

The effects of recombinant human epithelial growth factor and protein-free calf blood extract for recovery of corneal mechanical epithelial defects healing and neovascularization

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Abstract. – OBJECTIVE: This study was undertaken to investigate the effects of recombinant human epidermal growth factor (rhEGF) and protein-free calf blood extract on corneal wound healing and neovascularization.

MATERIALS AND METHODS: An rabbit central corneal wound and neovascularization model was established in rabbits. One eye of each group was chosen randomly for topical administration of protein-free calf blood extract, rhEGF, or NS (physiological saline), and variability in the area of corneal epithelial wound healing and neovascularization was observed.

RESULTS: On days 1 and 2, the healing rate of corneal epithelium was various among the protein-free calf blood extract group, rhEGF group and NS group ($F = 6.475$, $p = 0.012$). The healing rate of corneal epithelium in the rhEGF group was better than the protein-free calf blood extract group ($p = 0.004$) and NS group ($p = 0.041$) on day 1. The corneal neovascularization area in the protein-free calf blood extract group was less than that of rhEGF group ($p = 0.04$) and NS group ($p = 0.008$) on day 18.

CONCLUSIONS: rhEGF had better promotive effect on corneal epithelial wound healing than the protein-free calf blood extract in the advanced phase (within 2 days). Both rhEGF and protein-free calf blood extract were not found to promote the growth of CNV (corneal neovascularization).

Key Words:

Cornea, Wound healing, Neovascularization, Growth factor.

Introduction

The corneal epithelial injury, which may be caused by oculopathy, ocular injury or operation, is a frequent ocular surface disease¹. It can increase the risk of the corneal infections, perforations and

neovascularization, result in many unwell complications, including irritative sensation and low visual acuity^{1,2}. Protein-free calf blood extract and rhEGF (recombinant human epithelial growth factor) are commonly used in the clinic due to their promotive effect on corneal epithelium repair³. It remains to be studied that whether protein-free calf blood extract and rhEGF could induce corneal neovascularization and which would have a better effect on corneal injury repair⁴. Therefore, it is still very useful to search the optimal drugs of corneal wound healing for lessening the side effect.

Protein-free calf blood extract, also named deproteinized calf blood extract, mainly consists of many kinds of free amino acids, small molecular peptide and oligose, which can stimulate cytothesis and tissue repair by promoting ocular superficial histiocyte to uptake glucose and oxygen, and lessen scar formation by making collagen fiber restructuring². Since 1995, Protein-free calf blood extract (solcoseryl) produced in Switzerland was applied to cure various kinds of corneal epithelial wound. As solcoseryl eye gel gradually vanished in Chinese market due to the outbreak of mad cow disease in European, other kinds of protein-free calf blood extract eye gel were still applied widespreadly in China². However there are few reports systematic assessing them on corneal epithelial repair and neovascularization.

In order to assess the efficacy and safety of protein-free calf blood extract and rhEGF on corneal epithelial wound repair objectively, to identify their optimal applied opportunity, and to lessen their side effect, a study was designed to assess the effects of protein-free calf blood extract and rhEGF for recovery of mechanical corneal epithelial defects healing and neovascularization in vivo, and it is reported as follows.

Materials and Methods

Animals

White New Zealand rabbits (purchased from Guangdong Medical Laboratory Animal Center, Guangdong, China), weighing between 2.0 and 2.5 kg, were used for this study and were randomly divided into 3 groups: DCBE (protein-free calf blood extract eye gel, or Deproteinized Calfblood Extract), rhEGF (recombinant human epithelial growth factor eyedrops), and NS (physiological saline eyedrops), five rabbits for every group. The rabbits were quarantined and acclimatized one week before the experiments in the Ophthalmic Animal Laboratory of Zhongshan Ophthalmic Center at Sun Yat-sen University, Guangzhou, China. The experiments were performed on the rabbits under standard conditions throughout the study as follows: room temperature $23 \pm 2^\circ\text{C}$, relative humidity $60\% \pm 10\%$, and alternating 12-hour light-dark cycles (8AM to 8PM). All experimental procedures conformed to the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Medical Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University.

Animal Experiments Procedure

A central corneal epithelial wound mode was created as previously described⁵. Rabbits were anesthetized by an intramuscular injection of ketamine hydrochloride (10 mg/kg) and xylazine (6 mg/kg) and by topical administration of 1% lidocaine hydrochloride. The ocular surface was disinfected using a 1% povidone-iodine solution. The upper and lower eyelids were braced using an eyelid speculum. Excessive moisture on the corneal surface was absorbed using a sterile cotton swab, and two sterilized surgical sutures were sutured in the upper and lower cornea-scleral limbi for traction. A 7.5 mm diameter corneal trephine was placed on the cornea with the pupil at the center, and the traction sutures were pulled by an assistant to make the corneal trephine touch the surface of the cornea. A circular incision was made on the cornea by turning the trephine to a depth of approximately one-third of the thickness of the cornea. An excisional trephine wound was created by removing the epithelium and superficial stroma at the center hole of the corneal trephine using a micro-smooth forceps⁶. Six sterilized surgical sutures were sutured in the peripheral cornea at a distance of 2mm to the cornea limbus at 2, 4, 6, 8,

10, and 12 points. The rabbit eyes were treated postoperatively with topical administration of DCBE, rhEGF, and NS drops four times per day. Variety in the area of corneal epithelial wound healing and neovascularization was observed using fluorescein staining followed by photography and was analyzed with Image J software.

Each area of wound healing was analyzed against the wounded cornea at zero time (0% healing) with an un-injured control cornea (100% healing) as the positive reference. The degree of wound healing was expressed as the ratio of the difference of the means of the total pixels of the wound areas at control 0 time (A) and the means of the total pixels of the wound area remaining in the experimental cornea (B) over the control, or $(A-B)/A\%$ ⁷. The extent of healing (%) was calculated as the mean \pm SD from 5 corneas: Corneal epithelial healing rate = $(0\text{d coloring area} - \text{observation coloring area}) / (0\text{d coloring area}) \times 100\%$. Corneal CNV areal formula: $A = C / 12 \times 3.1416 [r^2 - (r-1)^2]$, where C is related corneal points, l is the length of CNV, and r is 7 mm; the CNV area included six quadrants and was calculated at 3, 4, 5, 6, 8, 10, 12, 15, 18, and 21d after the operation⁸.

Statistical Analysis

All values were presented as the mean \pm SD. All statistical analyses were performed with SPSS software, version 13.0 (SPSS Inc., Chicago, IL, USA). Repeated measures analysis of variance for the healing rate of the rabbit corneal epithelium, and corneal neovascularization area, one-way analysis of variance for the rest statistical analyses, all tests were two-tailed, and a probability value of 0.05 was considered statistically significant.

Results

Comparison of DCBE and rhEGF on the Healing Rate of the Rabbit Corneal Epithelium

On day 1 and 2, the corneal epithelial healing rate varied in the DCBE, rhEGF, and NS groups ($F = 6.475, p = 0.012$), as the rabbit corneal central epithelium was scraped the first time. The healing rate in the rhEGF group was higher than in the DCBE group ($p = 0.0040$) and NS group ($p = 0.041$) on day 1. On day 3, the healing rates of DCBE, rhEGF, and NS were $98.40\% \pm 1.52\%$, $100\% \pm 0.00\%$, $99.50\% \pm 0.70\%$, respectively. For simulation of chronic corneal ulcer, the rabbit corneal central epithelium was scraped twice on day 5, the healing

Table 1. Overall healing rate of rabbit cornea in different groups at different time (% , $\bar{X} \pm S$, n = 5).

| Group | N | Day 1 | Day 2 | Day 3 |
|-------|---|-------------|------------|------------|
| DCBE | 5 | 39.35±6.92 | 87.19±4.74 | 98.40±1.52 |
| rhEGF | 5 | 51.83±8.87* | 95.38±3.17 | 100±0.00 |
| NS | 5 | 43.32±5.42 | 90.51±4.57 | 99.50±0.70 |

* $p < 0.05$ versus DCBE and NS

rate in the rhEGF group was higher than in the NS group ($p < 0.05$), and the results of twice abrading the corneal central epithelium indicated that rhEGF promoted corneal epithelial injury repair in early phase, DCBE had not an effect within 2 days (Table 1, Figure 1A and B).

Comparison of DCBE and rhEGF on corneal neovascularization area

On day 3, there was a neovascularization bud born from the corneal limbus in the three groups.

On day 13, the corneal neovascularization in the three groups reached peak levels at this time, and the stitches were removed. Corneal neovascularization in the three groups started to regress, as the corneal chronic sutural stimulus was eliminated. On day 18, the corneal neovascularization area in the DCBE group was much smaller than the rhEGF ($p = 0.04$) and NS groups ($p = 0.008$). The corneal neovascularization area in the rhEGF group was not significantly different, compared with that of the NS group. The regression velocity

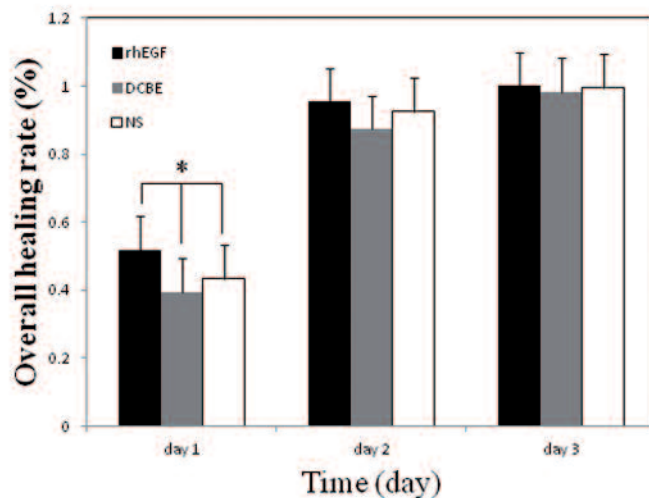
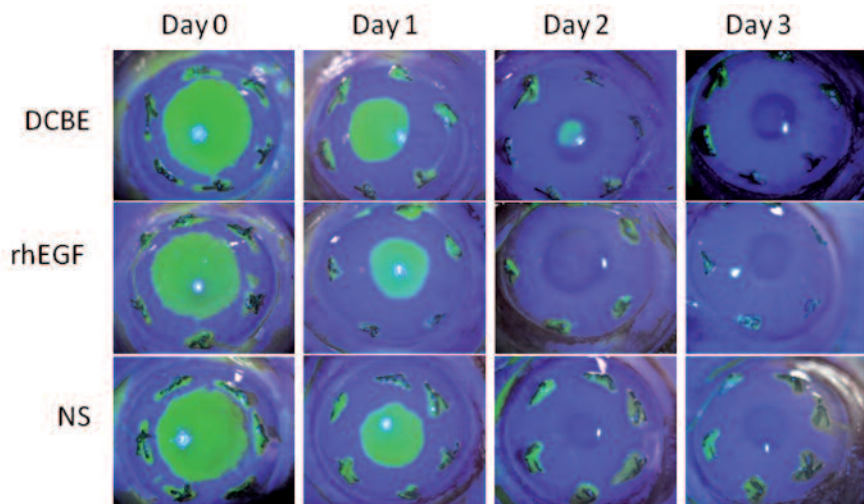


Figure 1. Comparative the effects of DCBE and rhEGF on the healing rate of the rabbit corneal epithelium (A-B). **A**, It showed the effects of DCBE and rhEGF on the wound healing of rabbit corneal epithelium on days 0, 1, 2, 3 (on the upside). **B**, It indicated the effect of rhEGF on the healing rate of corneal epithelium was better than DCBE and NS on day 1 ($p < 0.05$) (on the downside).

Table II. The corneal neovascularization area in different groups at different time (mm², $\bar{X} \pm S$, n = 5).

| Group | N | Day 8 | Day 12 | Day 15 | Day 18 | Day 21 |
|-------|---|---------------|---------------|---------------|---------------|---------------|
| DCBE | 5 | 57.550±5.511 | 95.624±9.006 | 86.314±16.726 | 58.267±5.421 | 62.780±3.924 |
| rhEGF | 5 | 53.792±14.995 | 95.484±13.971 | 76.392±19.249 | 79.978±8.627 | 63.897±7.089 |
| NS | 5 | 56.467±16.202 | 92.510±12.660 | 84.000±18.351 | 77.156±12.588 | 57.812±13.497 |

of corneal neovascularization in the DCBE group was much faster than that of the rhEGF group. These findings indicated that DCBE and rhEGF group did not promoted corneal neovascularization by chronic corneal sutural stimulus, compared with NS group (Table 2, Figure 2A and B).

Discussion

Clinically, how to choose the correct usage of medicine in corneal epithelial injury repair is consid-

ered as a long-term concern in clinical practice research⁹⁻¹². Up to now, the medication of ocular surface injury repair mainly includes: corneal lubricant, corneal pleriosis, and corneal nutrition². DCBE (deproteinized calfblood extract) and rhEGF have been used widely in the clinic, which possess a certain therapeutic effect with different repair mechanisms¹³⁻¹⁷. Autoserum can promote corneal epithelial wound healing, containing different kinds of nutritional components, such as growth factors, vitamin A, glucose, but it is limited to draw the patients with periphery venous blood frequently in the clinic^{18,19}.

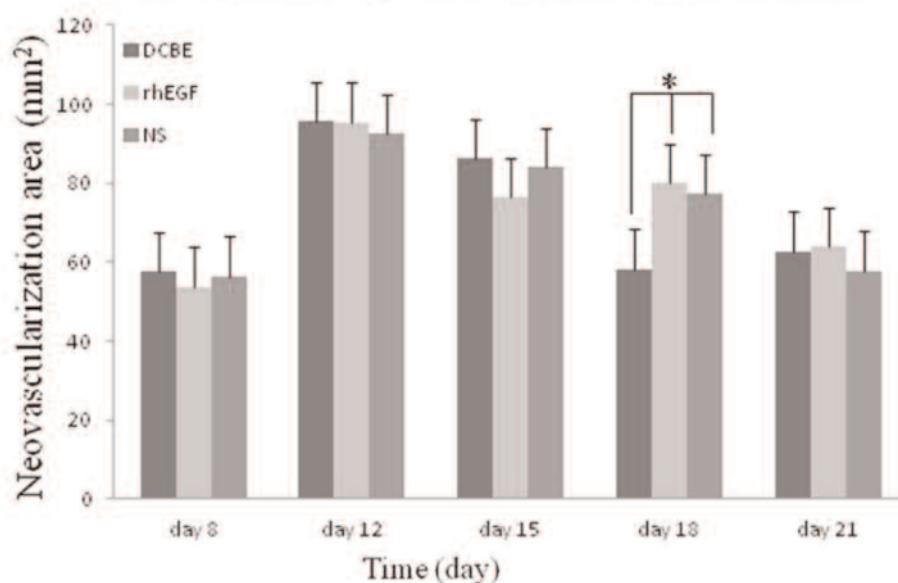
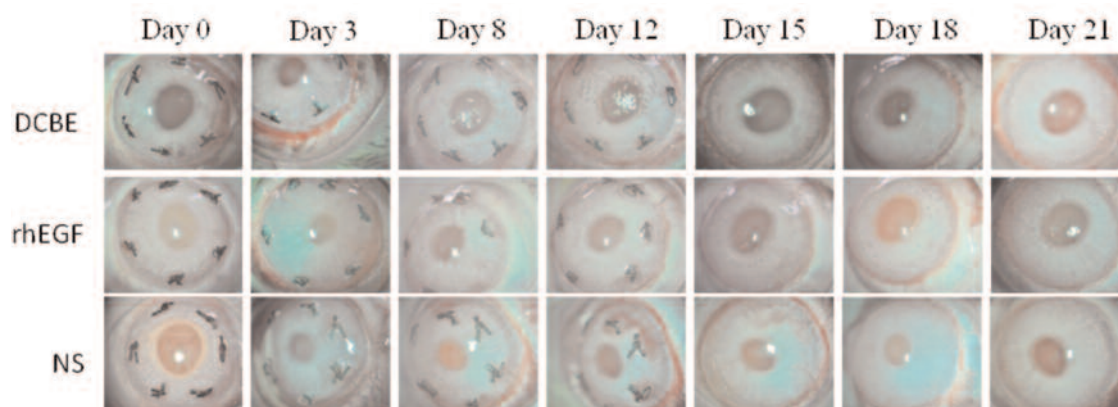


Figure 1. Comparative the effects of DCBE and rhEGF on the healing rate of the rabbit corneal epithelium (A-B). **A**, It showed the effects of DCBE and rhEGF on the wound healing of rabbit corneal epithelium on days 0, 1, 2, 3 (on the upside). **B**, It indicated the effect of rhEGF on the healing rate of corneal epithelium was better than DCBE and NS on day 1 ($p < 0.05$) (on the downside).

However, DCBE as one of the substitute, could eliminate etiological factor as well as ameliorate the nutritional status of corneal surface, but the therapeutic effect and whether to promote CNV (corneal neovascularization) remains to be studied furtherly. To guide clinical treatment, we designed the study for observing the effects of rhEGF and DCBE on corneal epithelial repair and neovascularization.

DCBE was found to be one kind of compound medicine with multiple function including corneal lubricant, plerosis, and nutrition, which could be applied to cure mechanical corneal epithelial injury effectively with the feature of stable therapeutic effect, absolute safety, and enough tolerance²⁰. The effect of protein-free calf blood extract for recovery of corneal nerve after LASEK (Laser Assisted Sub-Epithelial Keratomileusis) and LASIK (Laser Assisted in Situ Keratomileusis) was found, and it also could make histiocyte fully uptake and utilize glucose and oxygen as well as provide them nutrition, as the histiocyte respiratory and activated agent²⁰. Otherwise, protein-free calf blood extract eye gel could form protective membrane to lubricate ocular surface, lessen ocular uncomfortable and the mechanical friction between eyelid and cornea, magnify the patient's comfortable sensation, and promote corneal epithelial wound repair^{18,19}.

To simulate the symptom of repetatus cornea erosion, the central corneal epithelium was scraped twice at day 5 post-operation. The results indicated that DCBE did not stimulate corneal epithelium repair in the short term (within 2 days), but rhEGF did. In addition, both DCBE and rhEGF were not found to promote the growth of CNV, compared with physiological saline. In short, with chronic stimulus, DCBE could release patients with ocular comfortable sensation without the promote effect of CNV, although the effects presented more slowly on corneal epithelial injury repair in short-term. rhEGF has a good effect on corneal epithelial wound healing, without the promote effect of CNV too. They could be one choice of long-term medication for the chronic corneal erosion, especially for the treatment of chronic exposure keratitis.

The animal model constructed in this study combined classical suture-induced neovessels and corneal central epithelium abrasion, which was important for studying both corneal epithelium injury repair and corneal neovessels²¹. Because a suture-induced animal model could control the scope, depth, and length of corneal neovessels more easily than an alkali burn animal model. Also, alkali burns involve masses of immunopathology processes: neutrophilic granulocyte infiltration as

well as the release of collagenase, which dissolves corneal collagen structures, even corneal stroma solution or perforation²²⁻²⁵. It was clear that corneal epithelial abrasion had some interaction with suture-induced methods, which had an influence on the speed of corneal wound healing and CNV. However, the result above was very significant for the study of corneal epithelial wound healing and CNV at the same time in the short term. In animal experiments, on day 13 after operation, when the length of one corneal neovessels achieved the corneal radius, the sutures were removed in the three groups and, then, the regression velocity of CNV was observed. The regression of CNV in the DCBE and rhEGF groups was found, which was not significant difference compared with NS group. It indicated that both DCBE and rhEGF did not promote CNV obviously.

It is known that, rhEGF and DCBE with different repair mechanism and speciality, have been widely applied in China.

Conclusions

Oculists should choose suitable type of medicine according to different property of corneal epithelial injury to achieve better curative effects, depending on three major factors: position, depth and property. DCBE could obviously release patients with ocular uncomfortable sensation-especially without the promote effect of CNV. rhEGF has promotive effects on corneal epithelium injury repair, without the promote effect of CNV too. Therefore, both DCBE and rhEGF can be administered for simple mechanical corneal epithelial injury. However, many ocular surface disease with chronic inflammatory stimulus or foreign body sensation, demanding repetatus treatment such as chronic corneal ulcers, keratoconjunctivitis sicca, dry eye and corneal epitheliopathy, rhEGF combined with DCBE is suggested for them.

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Conflict of interest

The Authors declare that they have no conflict of interests.

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