A comparison of the effect of hyaluronic acid versus gentamicin on corneal epithelial healing

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CLINICAL STUDY

Abstract

Purpose **An animal model study was conducted to compare the efficacy of recurrent topical applications of hyaluronic acid and gentamicin ointment for the treatment of non-infected, mechanical corneal erosions.**
Methods **An artificial, controlled wound of identical size and depth was inflicted to the corneas of three groups of rabbit eyes in order to measure their healing rates. One group was treated with hyaluronic acid (10 eyes) while the second group received gentamicin ointment (10 eyes). The third group remained untreated and served as the control (10 eyes). The rate of re-epithelisation was measured at 8-h intervals until complete re-epithelisation was observed. After complete wound closure, the rabbits were killed, and comparative histological examinations were performed.**
Results **Rabbit eyes treated with hyaluronic acid showed a significantly enhanced rate of epithelial defect closure compared with untreated eyes and a similar rate to that achieved with gentamicin ointment. In the eyes treated with hyaluronic acid a normal, multilayered epithelium was observed 48 h after complete healing, whereas the gentamicin-treated eyes showed an imperfectly layered epithelium, with irregularity of the cuboidal cells.**
Conclusion **While both hyaluronic acid and gentamicin enhance corneal epithelial healing at comparable rates, our study suggests that hyaluronic acid may have a more favourable effect on the structure of the healing epithelium, and can offer an alternative mode of therapy for non-infectious corneal erosions.**

Key words Hyaluronic acid, Gentamicin, Corneal erosions

Hyaluronic acid is a non-immunogenic,[1] viscoelastic substance commonly used in ophthalmic surgery to protect the corneal endothelium and to maintain anterior chamber depth during manipulation. The main indications for its use are cataract surgery, corneal grafting, glaucoma and vitreo-retinal operations.[2–7]

While the use of hyaluronic acid for minimising endothelial damage is widely documented,[2–7] its effect on the epithelium is less well studied. Topically applied hyaluronic acid for graft epithelial preservation was described by Reed *et al*.[8] in 38 patients undergoing penetrating keratoplasty, with favourable results. Reim[9] described its use as an aid in the treatment of severe burns.

Antibiotic ointments are commonly used in cases of epithelial erosions and abrasions, both traumatic and iatrogenic, to enhance the rate of healing.[10,11] The present masked, controlled study compared the effect of hyaluronic acid with that of an antibiotic ointment (gentamicin) in an animal model of corneal re-epithelisation.

Materials and methods

Twenty New Zealand rabbits, 2–4 months of age, were anaesthetised with an injection of 1 ml xylazine (1%) and 1 ml ketamine (10 mg/ml). A controlled corneal wound, using a 7.12 mm rotating disc, was inflicted to 30 rabbit eyes, causing an identical epithelial defect (surface area 40 mm^2, depth 40 μm). The depth of the wound was controlled by an adjustment knob on the rotating disc so that the epithelial basement membrane remained intact, in a method similar to corneal trephination.

After wound infliction, the right rabbit eyes were randomly divided into two groups using a random number system. Group 1 (10 eyes) was treated with an application of hyaluronic acid, 10 mg/ml (Biolon, Biotechnology General, Israel) and group 2 (10 eyes) with gentamicin ointment, 0.3% (Garamycin Ophthalmic, Schering, USA). Both Biolon and gentamicin were administered so that the entire corneal surface was covered, from the superior to the inferior conjunctival sacs. Group 3 served as a control, and consisted of 10 randomly selected left eyes from groups 1 and 2. The control group eyes were wounded by the same method but

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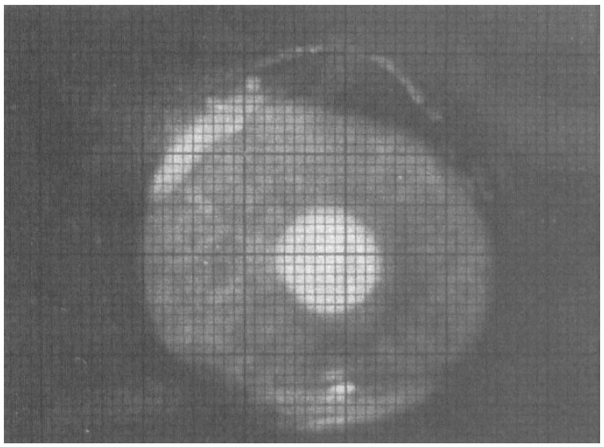

Fig. 1. *Photograph of a rabbit eye with an epithelial defect projected onto a millimetric scale. ×100.*

untreated after corneal injury. Timing of the treatment was planned so that during the acute phase of healing (the first 48 h) medication was administered four times daily, and twice daily at the end of the process, as common in everyday treatment.

The rabbit eyes were examined at 8-h intervals by slit-lamp microscopy and colour photographs taken. Before each examination the rabbits were anaesthetised and their corneas stained with a 2% fluorescein solution. The photographs were projected from a fixed distance onto a millimetric grid scale, and the area of erosion that had closed was measured in square millimetres (Fig. 1). The photographs were taken at the same magnification (×100) throughout the study. The examination of the wound was performed in a masked fashion; the examiner was unaware of the treatment modality as the treatment was administered by another study participant.

After complete re-epithelisation had occurred the rabbits were killed; 50% of the eyes in each of the three groups were examined 24 h after complete healing, and the remainder after 48 h. The eyes were fixed and stained with haematoxylin–eosin for microscopic examination.

Statistical analysis of the results was performed with analysis of variance with repeated measures. Probability values less than 0.05 were considered significant.

Results

Statistical

Comparison of average wound closure areas, in square millimetres, between the Biolon-treated (group 1) and untreated eyes (group 3) is shown in Table 1. The corneal wound in the eyes treated with Biolon healed at a faster rate than the wound in the untreated eyes. This difference was found to be statistically significant ($p = 0.0175$). Fig. 2 depicts the rate of healing differences between the Biolon-treated eyes and the untreated controls.

Comparison of the average wound closure areas in the gentamicin-treated eyes (group 2) and the control group (group 3) is shown in Table 1. The eyes treated with gentamicin ointment showed more rapid wound closure rates than the untreated eyes; this difference also proved to be significant ($p = 0.0169$).

When the rates of wound healing between the eyes treated with Biolon and those treated with gentamicin were compared, no statistically significant difference was observed ($p = 0.5$). Fig. 3 demonstrates the difference in rate of healing between eyes treated with gentamicin and untreated controls.

Histological

Histological examination of the corneal epithelium was performed for half the rabbits at 24 h after complete healing, and for the rest at 48 h.

After 24 h of wound closure all untreated corneas, as shown by haematoxylin–eosin staining, had a single-layered epithelium, while most of the group 1 and 2 eyes, whether treated with Biolon or gentamicin ointment, showed the development of a multi-layered epithelium in most areas of the cornea.

Table 1. *Average healed area in rabbit corneas treated with Biolon (group 1) or gentamicin (group 2) and in untreated controls (group 3)*

	Healed area (mm^2)					
	Biolon-treated (group 1)		Gentamicin-treated (group 2)		Untreated (group 3)	
Time (h)	Ave	(SD)	Ave	(SD)	Ave	(SD)
0	0	(± 0)	0	(± 0)	0	(± 0)
8	4.9	(± 0.4)	4.6	(± 0.3)	4.4	(± 0.6)
16	16.3	(± 2.4)	18.3	(± 3.7)	12.7	(± 3.0)
24	28.2	(± 2.8)	27.6	(± 4.2)	18.4	(± 3.9)
32	30.7	(± 3.5)	30.0	(± 5.6)	25.2	(± 3.3)
40	34.4	(± 7.3)	32.8	(± 3.7)	29.2	(± 5.8)
48	36.1	(± 6.3)	35.8	(± 5.2)	32.8	(± 7.3)
56	37.0	(± 4.1)	36.9	(± 3.9)	35.6	(± 6.5)
64	37.8	(± 9.0)	38.1	(± 8.2)	37.7	(± 5.1)
72	38.8	(± 4.3)	39.0	(± 5.5)	39.1	(± 9.2)
80	39.5	(± 7.3)	39.4	(± 8.9)	39.4	(± 7.7)
88	39.8	(± 5.7)	39.7	(± 7.3)	39.5	(± 6.4)
96	40.0	(± 4.4)	40.0	(± 8.7)	39.6	(± 8.5)

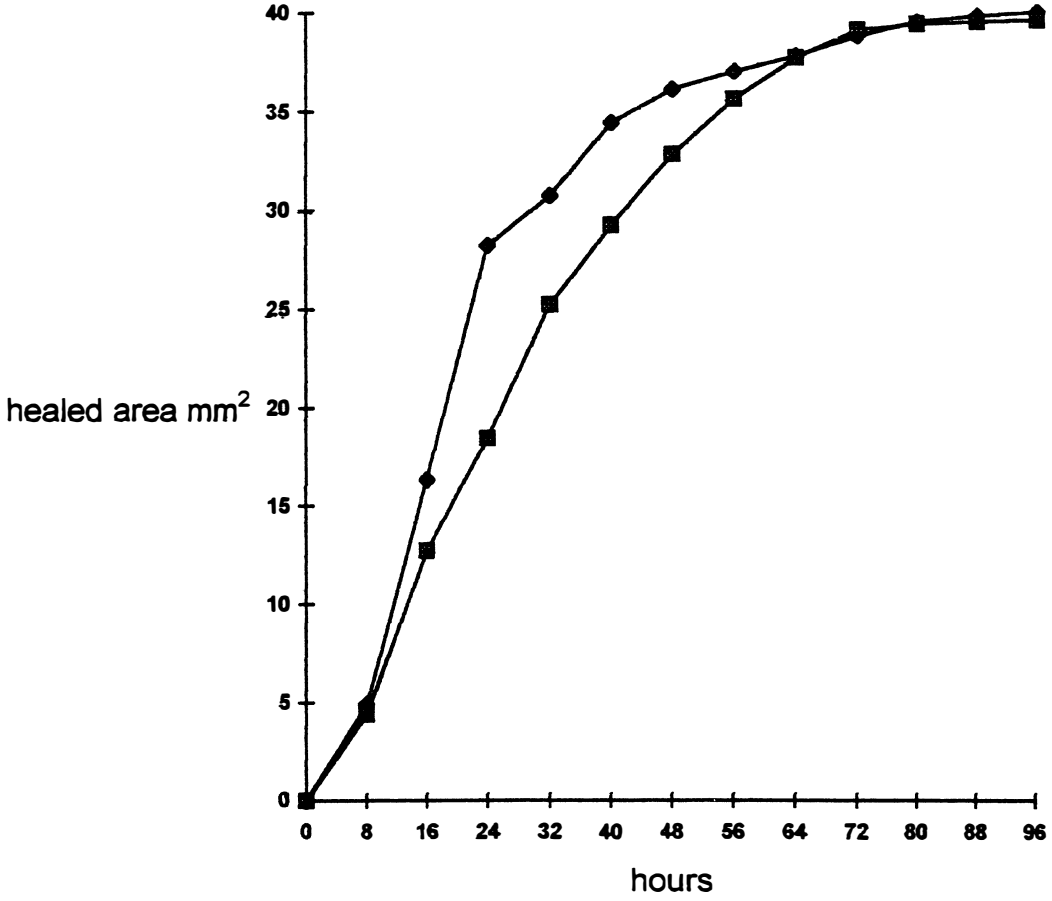

Fig. 2. *Rate of wound closure in rabbit eyes treated with Biolon (diamonds) and untreated controls (squares).*

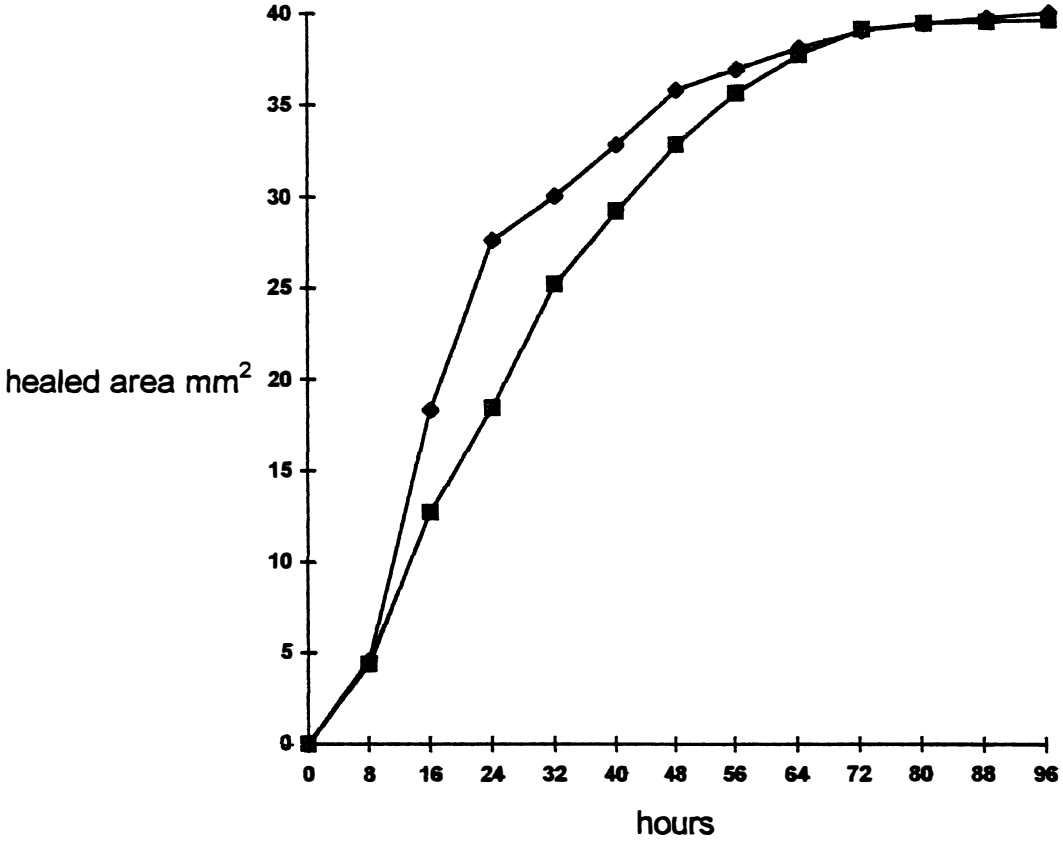

Fig. 3. *Rate of wound closure in rabbit eyes treated with gentamicin (diamonds) and untreated controls (squares).*

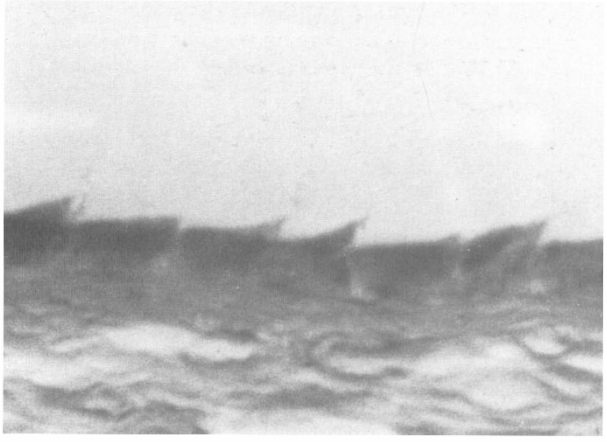

Fig. 4. *Histological section of an untreated rabbit cornea 48 h after complete wound closure. Note the single-layered, ragged epithelium. Haematoxylin–eosin, ×100.*

After 48 h the untreated corneas remained single-layered, although the cells were larger and nucleated (Fig. 4), whereas the eyes treated with Biolon showed the fully organised and multi-layered appearance of normal epithelium (Fig. 5). In the eyes treated with gentamicin, an imperfectly layered epithelium had developed, with irregularity of the cuboidal cell layer (Fig. 6).

Discussion

The high incidence of corneal erosions and abrasions has led to a search for new and better therapeutic strategies.[12–14]

According to the results of this study, the rate of epithelial defect closure was similar in eyes treated with hyaluronic acid and those treated with gentamicin. However, at wound closure the eyes treated with hyaluronic acid revealed a well-developed, multi-layered epithelium, whereas the eyes treated with gentamicin had an imperfectly layered epithelium with irregular and relatively flat cuboidal cells. This difference may provide evidence that while both these substances enhance

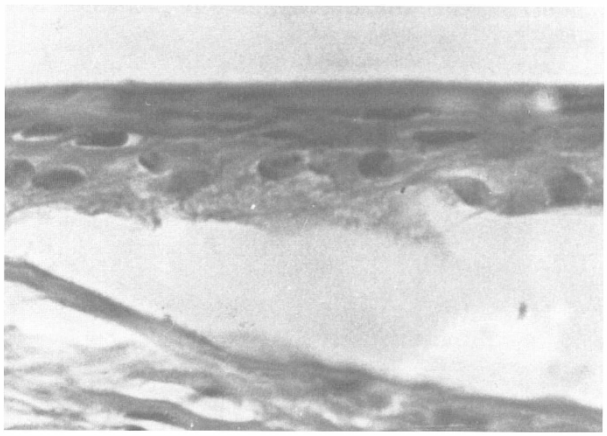

Fig. 5. *Histological section of a rabbit cornea treated with Biolon 48 h after complete wound closure. A fully organised, multi-layered epithelium has formed, with well-developed cuboidal cells. Haematoxylin–eosin, ×100.*

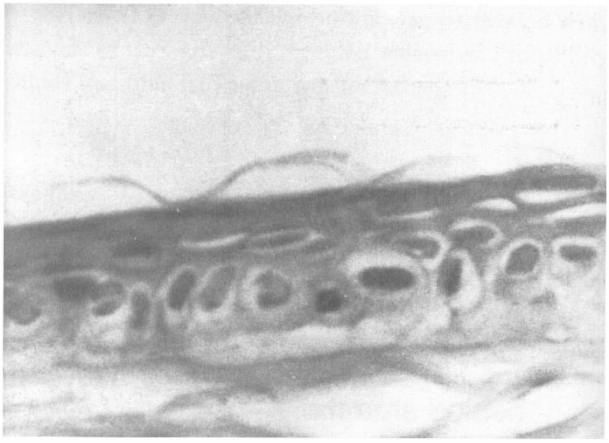

Fig. 6. *Histological section of a rabbit cornea treated with gentamicin 48 h after complete wound closure. Note that an imperfectly layered epithelium has developed, with irregularity of the cuboidal cells. Haematoxylin–eosin, ×100.*

wound closure, hyaluronic acid has a favourable effect on cell development and layering, as opposed to gentamicin's known deleterious effect on cell structure.[15]

To the best of our knowledge, the present study is the first comparing the effects of hyaluronic acid and gentamicin on corneal epithelial healing rate and quality. Antibiotic ointments remain an important element in the treatment of infected corneal abrasions. Our results suggest that hyaluronic acid has a favourable effect on the quality of epithelial healing. This may prove to be especially pertinent to non-infected epithelial erosions, and in cases of recurrent corneal erosions, in which both rate and quality of epithelial healing are crucial.

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