DESORPTION OF CALCIUM FROM THE CORNEA BY MEANS OF CHELATING AGENTS

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UDC 617.713-008.924.1-008.61-085.272.2

KEY WORDS: cornea; calcium; sorption; chelating agent.

An excess of bivalent ions, especially calcium ions, in the tissues of the cornea is often accompanied by damage to the organ of vision. This type of pathology of the cornea is a feature of band keratitis, burns of the eyes with slaked and unslaked lime, calcium carbide, and so on [5]. These lesions account for up to 20% of the total number of cases of trauma to the eye. The calcium cation, unlike other cations, has special affinity for corneal proteins, and for that reason the harmful action of lime, for example, is not confined to alkaline solubilization of proteins. The calcium ion causes additional injury to the tissue by interacting with corneal proteins to form Ca-albuminates or Ca-collagenates. Under these circumstances foci of calcification appear in the cornea [4]. Attempts to lower the calcium ion concentration in the cornea have been made by a number of workers [1, 2, 6], but the mechanism of sorption and desorption of calcium, their dynamics, their quantitative characteristics, and the effectiveness of use of various chelating agents have not been studied. In the investigation described below an attempt was made to remedy this omission.

EXPERIMENTAL METHOD

Experiments were carried out on healthy adult Chinchilla rabbits. After decapitation of the animals the eyes or the isolated corneas were used. The tissues were dissected in the cold. The isolated corneas were placed in 0.01 M $CaCl_2$ solution containing the radioactive isotope ^{45}Ca. The incubation time varied. After incubation, ^{45}Ca activity in the cornea was measured. The tissue was then placed in solutions containing 0.01 M concentrations of chelating agents (EDTA — the disodium salt of ethylenediamine tetraacetic acid, and DTPA — the trisodium salt of diethylenetriaminopentaacetic acid) for different times. Periodically samples were taken from these solutions, in which ^{45}Ca activity was determined, just as also in the tissue itself. In parallel experiments on the isolated eyes of the animals the outflow of ^{45}Ca from the corneas in response to the combined action of the chelating agent and papain was investigated. In this series of experiments the possibility of elution of the ^{45}Ca through the lateral section in the cornea was ruled out.

Radioactivity was measured on an Intertechnique (France) liquid scintillation counter, in the form of β-radiation, in Bray's standard scintillation fluid [3]. For this purpose, 0.5 ml of the solution to be measured was added to 5 ml of the fluid, or pieces of cornea of equal weight were dropped into it.

EXPERIMENTAL RESULTS

In the experiments of series I saturation of the cornea with Ca^{++} ions was investigated as the simplest experimental model of Ca-dependent keratopathy. Incubation lasted 1.5-2 h until equilibrium for interaction between Ca^{++} ions and the cornea was reached.

The experimental results were expressed in millimoles Ca bound by 1 g of cornea. As Fig. 1 shows, the normal cornea actively absorbs Ca^{++} ions; some opacity of the tissue was observed under these circumstances, and the higher the calcium content in the cornea, the more opaque it became. The degree of opacity was determined visually according to the scale: transparent (T), slightly opaque (SO), and opaque (O). Opacity of the tissue was not observed in the presence of Ca^{++} in concentrations a little above the physiological norm. The lower limit of the norm, as our observations showed, was a calcium concentration of about 20-30 μeq/liter, at which about 0.04 nmole Ca^{++} is adsorbed by 1 g cornea.

N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 9, pp. 299-301, September, 1981. Original article submitted September 15, 1980.

0007-4888/81/9209-1191$07.50

TABLE 1. Desorption of ^{45}Ca from Cornea by EDTA and DTPA Solutions

Weight of tissue, mg	Desorbent	Residual activity of cornea, counts/100 sec/g tissue	Quantity removed, %	Degree of opacity of tissue
	EDTA:			
101,8	0,01 M	197	94,0	SO
93,7	0,005 M	160	95,1	SO
102,9	0,0035 M	233	93,0	O
	DTPA:			
131,7	0,01 M	144	96,6	T
121,0	0,005 M	231	93,0	T
130,3	0,0035 M	228	93,1	T
103,2	0,85% NaCl	468	85,8	SO

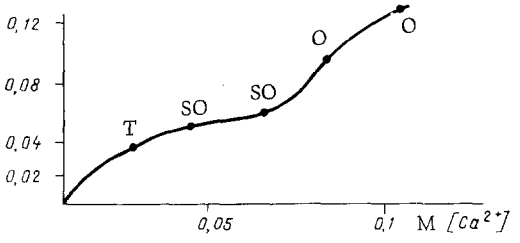

Fig. 1

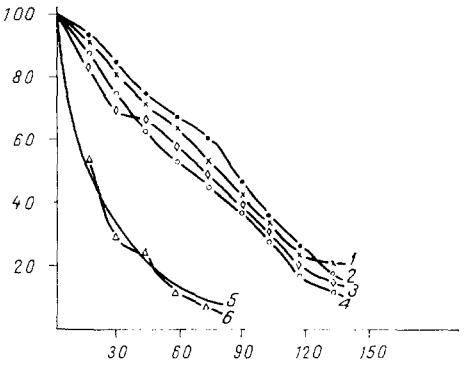

Fig. 2

Fig. 1. Absorption isotherm for Ca^{++} ions from the isolated cornea. Abscissa, concentration of $CaCl_2$ solution in which cornea was incubated, in M; ordinate, quantity of ^{45}Ca bound with 1 g of cornea (in mmoles).

Fig. 2. ^{45}Ca desorption curves from the isolated eye. 1) 0.01 M EDTA, 2) 0.005 M EDTA, 3) 0.01 M DTPA, 4) 0.005 M DTPA, 5) papain + 0.01 M EDTA, 6) papain + 0.01 M DTPA. Abscissa, time (in min); ordinate, desorption (in %).

In the experiments of series II the aim was to discover whether excess calcium could be removed from the cornea previously saturated with it. Tissue samples were placed in solutions of EDTA (0.01, 0.005, and 0.0035 M), DTPA (0.01, 0.005, and 0.0035 M), and in physiological saline (0.85% NaCl) at pH 7.4. The solutions with the corneas were exposed for 15 min with stirring. The results of these experiments showed that both chelating agents desorb calcium absorbed by the cornea sufficiently effectively; the effectiveness of action of the substances used, moreover, did not differ significantly (Table 1). Physiological saline, incidentally, also had some ability to "elute" Ca^{++} ions from the cornea. Removal of Ca^{++} ions from the cornea was accompanied by a decrease in its opacity. In the case of DTPA this effect was more marked, despite the fact that the character of desorption was evidently the same when both EDTA and DTPA were used. This can be explained by the specific effect of DTPA on processes taking place in the deep layers of the cornea, but these processes have not yet been studied in detail.

To rule out the possibility that calcium could have escaped through the lateral incisions in the cornea, experiments were carried out with isolated eyes and without removal of the corneas. For this purpose, 0.1 ml of a 0.01 M solution $^{45}CaCl_2$ was injected intravitally into the stroma of the cornea. The animals were decapitated and the eyes enucleated and placed in solutions of EDTA and DTPA. Solutions of the chelating agents were changed every 15 min. The dynamics of removal of ^{45}Ca from the isolated eye (Fig. 2) indicates that the chelating agents used are highly effective. Solutions of chelating agents, in the chosen concentrations, had about equal action on desorption of Ca^{++} ions from the isolated eye; op-

timal effectiveness of action of the preparations was observed after two or three changes of solutions. It is known that EDTA [6] and, probably, DTPA also, do not penetrate through the corneal epithelium, and for that reason reactions of complex formation between Ca^{++} and EDTA (or DTPA) proceed at the boundary solution — epithelium, and loss of reacting calcium from the cornea depends on its diffusion toward the solution from other layers of tissue. Because of the closeness of the chemical structure, molecular weight, and properties of the chelating agents there are no grounds for suggesting any different mechanism of action between DTPA and the epithelial tissue.

The dynamics of the process is thus limited by diffusion of calcium in the tissues and it takes place relatively slowly. To increase the velocity and effectiveness of the reaction, the necessity for which is dictated by the use of these preparations in clinical practice, the combined action of the chelating agents with papain was studied. Papain is known to loosen the epithelial membrane and also, evidently, the deeper membranes of the cornea, and this facilitates the processes of diffusion in general and of Ca^{++} ions in particular.

Moreover, the possibility cannot be ruled out that after the action of papain the chelating agents penetrate through the epithelial membrane into the depth of the cornea and, as a result, the whole process of complex formation was more effective.

The dynamics of calcium removal following the combined action of EDTA and papain (Fig. 2, 5) indicates that this combined treatment is more effective than the action of solutions of EDTA and DTPA alone.

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