of rat bladders with urothelial lesions (score, 1–5) following perfusion using different solutions and conditions are listed in Tables 1 and 2. Lesions found in the ureter and bladder of the five dogs were similar to those observed in rats. A score of 4 was established in three ureters and two bladders.

In six rats that received a 6-h bladder perfusion with 12.5 m*M* K_2-EDTA + 0.2 *M* TEA at pH 8.5 serum levels of Ca^{2+} were measured at the end of the experiment, revealing values of 3.12, 3.31, 3.54, 3.91, 4.14 and 4.20 mEq/l. This clearly indicates a tendency toward hypocalcemia after 6 h perfusion with the chemolytic solution, as normal calcium levels measured in our laboratory lie above 4.50 mEq/l.

The K_2-EDTA solution induced fewer urothelial lesions when used at a concentration of 12.5 m*M* (Table 3) and produced better results as compared with Suby solution. No differences could be detected at pH 8 vs pH 8.5. Nevertheless, the lesions that were induced by this

Table 2. Bladder lesions in rats following perfusion with K_2-EDTA buffered with 0.2 *M* TEA under at pH 8.5 under different perfusion conditions

Perfusion solution	Perfusion conditions	Score of bladder lesions					Total number	*P* value when compared with 6 h normal perfusion
		1	2	3	4	5		
K_2-EDTA 25 m*M* + TEA 0.2 *M*, pH 8.5	6 h at 0.37 ml/h = 1/4 of the normal flow rate	0	1	6	5	0	12	*
K_2-EDTA 12.5 m*M* + TEA 0.2 *M*, pH 8.5	1 h, alternating 6× with multi-ionic physiologic solution for 0.5 h. Total duration, 9 h	0	2	11	11	2	26	*
K_2-EDTA 25 m*M* + TEA 0.2 *M*, pH 8.5	0.5 h, alternating 12× with multi-ionic physiologic solution for 1 h. Total duration, 18 h	0	0	2	16	3	21	*

Statistical comparison: * $P > 0.05$; ** $0.01 < P < 0.05$, statistically significant; *** $P < 0.01$, highly significant

Table 3. Cross-comparison of different concentrations of K_2-EDTA solution buffered with 0.2 *M* TEA at pH 8 and 8.5

K_2-EDTA + 0.2 *M* TEA		3.125 m*M*	6.25 m*M*		12.5 m*M*		25 m*M*		50 m*M*	100 m*M*
		8.5	8.0	8.5	8.0	8.5	8.0	8.5	8.5	8.5
3.125 m*M*	8.5	–	**	*	*	*	**	**	*	***
6.25 m*M*	8.0	**	–	*	**	**	*	*	*	*
	8.5	*	*	–	*	*	*	*	*	*
12.5 m*M*	8.0	*	**	*	–	*	*	**	*	***
	8.5	*	**	*	*	–	*	*	*	***
25 m*M*	8.0	**	*	*	*	*	–	*	*	*
	8.5	**	*	*	**	*	*	–	*	**
50 m*M*	8.5	*	*	*	*	*	*	*	–	*
100 m*M*	8.5	***	*	*	***	***	*	**	*	–

Statistical comparison: * $P \geqslant 0.05$; ** $0.01 < P < 0.05$, statistically significant; *** $P < 0.01$, highly significant

solution were severe and their incidence was unacceptably high after 24 h perfusion.

Discussion

When these animal experiments were started, no serious local toxicity was expected. Indeed, EDTA solutions have long been used clinically by other investigators [4], and obvious side effects have never been mentioned. The concentrations of EDTA used by Timmerman and Kallistratos in their vast experience with chemolysis in man [4] were even higher than those used in the present study. The main difference between the solutions tested by these authors and those evaluated in the present study involved the addition of the buffer TEA and the high pH in the latter experiments. Our rat experiments were started with 100 m*M* K_2-EDTA + 0.2 *M* TEA at pH 8.5. Half of the animals died within 6 h and the bladder lesions were pronounced.

At first, the buffer TEA was suspected to be responsible for this noxious effect; consequently, solutions containing only TEA were tested. However, these solutions induced only slight lesions comparable with those produced by physiologic solutions. The latter are generally caused by nonsterile surgical procedure as well as by the presence of tubes inside the bladder for a long period.

Progressively lower concentrations of K_2-EDTA were tested, but even very low concentrations such as 3.125 m*M* resulted in severe lesions in the bladder mucosa. At this concentration, the chemolytic efficiency of the solution becomes questionable; therefore, we did not test lower concentrations.

Next, the pH of the solution was lowered to 8 but, again, no improvement could be observed. Lower pH values were not studied, as previous in vitro experiments [5, 6] have clearly demonstrated that the chemolytic capacity of the solution drastically decreases below a pH of 8. It is also essential that the solution be buffered so as to keep the pH above 8. In fact, both admixing with acidic urine and the increase in proton concentration caused by the chemolytic process itself rapidly decrease the pH of unbuffered solution.

To improve the tolerance by the urothelium of the EDTA solutions, in one experiment we diminished the flow rate to one-fourth of that normally used for perfusion, which amounts to 1.4 ml/h. The capacity of a rat bladder lies between 0.5 and 1 ml. Translated into human values, the normal perfusion rate used in our experiments corresponds to approximately 500 ml/h. Reducing the flow rate had no influence on the severity of the lesions. The rapid appearance of lesions was astonishing; severe lesions were noted as early as 1.5 h following perfusion in 8 of 16 rats.

In an attempt to obtain the fastest possible recovery of the bladder mucosa, we decided to determine whether alternating regimens of K_2-EDTA solution vs a physiologic multi-ion solution could reduce the severity of the bladder lesions. Two regimens were studied. The first consisted of six cycles of 1 h perfusion with K_2-EDTA followed by a 0.5-h perfusion with the multi-ion solution, and the second comprised six cycles of 0.5 h perfusion with K_2-EDTA followed by 1 h perfusion with the physiologic solution. In the cases the urothelial lesions induced were as severe as those found after a continuous 6-h perfusion with K_2-EDTA.

The slight to severe hypocalcemia that was noted in rats after 6 h bladder perfusion suggests a possible exchange of Ca^{2+} between the body fluids and the chelator. This exchange is enchanced after partial or total destruction of the urothelium. On this basis, a solution with a lower chelating capacity would be expected to be less harmful. For this reason, the toxicity of the K_2-EDTA solution was compared with that of a well-known and generally accepted litholytic agent, Suby solution. Although the latter is inefficacious for the dissolution of calcium oxalate, it can apparently be successfully used for calcium phosphate stones [3]. Although Suby solution provokes significantly less damage than K_2-EDTA, it is certainly not harmless, as it induced severe lesions in several rats. Nevertheless, the difference between Suby solution and physiologic solution was found to be statistically insignificant. A larger number of experiments could possibly reveal a difference.

Among the different K_2-EDTA concentrations tested, that of 12.5 m*M* was found to be the least toxic; this effect was independent of the pH. Although there is no logical explanation for this phenomenon at first sight, it may be caused by the broad variations that are often noted in animal studies. Insufficient irrigation of some part of the bladder by a two-way catheter or a lesion induced by other factors (distention, mechanical irritation) could also be responsible for the severity of some bladder lesions. Nevertheless, it is obvious that concentrations of 100 m*M* K_2-EDTA are much too high, as they not only caused extremely severe lesions but also resulted in 50% mortality.

After the rat experiments, an extension of the study to other animals seemed necessary. Although restricted in number, our canine experiments clearly indicate that severe lesions also occur in the perfused ureter. The lesions were less pronounced in the bladder because the solution was mixed with urine coming from the other kidney. It is also noteworthy that dogs as well as rats started urinating as soon as the bladder mucosa came into contact with the K_2-EDTA solution. Pronounced pollakiuria was observed throughout the study period.

Finally, the solution comprising 12.5 m*M* K_2-EDTA + 0.2 *M* TEA at pH 8.5 was tested in two patients. The drug was given through a nephrostomy tube so as to dissolve ureteral stones in both cases. Administration was continued until patients complained of pain. Symptoms of bladder irritation appeared after 30 min and continued for up to 3 h during perfusion. Treatment of the patients could be switched to a physiologic solution until the bladder irritation had improved, at which time the administration of chemolytic solution could be restarted. Bladder pain reappeared quite rapidly; thus, the K_2-EDTA solution could maximally be given over 5 of the 24 h, during which time only 1 l chemolytic solution was perfused through the kidney. Chemolysis had to be discontioned due to bladder irritation after 2 days and 1 day, respectively. In a recent study using K_2-EDTA at pH 7.5 in rabbit bladders, Kane et al. [2] reached the same conclusion.

Conclusion

Although K_2-EDTA solutions buffered with 0.2 *M* TEA at pH 8.5 and 8 are ideal chemolytic solutions for calcium-containing stones from the chemical point of view, they are unsuitable for clinical use because they induce severe urothelial lesions in the rat and the dog. These lesions appear rapidly, as they could be demonstrated as early as 1 h following perfusion. The number and severity of the lesions is independent of the K_2-EDTA concentration and of the flow rate. Regimens alternating K_2-EDTA solutions with physiologic solutions are as noxious as a continous perfusions. The toxicity was not attributable to the buffer TEA.

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