Regenerative Processes in the Cornea after Erosion and Effects of Adhelon on These Processes

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Adhelon markedly stimulated regeneration of erosive defects in the corneal epithelium. Under the effect of adhelon they healed more than 2-fold more rapidly after subtotal chemical torpid erosion than after total mechanical erosion of the cornea.

Key Words: *cornea; reparative processes; injury; torpid erosion; Adhelon*

Ocular injuries in humans are responsible for up to one-third of all traumas, the overwhelming majority of them, including corneal erosions, are the most incident among outpatient injuries. However, wide spectrum of factors causing corneal erosions (CE), their frequent complications and possible unfavorable outcomes necessitate the search for drugs stimulating the repair processes in the cornea [1]. Adhesive proteins stimulating regeneration in various tissues recently attracted special attention in this respect. One of them is a new regulatory low-molecular-weight protein adhelon isolated from bovine and human serum [5,6]. It stimulates regeneration processes and is effective in the treatment of gastrointestinal diseases, various limb injuries and fractures, articular injuries, *etc.* [3,4]. We hypothesized that it could stimulate corneal recovery after its erosion and experimentally investigated this problem.

MATERIALS AND METHODS

In series I we studied the dynamics of regenerative processes in the cornea of adult rabbits (8 animals, 16 eyes) after total mechanical CE in both eyes [2] and the effect of adhelon eye drops (control and experimental groups, respectively). Controls were instilled with placebo, experimental animals received adhelon into both eyes (50 μl - 2 droplets) 6 times daily for 10 days. The eyes were examined starting from day 1 (6 h after development of CE) daily until the end of the experiment. Lateral focal illumination of the eye and biomicroscopy with fluorescein test (2% sodium fluorescein solution) were used.

In series II (20 rabbits, 40 eyes) torpid (slowly progressing) subtotal CE was induced by 99% heptanol ($C_7H_{16}O$) solution [7]. The dynamics of corneal healing was evaluated 3 times daily by biomicroscopy and fluorescein test over 10 days after induction of CE. Placebo or adhelon (50 μl - 2 droplets into both eyes 6 times daily) was administered to control and experimental animals, respectively. The longer and shorter diameters of CE were measured by a calibrometer inserted into the microscope ocular. The mean area of corneal erosion (MAE) was estimated by the formula: $MAE=\pi r^2$, where r is the mean radius.

The data were processed by methods of variation and alternative statistics, the significance of differences was evaluated by Student's test (the differences were considered significant at $p<0.05$).

RESULTS

In series I, the size of epithelial defects of the cornea in rabbits instilled with adhelon decreased significantly starting from day 3 of the experiment in comparison with the control (Fig. 1). The difference in the rate of epithelialization between the control and experimental group increased: 5% on day 2, 19% on day 3, 32% on day 4, and 60% on day 5. In rabbits treated with adhelon wound epithelialization was completed on day 6, while in the control group it was completed only on day 8 (Fig. 1). Analysis of individual rate of total CE

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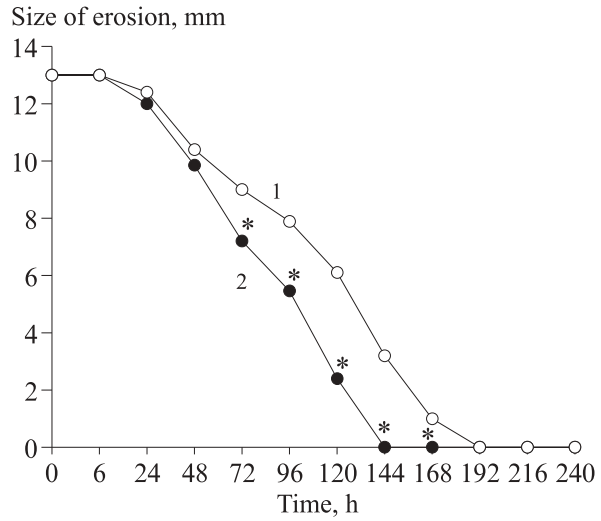

Fig. 1. Dynamics of corneal epithelialization in control and experimental rabbits after induction of total mechanical erosion. *1*) control; *2*) experiment. Here and in Fig. 2: **p*<0.05 compared to the control.

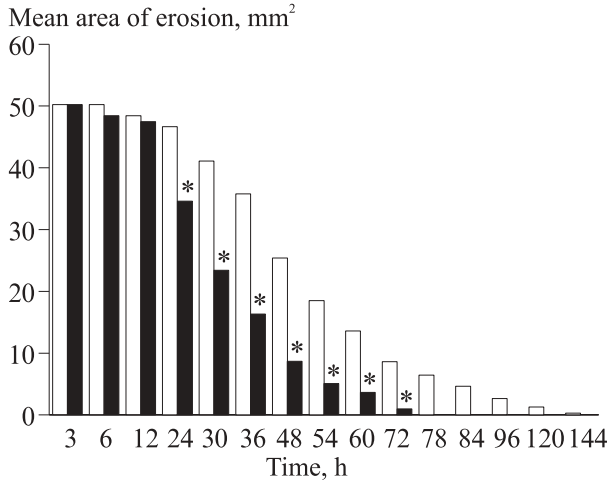

Fig. 2. Dynamics of healing of corneal epithelial defect in control and experimental rabbits after induction of subtotal chemical torpid erosion. Light bars: control; dark bars: experiment.

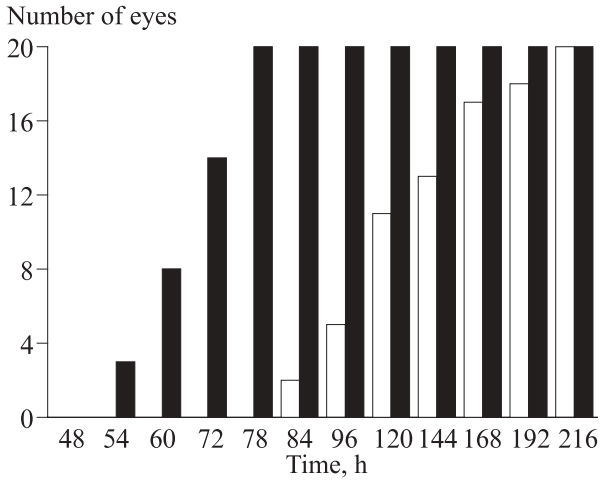

Fig. 3. Duration of corneal epithelialization in control (light bars) and experimental (dark bars) rabbits (number of eyes).

healing showed that the defects healed on day 6 in all experimental animals treated with adhelon, while in the control group the defects healed only in 2 of 8 eyes (25%) by this time. In the control group epithelialization of the corneal defect on day 7 after induction of total CE was noted in only 5 eyes (62.5%), and only on day 8 the epithelial layer was restored in all eyes.

Hence, adhelon promoted (by 25%) regeneration of the corneal epithelium in rabbits with total mechanical CE.

Series II showed that adhelon accelerated regeneration of rabbit corneal epithelium after induction of subtotal torpid chemical CE. During the first 12 h of the experiment the mean areas of epithelial defects were virtually the same in the control and experimental groups (Fig. 2). But after 24 h the cornea healed more rapidly in rabbits treated with adhelon compared to controls. Later the differences by MAE between controls and animals treated with adhelon became more pronounced. In rabbits treated with adhelon the cornea healed by 138 h sooner (2.8 times) than in the control due to intensification of regeneration processes (Fig. 3). Posttraumatic inflammation in the eye was arrested earlier (by 18 h, or by 1.3 times) than in the control (54 and 72 h, respectively).

Adhelon activated regeneration of rabbit corneal epithelium after infliction of subtotal torpid chemical CE. The treatment accelerated healing of CE by 74% (216 h in the control *vs.* 78 h in experiment) and disappearance of inflammatory reaction in CE by 25%.

Hence, adhelon, a bioregulatory protein with adhesive characteristics, had a pronounced stimulatory effect on the course of regeneration processes in the cornea after CE. Its effect on epithelialization was 2-fold more pronounced in subtotal torpid chemical CE than in total mechanical CE. Presumably, this difference is due to the fact that reproduction of subtotal CE does not involve some zones in the corneal epithelium, which then participate in its repair. In case of subtotal CE cells in these zones are activated by adhelon and provide more rapid epithelialization in comparison with total CE.

These data open new vistas for the use of adhelon for the treatment of CE in humans.

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