Urol Res (1992) 20:19–21

Urological Research

Rationale for local toxicity of calcium chelators*

W. Oosterlinck[1], R. Verbeeck[2], C. Cuvelier[3], and D. Vergauwe[2]**

Departments of [1]Urology, [2]Analytical Chemistry and [3]Pathology, State University of Ghent, Ghent Belgium

Accepted: June 1, 1991

Summary. Calcium chelating agents, such as ethylenediaminetetraacetate are toxic to urothelium. Their capacity to form complexes with calcium ions, which is the basis for their chemolytical effectiveness, also determines their toxicity. A decrease of chemolytical effectiveness by prior saturation of the chelator with Ca^{2+} or by lowering the pH to levels unfavourable for calcium binding significantly diminishes tissue injury. Exchange of Mg^{2+} ions does not, however, diminish tissue damage. The clinical use of calcium ligands is therefore unsafe.

Key words: Calcium oxalate – Renal lithiasis – Chemolysis – Ethylenediaminetetraacetate

We have recently demonstrated [5] that dipotassium ethylenediaminetetraacetate (K_2EDTA) buffered with triethanolamine (TEA) at pH 8 or 8.5 induced severe lesions to urothelium of the rat and dog. Although K_2EDTA has excellent chemolytic properties in vitro for calcium-containing stones [9, 10], it cannot be used clinically. Even a low concentration of 3.125 mM, K_2EDTA produces severe lesions of the bladder mucosa. Slow perfusion rates or alternating regimens of K_2EDTA with a physiological solution do not diminish toxicity to an acceptable degree.

The data of Kane et al. [3], although based on a very limited number of rabbits, suggested that the disodium salt of EDTA at pH 7.5 was harmful. In another study [6], it was found that other calcium chelators were not tolerated by rat bladder mucosa, irrespectively of the salt (Li, Na, K or Cs) used. Contrary to other reports [2, 4, 8], these studies apparently suggest that the calcium binding capacity of the chelator itself is responsible for damage to bladder mucosa. Therefore, the clinical use of calcium chelating agents as urothelial irrigants would generally be limited by their local toxicity.

In the present study, the effect of calcium binding capacity on urothelial injury was investigated, and experiments were performed to help reduce such injury by changing the composition of the chemolytic solution.

Materials and methods

The calcium binding capacity of a chemolytic solution can be varied in several ways [9, 10]. By using a weak chelator such as citric acid or EDTA solutions at low pH, the binding of Ca^{2+} is strongly reduced. In addition, chelation of calcium is virtually zero when the calcium salt of the chelator is used. Although solutions based on such calcium salts are useless as chemolytic agents, a comparison of the effect on bladder mucosa by calcium salts of different chelators then enables a control of the toxicity due to the chelator molecule itself.

Finally two chelators, ethyleneglycol-bis (2 aminoethyl)-tetraacetic acid (EGTA) and hydroxyethylenediaminetetraacetic acid (HEDTA) were tested because they have the capacity to exchange Mg^{2+} ions. It was hoped that exchanging a Ca^{2+} for a Mg^{2+} ion at the level of the urothelium could reduce destruction of the urothelial membrane.

Experiments in rats

The experimental protocol was performed as previously described [5, 6]. Briefly, the rat bladder was perfused with a two-way catheter with different solutions for 6 h at 1.4 ml/h. The bladder was then excised for histological examination. The lesions were scored semi-quantitatively from 1 (no lesions) to 5 (complete destruction of the urothelium with strong inflammation in the submucosal layers).

Solutions

The citrate solutions contained 100 mM citric acid (Merck 244; Darmstadt, FRG), 10.90 mM Na^+, 8.40 mM K^+ and 147.4 mM Cl^-. The pH was adjusted with TEA (Merck 8379). The TEA concentration was 0.128 M for the pH 4 solution and 0.446 M for the pH 7 solution.

* This work was supported by the Fund for Medical Scientific Research (FGWO) Belgium
** Research Associate of the NFSR Belgium

Table 1. Score of bladder lesions after perfusion of the bladder with different solutions

Solution	pH	Score of bladder lesions					*n*	Comparison to NaCl 0.9%
		1	2	3	4	5		
NaCl 0.9% in water	7.5	2	23	9	2	0	36	0
Citric acid 100 mM + TEA	4.5	0	3	6	3	0	12	+
	7.5	0	2	4	5	1	12	++
K_2EDTA 12.5 mM	4.5	6	6	1	0	0	13	0
	8.5	9	6	5	4	0	24	0
K_2EDTA 12.5 mM + TEA 0.2 M[a]	8.0	2	5	7	7	1	22	+
	8.5	3	3	8	9	1	24	++
Na_2EDTA 12.5 mM	5.0	0	2	6	5	0	13	++
	6.1	1	3	12	6	1	23	++
	7.0	0	1	8	4	0	13	++
Na_2EDTA 12.5 mM + TEA 0.2 M[a]	8.0	2	5	7	7	1	22	+
	8.5	3	3	8	9	1	24	++
CaEDTA 50 mM	5.6	3	8	1	0	0	12	0
CaEDTA 50 mM + TEA 0.2 M	8.5	1	8	2	1	0	12	0
K_2EGTA 12.5 mM + TEA 0.2 M	8.0	0	0	4	7	1	12	++
	8.5	0	0	2	8	2	12	++
CaEGTA 50 mM + TEA 0.2 M	7.9	1	6	14	4	0	25	+
K_2DTPA 12.5 mM + TEA 0.2 M[a]	8.5	0	0	5	4	3	12	++
CaDTPA 50 mM + TEA 0.2 M	8.5	1	12	9	1	0	22	0
MgEGTA 50 mM + TEA 0.2 M	8.0	0	0	3	4	1	8	++
	8.5	0	1	3	5	3	12	++
MgHEDTA 25 mM + TEA 0.2 M	8.5	0	0	7	18	0	25	++
50 mM + TEA 0.2 M	8.2	0	2	12	10	0	24	++
100 mM + TEA 0.2 M	8.0	0	7	12	6	0	25	++
200 mM + TEA 0.2 M	8.0	0	0	9	17	0	26	++

Statistical comparison of the solutions with NaCl 0.9%: 0, no significant difference $P < 0.05$; +, statistical significant difference $0.01 < P < 0.05$; ++, highly significant difference $P < 0.01$

[a] Data taken from [5] and [6]

The calcium and magnesium salts of EDTA, diethylene triaminepentaacetatic (DTPA), HEDTA and EGTA were prepared by mixing $CaCO_3$ (Carlo Erba 433187, Milan, Italy) or $MgCO_3$ (Merck 5827) with EDTA (Kestranal, UCB 1411, Leuven, Belgium), DTPA (Titriplex V, Merck 8426), HEDTA (Janssen 12.117.89, Beerse, Belgium) or EGTA (Fluka 03780, Bucks, Switzerland) in a small amount of water. The molar ratio of carbonate to acid was 1:1. When the formation of carbon dioxide stopped, more water and TEA were added. The pH was adjusted to pH $8 \leq$ pH ≤ 8.5 with NaOH or HCl when necessary.

The Na_2- and K_2 EDTA solutions were prepared with $Na_2EDTA.2H_2O$ (Titriplex III, Merck 8418) and $K_2EDTA.2H_2O$ (Fluka 03660) respectively. After dissolution of the EDTA salt, the pH was adjusted with HCl or KOH. To the buffered solution, 0.2 M TEA was added and the pH adjusted with HCl.

Statistical analysis of the results

The effect of the solution composition on the frequency of lesion classes was evaluated on the basis of a 2×5 contingency table using Fisher's exact probability test. All solutions were compared individually with the physiological solution.

Results

The scores of the bladder lesions caused by the different solutions together with the results of the statistical comparison of the global injury with respect to that caused by the physiological solution are summarized in Table 1.

In addition, the effects of the Na_2-, K_2- and CaEDTA solutions buffered with 0.2 M TEA at pH 8 or 8.5 were compared with those caused by their unbuffered solutions. The differences were highly significant ($P < 0.01$) for K_2EDTA at pH 4.5 or 8.5 and for CaEDTA, but no significant differences ($P > 0.05$) were found between the Na EDTA solutions. Whereas the 50 mM CaEDTA and CaDTPA were not significantly different from the physiological solution, the 50 mM CaEGTA appeared to be more toxic. However, a comparison with the K_2EGTA solutions showed that CaEGTA was significantly less toxic ($P < 0.01$).

Discussion

Even a weak chelator such as citrate produced relatively severe damage to the urothelium of the rat [7]. This toxic effect was more pronounced at pH 7.5; this might be due to a slight increase in the affinity for Ca^{2+} at this pH [9].

The addition of the buffer TEA, which also reduces the surface tension, may play a role in enhancing the contact between urothelium and the calcium ligand, thus increasing toxicity. In previous experiments our group has shown [5] that TEA alone is not toxic to the urothelium.

Other workers [9, 10] have demonstrated that the calcium binding capacity is strongly pH dependent. Very low activity can be expected at pH 4.5, while optimal activity of maximal complexation occurs at pH 8.5. This corresponds to the absence of toxicity of K_2EDTA at pH 4.5, but at pH 8.5 it was also found to be atoxic to the urothelium. On the other hand, we did not find a reduced toxicity for Na_2EDTA in unbuffered solutions, indicating that the exchange cation Na^+ or K^+ probably also plays a role in toxicity.

The prior saturation of the chelators EDTA and DTPA at 50 mM with Ca^{2+}, with or without addition of TEA, completely eliminated tissue injury. Fifty mM CaEGTA was not atoxic but did, however, produce less damage than a four times more diluted K_2EGTA solution. This suggests that the same calcium chelating property which makes calcium ligands chemically so effective also makes them toxic. This confirms the restricted observations made by Kane et al. [3].

As the Mg salts of EGTA and HEDTA are capable of exchanging Mg^+ for Ca^{2+}, they have some binding capacity for Ca^{2+}. However, as this capacity is much lower than that of Na_2 or K_2 salts, higher concentrations must be used in order to obtain an acceptable therapeutic effect. Therefore, higher concentrations of the Mg salt of EGTA and HEDTA were tested. However, decreasing concentrations of Mg HEDTA down to 25 mM did considerable harm to the urothelium (Table 1). Lower concentrations of this chelator were ineffective from the chemical point of view.

It is astonishing that EDTA and other calcium ligands have been used in the past without major animal experimental work to assess their local toxicity, Indeed, Timmerman and Kallistratos [8] have used EDTA solutions at concentrations of 5–7% extensively in a clinical setting without reporting major side-effects. Gehres and Raymond [2] reported that a 6% EDTA solution showed moderate inflammatory changes in the bladder of rabbits and concluded that the optimum concentration appeared to be 1.5%. The data from this older literature were often very inaccurate, which makes them difficult to repeat. Kuwahara et al. [4] observed that bladder urothelium of rat was well preserved by 5% Na_4EDTA. However, they only examined four bladders.

From our experiments it is clear that there is no place for the clinical application of calcium chelators such as EDTA and similar products. Evidence that local toxicity is due to the strong calcium binding capacity of chelators is supported by the following facts:

1. The calcium ligands can become less toxic when their calcium binding capacity is decreased unfavourable pH.
2. Several calcium salts of chelators are less toxic or atoxic.
3. Exchanging Ca^{2+} ions for Mg^{2+} ions does not diminish the tissue damage.

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Dr. W. Oosterlinck
Department of Urology
University Hospital
De Pintelaan 185
B-9000 Gent
Belgium