Human Recombinant Epidermal Growth Factor in Experimental Corneal Wound Healing

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Human recombinant epidermal growth factor (hEGF) was evaluated in various corneal wound healing models in the rabbit. Human EGF accelerated epithelial wound healing in corneal reepithelialization, anterior-keratectomy, and alkali-burn models at concentrations of 10–500 μg/ml given four times daily (qid). In the corneal reepithelialization model, 100 μg/ml of hEGF qid produced a 45% increase in the wound-healing rate compared with control (0.13 versus 0.09 mm/hr) with a similar response at 500 μg/ml qid. In the anterior-keratectomy model, 500 μg/ml of hEGF qid accelerated healing by 40% (0.07 versus 0.05 mm/hr), although the 100 μg/ml dose was not active in this model, and 1 μg/ml of hEGF actually slowed the healing rate. In the alkali-burn model, 10 and 100 μg/ml of hEGF qid for 32 days appeared to produce faster initial healing of the wound compared with control, although the wound recurred in both hEGF and control groups. These results suggest that hEGF may be helpful in some epithelial disorders in humans, although considerations of dose response and optimal dosing regimens must be addressed.

Epidermal growth factor (EGF) was first isolated from mouse submaxillary glands in the early 1960s.1 Mouse EGF (mEGF), a polypeptide of 53 amino acids with a molecular weight of 6045 D,2-4 has been shown to stimulate the in vitro uptake of the precursors of protein, DNA and RNA synthesis by ectodermal cells,5,6 to stimulate growth of epidermal cells in organ culture,7 and to enhance epidermal growth and keratinization in vivo.8

Savage et al9,10 first demonstrated the stimulatory effect of mEGF on corneal epithelial proliferation, and this was confirmed in a number of experimental systems.11-17 Frati et al18 demonstrated in rabbits that 2 mg/ml of mEGF accelerated epithelial healing of nonperforating corneal scrape wounds by approximately 30% compared with control. Ho et al12 found that topical mEGF given four times daily (qid) to rabbits at doses of 0.05–2 mg/ml increased epithelial healing of central corneal epithelial wounds, although the effect did not appear to be dose dependent. In addition, mEGF has also been shown to accelerate healing in alkali burns of rabbit corneal epithelium at doses ranging from 0.05–2 mg/ml qid.13,16

In humans, 2 mg/ml of mEGF dosed every 4 hr doubled wound healing rates in various nンドystrophic diseases of the corneal epithelium, including traumatic epithelial loss, septic ulcers, and corneal burns.18 However, there appeared to be no improvement in herpetic or stromal keratitis or in bullous keratopathy, and the effect of mEGF was reduced as stromal damage increased. In patients undergoing penetrating keratoplasty, mEGF given at 1–2 mg/ml eight times daily had no effect of epithelial healing rates compared with control.19

Human EGF (hEGF) was first isolated in 197520-22 and has been shown to contain 53 amino acids with the same three disulfide bonds as mEGF, although the hEGF sequence differs from mEGF in 16 positions.20,21 The biologic activity of hEGF appears to be identical to mEGF and both bind to the same membrane receptors.20,21,23 The hEGF has been identified in various human tissues and fluids including urine, saliva, plasma, and breast milk24,25 although not in corneal epithelium or conjunctiva.26 Human EGF has recently been synthesized using recombinant DNA techniques, which has provided sufficient quantities of hEGF for preclinical and clinical evaluation.27

To date, there have been few published reports concerning the effect of hEGF on corneal healing. Topical application of 0.1 mg/ml of hEGF three times a day to primates in combination with an antibiotic and a corticosteroid was shown to accelerate the epithelial healing rate of nonpenetrating scrape wounds and to increase significantly the wound strength of full-thickness stromal incisions.28 This report discusses the results of a number of preclinical

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evaluations of hEGF which were conducted in an attempt to understand the potential of this exciting molecule better and to determine which clinical situations might benefit most through its use.

**Materials and Methods**

**Materials**

Human EGF was produced by Creative Biomolecules (Hopkinton, MA) using genetic recombination in *Escherichia coli*. The material was highly homogeneous, had a purity of greater than 99% as determined by high-performance liquid chromatography, and was shown to be fully equivalent to purified mEGF in a radioreceptor assay using A431 cells. The material was stored frozen as a lyophilized powder and was reconstituted with phosphate-buffered saline (PBS), pH 7.0, before each study day.

**Animal Studies**

All procedures described in these experiments conformed to the ARVO Resolution on the Use of Animals in Research.

*Corneal reepithelialization (study 1)*: New Zealand albino rabbits weighing 2–3 kg were anesthetized by intramuscular injection of ketamine hydrochloride (30 mg/kg) and xylazine (6 mg/kg) and by topical administration of proparacaine. The entire corneal epithelium was removed using a corneal gill knife, leaving the basement membrane intact, resulting in an epithelial scrape wound with a radius of approximately 6 mm. The wound size was determined by staining the surface of the eye with fluorescein and photographing the cornea with a slit-lamp camera equipped with a cobalt exciter filter. The area of the corneal scrape wound was quantitated from the photographs using computer imaging analysis (R. M. Biometrics, Nashville, TN). Three groups of four rabbits each were used in the study with each group being dosed with either PBS vehicle or 100 or 500 μg/ml of hEGF. Each dose was administered qid to both eyes with the first dose being administered immediately after the initial measurement of wound size. The eyes were photographed before the first dose and at 24, 32, 48, 56, 60, and 72 hr after initiation of dosing.

*Anterior keratectomy (study 2)*: New Zealand albino rabbits weighing 2–3 kg were anesthetized similarly to the previous studies. A central corneal epithelial wound extending into the stroma was produced in the left eye of each animal using a filter-paper disc (6 mm in diameter) soaked in 1 N NaOH applied to the corneal surface for 60 sec. Two groups of eight rabbits each were dosed qid for 32 days with either 10 or 100 μg/ml of hEGF to the wounded eye with a third group of animals being treated similarly with PBS vehicle. In addition, each animal was treated with topical gentamicin solution qid. The wound area was measured daily for the 32 days of dosing using the method previously described.

**Results**

The kinetics of reepithelialization of wounded rabbit corneas dosed qid with PBS vehicle after corneal reepithelialization and anterior-keratectomy wounds are presented in Figure 1. The wound radius versus time curves showed a biphasic decrease in wound size after both procedures. In the corneal-reepithelialization model, an initial lag phase of 24–32 hr was observed, during which time healing was relatively slow. This was followed by a faster linear healing phase with the wound radius decreasing at approximately 0.09 mm/hr. Extrapolation of this healing phase to zero radius suggested that complete healing of the...
wound occurred at 80–90 hr. With the anterior-keratectomy model, the initial wound size was smaller (Fig. 1), and a shorter lag time was observed (approximately 10 hr). Healing of the anterior-keratectomy wound was somewhat slower than the corneal-reepithelialization wound, with a healing rate of 0.05 mm/hr and complete healing occurring after 80 hr.

The effect of hEGF treatment on the wound-healing rate for both these models is presented in Figures 2 and 3. Slopes for the linear healing phase of the various treatment groups for both models is presented in Table 1. Comparison of the slopes of the two control groups (Table 1) suggest that the rate of healing of the epithelial scrape wound was approximately twice that of the keratectomy wound (0.089 versus 0.048 mm/hr). With the anterior-keratectomy model, only the 500 µg/ml dose appeared to increase healing rate, whereas for corneal reepithelialization, both the 100 and 500 µg/ml doses produced an increased healing rate.

Statistical analysis of these data was approached in two ways: one compared the wound radius of the various treatment groups at each observation time, and the other used regression analysis and statistical comparison of the healing rate between treatments. Both statistical analyses were done using SAS software. For the corneal-reepithelialization model, the wound sizes for the groups that received the 100- and 500-µg/ml doses of hEGF were significantly smaller (P < 0.05) than the PBS-treated control group at 48 hr and all times thereafter. Regression analysis showed both 100 and 500 µg/ml of hEGF reduced wound size statistically faster than untreated control, although separation between the regression lines did not occur until after 32 hr, suggesting a lag phase in the onset of activity.

In the anterior-keratectomy model, the 500-µg/ml hEGF dose was significantly better in reducing wound size than any other treatment at 46, 53, 71, and 78 hr (P < 0.05). The 10- and 100-µg/ml doses of hEGF were not significantly different from control at any time. However, the 1-µg/ml hEGF group healed significantly slower (P < 0.05) than did the control group at all times after 24 hr. Regression analysis of these data showed the 500-µg/ml hEGF treatment to be significantly better in reducing wound size than any of the other treatments, including PBS control. These analyses also showed that the 1-µg/ml hEGF group was significantly slower in healing than both control and the 10- and 500-µg/ml groups.

In the alkali-burn model, the initial wound was completely healed by day 3 in all animals in the 10- and 100-µg/ml hEGF treatment groups. With PBS control, complete healing occurred by day 3 in five of

<table>
<thead>
<tr>
<th>Dose</th>
<th>Healing rate (mm/hr)*</th>
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<tr>
<td>PBS control qid</td>
<td>0.048 ± 0.009</td>
</tr>
<tr>
<td>1 µg/ml hEGF qid</td>
<td>0.038 ± 0.009</td>
</tr>
<tr>
<td>10 µg/ml hEGF qid</td>
<td>0.051 ± 0.006</td>
</tr>
<tr>
<td>100 µg/ml hEGF qid</td>
<td>0.042 ± 0.008</td>
</tr>
<tr>
<td>500 µg/ml hEGF qid</td>
<td>0.067 ± 0.014</td>
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*Calculated as the slope of the linear regression analysis of wound radius vs. time.
eight animals but not until day 5 or 6 in the other three. Over the next week of dosing, the wounds remained healed in seven of eight animals at 100 µg/ml of hEGF, in five of eight animals at 10 µg/ml of hEGF, and in only three of eight animals in the PBS control. Thereafter, there was periodic recurrence of the wound in all treatment groups throughout the duration of the study, with neither hEGF dose preventing these recurrent erosions.

The mean defect areas for the three treatment groups from the alkali-burn model during the 32 days of the study are presented in Figure 4. The mean defect area for both the 10- and 100-µg/ml hEGF groups were smaller than that in the PBS control group throughout most of the observation period. Student t-test showed statistically significant differences (P < 0.05) between PBS control and the 100-µg/ml hEGF group on the fourth and fifth post-operative day. When the data from each group was organized in terms of the total numbers of observation with a defect present, chi-square analysis showed statistically significant differences (P < 0.05) between the PBS control and each of the hEGF treatment groups, with both doses of hEGF having fewer defects than control.

In the rabbit corneal-reepithelialization model, in which an epithelial scrape wound with a 6-mm radius was produced, 100 µg/ml of hEGF qid produced a 45% increase in the wound-healing rate compared with the PBS control (0.13 versus 0.09 mm/hr). A similar acceleration in healing was observed in the anterior-keratectomy model, which involved a 4-mm wound in which the epithelium, basement membrane, and anterior stroma were removed. In this model 500 µg/ml of hEGF given qid accelerated healing 40% compared with the PBS control (0.07 versus 0.05 mm/hr); the 10- and 100-µg/ml doses were not different from the control, and 1 µg/ml of hEGF actually decreased the healing rate relative to the control. The magnitude of the acceleration of healing was similar for the two models, although a higher dose was necessary to increase healing in the keratectomy model relative to corneal reepithelialization. Although the reasons for the differences in effective doses are not obvious from these data, the results are consistent with the observation made by Daniele et al18 that the response to EGF is reduced as stromal damage is increased.

In the anterior-keratectomy model, 1 µg/ml of hEGF appeared to produce a slight, yet statistically significant decrease in the rate of epithelial healing (0.38 versus 0.48 mm/hr). This suggests that the dose–response relationship for hEGF may be more complex than most pharmacologic agents and may be different than the typical sigmoidal relationship between dose and effect. More research into the nature of the dose–response relationship in each model is needed to understand the wound healing activity of hEGF better and to predict the optimal dosing strategies for further evaluation.

In the rabbit alkali-burn model, 10 and 100 µg/ml of EGF given qid over 32 days appeared to provide faster initial healing of the wound than the control group and also appeared to delay the recurrence of the wound after its initial healing. However, hEGF treatment did not prevent the recurrence of the wound, although the mean defect data (Fig. 4) suggests that the recurrence was less pronounced in the hEGF-treated groups than in the control group. These results are similar to that reported for mEGF in this model14,16 and suggest that hEGF alone may not be sufficient for healing wounds of this type. Singh et al16 suggested that attachment factors such as fibronectin and laminin may be needed in conjunction with EGF to heal alkali-burned corneas and recurrent erosions similar to those of the model studied here.

The combined results of these studies demonstrate that hEGF can increase the wound-healing rate in experimental models of corneal reepithelialization and that the effect is similar to that reported pre-

Discussion

The results from these investigations indicate that hEGF accelerates corneal reepithelialization in rabbit wound-healing models in a manner similar to that previously reported for mEGF.9-16 This agrees with previous reports that the biologic effects of the two growth factors are similar.20,21,23
viciously with mEGF. It is not known how these results will extrapolate to humans since all three models involve healthy corneal tissue which may not be representative of the pathologic conditions associated with many human corneal defects. Despite these limitations, these data support the human clinical evaluation of hEGF in various corneal defects and wounds, although considerations of dose response and optimal dosing regimens must be carefully addressed in such studies.

Key words: human epidermal growth factor, hEGF, cornea, wound healing, reepithelialization, rabbit

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References