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Effect of various eye drop carrier components on the fine structure of superficial corneal epithelial cells in rabbit eyes*

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Aim: *To study the effect of eye drops prepared with different preservatives and carrier fluid compositions on the fine structure of the superficial corneal epithelial cells in rabbit eyes.*

Materials and methods: *The eyes of twelve pigmented rabbits were treated with eye drops composed of various conventionally used preservatives in isotonic saline or with solutions which isotonic glycerine was added to twice a day for 3 months. Three animals which received no eye drops served as controls. At the end of the treatment period the animals were killed and samples were taken from the central part of their corneas which were examined using scanning electron microscopy.*

Results: *Chronic treatment using eye drops containing conventional preservatives in isotonic saline increased the percentage of damaged epithelial cells and decreased the average area of the epithelial cells. On the other hand, the corneas treated with solutions with added isotonic glycerine did not show significant difference to the controls in the percentage of pathological cells, and the average area of the epithelial cells was not smaller than in the controls.*

Conclusion: *Replacement of the isotonic saline with isotonic glycerine prevents the epithelial cell damage caused by the preservatives in saline-based solutions.*

Key words: *superficial epithelial cells of the rabbit cornea, damaging effects of the various vehicles of eye drops, scanning electron microscopy.*

The damaging effect of preservatives in eye drops solutions is a well known symptom. One of the measurement methods of the damaging effect is the morphology examination with scanning electron microscopy[12], another method is the examination of the effect of the preservatives on the permeability of the epidermis[3].

Damage to the epithelial cells has subclinical inflammation of the surface of the eye ball and the so called “dry eye” condition as a final result.[3]

The toxicity of preservatives was dealt with in a series of publications in the decades past, however in the recent years the results of the latest methods are mainly discussed.[1,2,7,8,9,10,14,15]

* in honour of Ildikó Süveges university professor

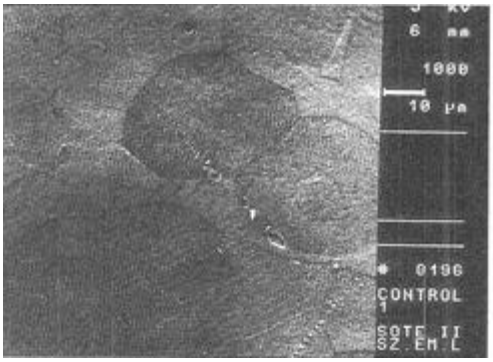

Figure 1 *Electron microscopic image of the centre of the cornea of the untreated (control) animal*

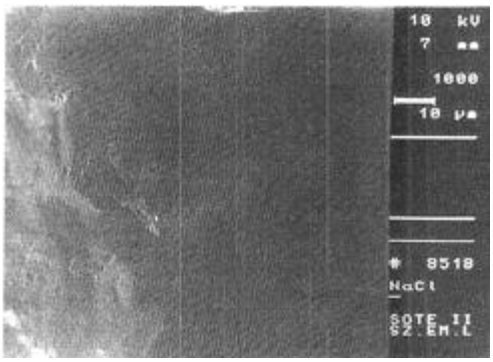

Figure 2 *Surface of the cornea treated with isotonic NaCl 0.9% solution*

In this study the damaging effect of different preservatives on the superficial epithelial cells of the centre of the cornea of the rabbits were examined. Three-three rabbits were treated by instillation of the individual vehicle solutions twice a day for 3 months, the kinds of the vehicles applied on the two eyes were different. Three animals were not treated, their corneas served as control. After treatment the animals were killed and the ratio of the sound and pathological cells found as well as the area of these cells were determined with electron microscope.

Materials and methods

Experiment 1: Vaccinated pigmented rabbits of the same age, originating from the same strain were used in the experiment, their average body weight was 1 kg. One of the materials was instilled into one of the eyes of the animals in group of three twice a day for 3 months (e.g. material 2) and the other material (e.g. material 3) was instilled into their other eyes, consequently 3 eyes received the same treatment (*Table 1*). Six eyes of 3 rabbits served as control. 15 rabbits in total were drawn into the experiment. The pH of each solution was adjusted to 7 by adding a few drops of sodium carbonate solution. After sterilization the solutions were stored in a refrigerator of +4 °C. The pH was measured at the end of the experiment as well and it was found not different from the initial value. The animals were killed by overnarcosis - by using Dormicum (midazolam) injection 5 mg/ml, Egis and Mildarine (saxamethonicum chloride) injection Br. Glaxo-Smith Kline.

Experiment 2: Before removal, 4% glutaraldehyde solution was instilled onto the corneas of the experimental animals in order to keep the structure of the corneas in the possible best condition. After taking out the eyeballs, the corneas were removed.

- Fixation: in glutaraldehyde 4% solution for 2 hours at +4 °C (composition of the fixing solution: 0.1 M Na-cacodylate buffer , glutaraldehyde 25%, 84 ml of 0.1 M Na-cacodylate, 16 ml of glutaraldehyde 25%).

- Washing: in 0.1 M Na-cacodylate buffer for 3x10 minutes.

- Last fixation: in OsO_4 1% fixing solution (made of OsO_4 5% solution diluted with 0.1M Na-cacodylate buffer to 1%) for 1 hour.

- Washing: in 0.1 M Na-cacodylate buffer for 3x10 minutes.

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Table 1: *Results of the examinations*

	Experimental composition	Area (μm^2)*	Damaged cells (%)	Result of the χ^2 probe as compared to the control
1	Untreated	590±16	16	
2	0.9% salt solution (isotonic)	542±10	28	p>0.01
3	0.01% benzalkonium chloride in isotonic salt solution	538±10	29	p>0.01
4	0.01% cetrimonium bromide in isotonic salt solution	591±16	27	p>0.01
5	0.1% ethylenediamine-tetraacetate (EDTA) in isotonic salt solution	531±21	15	p>0.5
6	2.5% (isotonic) glycerine	605±14	17	p>0.5
7	Material 3 in isotonic glycerine	699±18	14	p>0.5
8	Material 4 in isotonic glycerine	625±16	19	p>0.2
9	Material 5 in isotonic glycerine	616±19	17	p>0.5

* The t-probe ($p \leq 0.05$) shows that a) 2 differs from 1, b) 6 differs from 2 (but not from 1) c) 7 differs from 3, d) 9 differs from 5.

- Dehydration: in acetone 20% for 10 minutes, in acetone 40% for 10 minutes, in acetone 50% for 10 minutes, in acetone 70% for 10 minutes, in acetone 90% 3x10 minutes, in acetone 96% for 3x10 minutes, in absolute acetone for 3x10 minutes, in xylol for 1 day.

After draining off the xylol, the tissue parts were dried in an exsiccator. The preparation was stuck onto the Al-trunklets with Silver Print glue (GC Electronics). Following this, the surface was treated with carbon and then with gold. The examination was carried out with Zeiss DSM 940 scanning electron microscope. The images were made at a voltage of 20 KeV with 1000-fold magnification. The images were digitalized, then the area of the projection surface of the individual cells was measured, drawn around with the Scion Image program. After measuring the bar appearing on the scanning electron microscopic image, the data received in pixel were converted into μm^2. The data received were included in an excel table.

The eyes were coded, the electron microscopic examinations were carried out by two persons with masked method. The results of the two examiners did not differ significantly from each other.

The area of the projection surface of the cells was measured on 3x30=90 cells in total, and the percentage of the damaged-sound cells was calculated on all the three-three corneas. The statistical calculation was carried out with Person χ^2 probe, the area of the projection surface of the cells was evaluated with two-sample t-probe method, after giving the median and the standard deviation.

The clinical study was carried out with the authorisation of the Regional Research-Ethics Committee of the Semmelweis University (authorisation No. 105/2000) on the basis of the authorisation of the Animal Health and Food Control Station of Budapest Capital, registration No. 27-36-26/1999.

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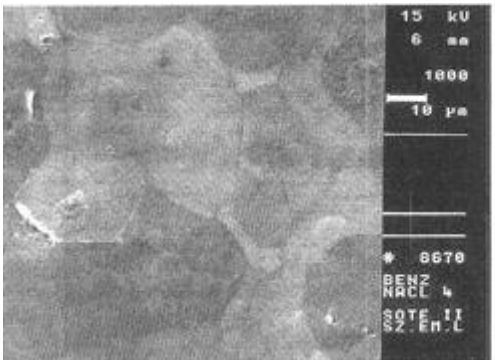

Figure 3 *Surface of the cornea treated with benzalkonium chloride 0.01% and isotonic sodium chloride*

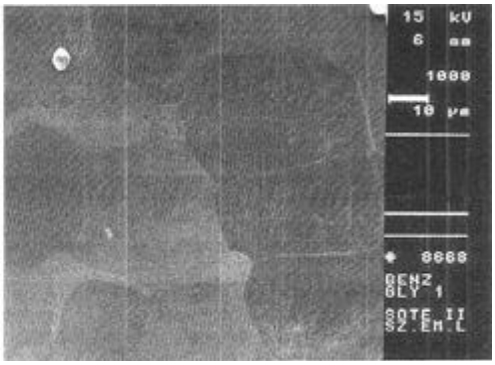

Figure 4 *Surface of the cornea treated with 2.5% isotonic glycerine+ benzalkonium chloride*

Results

Figure 1 shows the electron microscopic image of the centre of the cornea of an untreated animal. The intercellular connections are tight. Light and dark epithelial cells may be seen. In the plasma of a few cells there are crater-like formations.

Figure 2 shows the surface of the cornea treated with isotonic NaCl solution. The connections between the cells are interrupted in some places, the sizes of the cells are different, some of the cells are becoming detached.

Figure 3 shows a part of the cornea treated with benzalkonium chloride and isotonic sodium chloride, here the pathological changes shown on the previous figure are more expressed.

Figure 4 shows the corneal surface treated with isotonic glycerine+benzalkonium chloride. The cell connections are tight, no desquamating cell may be seen on the image.

The results are shown in *table 1*. The data of the treated animals were compared to the data of the control animals.

As it may be seen, the highest number of the pathological cells, cells with looser connection, breaking up, curling up of the cells occurred in the cases when eye drops with isotonic salt solution, or with salt solution with added benzalkonium chloride were used, while when cetrimonium bromide or EDTA was added to or eye drops with added glycerine were used, the occurrence of the pathological cells did not differ significantly from the control animals.

In the eyes treated with isotonic salt solution and salt solution+benzalkonium chloride the epithelial cells were smaller than in the controls. It is conspicuous that the addition of isotonic glycerine did not decrease the size of the cells.

The isotonic salt solution has a harmful effect on the human eyes as well[13].

Discussion

The examination results clearly show the harmful effect of the preservatives on the corneal epithelial cells. The question is that at the concentration used in general to what an extent the harmful side effect can be attributed to the preservative and to what an extent the isotonic salt solution itself causes the harm (**experiment 2** versus experiment 1).

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It was already communicated in our earlier lectures that if the isotonic salt solution is replaced with a highly moisturizing solution, glycerine, then according to the experimental models described here no harmful side effect is shown[7,8]. Of course, it may be as well that if the isotonic salt solution were replaced with a physiological salt solution, BSS Plus, then the damaging side effect of the sodium chloride would cease.

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