Safety, tolerability, and serum/tear pharmacokinetics of human recombinant epidermal growth factor eyedrops in healthy subjects

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Abstract

Aims: The aim of this study was to evaluate the safety/tolerability, and pharmacokinetics (PKs) of rhEGF eyedrops after administration of a single and multiple doses in healthy subjects. Methods: A phase 1, randomized, double-blind, placebo-controlled, and single ascending dose (SAD) and multiple ascending dose (MAD) study were conducted in 3 dose groups (10, 50, and 100 μg/mL). The subjects randomly received rhEGF eyedrops or their placebo in a 3:1 ratio. Serial blood and tear samples for PK analysis were collected up to 36 h and 180 h post-dose in SAD and MAD study, respectively. In addition, the serum and tear EGF concentrations were measured. Immunogenicity evaluations were conducted using serum anti-EGF antibody level. Results: A total of 50 subjects were enrolled and 48 subjects completed the study. Adverse drug reactions were mild and transient. There were no serious adverse events in this study. The tear EGF concentrations rapidly increased and returned to baseline after 4 hours without serum EGF level change after the administration of rhEGF eyedrops. Conclusion: rhEGF eyedrops were safe and well-tolerated in healthy subjects in a dose range of 10-100 μg/mL, which indicated it was suitable for further studies for corneal injury patients.

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*The authors confirm that the Principal Investigator for this paper is Jae-Yong Chung and that he had direct clinical responsibility for study participants.

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DATA SHARING: Please contact the corresponding author for questions concerning data sharing.

ABSTRACT

Aims: The aim of this study was to evaluate the safety/tolerability, and pharmacokinetics (PKs) of rhEGF eyedrops after administration of a single and multiple doses in healthy subjects.

Methods: A phase 1, randomized, double-blind, placebo-controlled, and single ascending dose (SAD) and multiple ascending dose (MAD) study were conducted in 3 dose groups (10, 50, and 100 μg/mL). The subjects randomly received rhEGF eyedrops or their placebo in a 3:1 ratio. Serial blood and tear samples for PK analysis were collected up to 36 h and 180 h post-dose in SAD and MAD study, respectively. In addition, the serum and tear EGF concentrations were measured. Immunogenicity evaluations were conducted using serum anti-EGF antibody level.

Results: A total of 50 subjects were enrolled and 48 subjects completed the study. Adverse drug reactions were mild and transient. There were no serious adverse events in this study. The tear EGF concentrations rapidly increased and returned to baseline after 4 hours without serum EGF level change after the administration of rhEGF eyedrops.

Conclusion: rhEGF eyedrops were safe and well-tolerated in healthy subjects in a dose range of 10-100 μg/mL, which indicated it was suitable for further studies for corneal injury patients.

What is already known

- Coneal injury is a common eye disease which may result in vision-threatening situation.
- Epidermal growth factor involves corneal healing, which may be a therapeutic option of corneal injury.
- Human recombinant epidermal growth factor eyedrops have shown the effect in vitro and animal study in the treatment of corneal injury, however, there are no published human data.

What this study adds

- Human recombinant epidermal growth factor eyedrops were safe and well-tolerated in healthy subjects.
- This study informed serum and tear pharmacokinetics after single and multiple administration of human recombinant epidermal growth factor eyedrops.
- This study provided information for further clinical study for corneal injury treatment.

Keywords: dry eye disease, epidermal growth factor, first-in-human study, pharmacokinetics

INTRODUCTION

Corneal injury is a common disease which is accounting for approximately 3% of emergency department visits. [1] Cornea can be injured from numerous causes including oculopathy, mechanical trauma, infection, inflammation, chemicals, and radiation. [2] Corneal injury is important because it may be vision-threatening. However, there are no absolute medical therapies for it.

Corneal healing consists of a complex process involving cell death, migration, proliferation, differentiation, and extracellular matrix remodeling. [3] In addition, limbal stem cells and basement membrane remodeling have key roles in corneal healing. [4] Numerous cytokines, chemokines, and growth factors are involved in corneal healing.

Epidermal growth factor (EGF) is one of the growth factors which is secreted from lacrimal gland. [5] EGF involves corneal healing; it regenerates limbal stem cells and regulates migration of corneal cells thus accelerating corneal healing. [6, 7] In addition, previous studies showed that tear EGF concentration was significantly decreased in dry eye syndrome which is the one of common causes of corneal injury. [8] Therefore, EGF has been thought a treatment option for corneal injury.

For the treatment of corneal injury, the topical eye administration of EGF has been used through cord blood serum, autologous serum, amniotic membrane extract, and amniotic membrane transplantation. [9-12] However, the currently implemented treatment options may not be wildly used because these mass produce for treatment options is difficult, not only infection risk is relatively high. Therefore, human recombinant EGF (rhEGF) eyedrops can be an appropriate treatment option to solve the above problems.

Some studies showed the effect of rhEGF eyedrops in vitro study and animal study. [7, 13] However, there are no published human data about rhEGF eyedrops. The purpose of this study was to evaluate the safety/tolerability, and pharmacokinetics (PKs) of EGF in serum and tear after topical administration of a single and multiple doses of rhEGF eyedrops in healthy subjects.

METHODS

This study was conducted at Seoul National University Bundang Hospital (SNUBH). The study protocol was approved by the Institutional Review Board of SNUBH (No. B-1902/522-005) This study was conducted according to the major ethical principles of the Declaration of Helsinki and Good Clinical Practice Guidelines. All subjects provided their written informed consent before any study-related procedures.

Study design and subjects

This study was a phase 1, randomized, double-blind, placebo-controlled, and single ascending dose (SAD) and multiple ascending dose (MAD) study. The subjects were enrolled and randomly assigned to the rhEGF eyedrops (10, 50, or 100 μg/mL) or placebo in a 3:1 ratio. The subjects received rhEGF or placebo twice a day for one day and 14 days in the SAD and the MAD study, respectively. The subjects who were assigned to the rhEGF eyedrops received rhEGF eyedrop in one eye which was randomly assigned and placebo in the other eye. Dose escalation was determeind based on the evaluation of safety/tolerability data from the previous dose groups. In addition, whether to proceed to the MAD study was determined based on the safety/tolerability results of the SAD study.

In this study, healthy male subjects who had bodyweight of 50-100 kg and body mass index more than 18.0 kg/m^2 with 19-50 years of age were eligible for enrollment into the study if no clinically significant abnormalities were observed in medical history, physical and ophthalmic examinations, clinical laboratory tests, vital signs, and 12-lead electrocardiograms (ECG).

Pharmacokinetic sampling and bioanalysis

For PK sampling, serial blood and tear samples of EGF were collected at the scheduled times; pre-dose and 0.25h, 0.5h, 1h, 2h, 4h, and 12h at -1d and 1d, and pre-dose and 12h at 2d after dosing for dose groups of SAD study; pre-dose and 0.25h, 0.5h, 1h, 2h, 4h, and 12h at -1d, 1d and 14d, pre-dose at 2d, 8d, and pre-dose and 12h at 15d after dosing for dose groups of MAD study. Tear samples were collected from the marginal tear strip of the lower lid near the medial canthus by using disposable microcapillaries.

Serum and tear concentrations of EGF were analyzed by a validated enzyme linked immunoassay (ELISA) using a human EGF Quantikine ELISA kit (R&D systems, Minneapolis, MN, USA). Serum and tear samples were diluted appropriately in the Assay Diluent RD1-6. EGF calibration standards were prepared at the

following concentrations: 3.91, 7.81, 15.6, 31.3, 62.5, 125, and 250 pg/mL. 200 μL of diluted sample or standard was added to the plate and incubated for 2 hours at room temperature.

After the incubation, the plates were washed three times with the wash buffer. 200 μL of human EGF conjugate was added to each well then incubated for 2 hours at room temperature. After the incubation, the plates were washed three times with the wash buffer. 200 μL of the substrate was added to each well and incubated for 20 minutes at room temperature. Then, 50 μL of reaction termination solution was added to each well to terminate the reaction. The subsequent absorbance was quantified by measurement at 450 nm using a VersaMax Microplate Reader (Molecular Devices, San Jose, CA, USA) and the results were analyzed using Softmax Pro 7.1 GxP (Molecular Devices, San Jose, CA, USA).

Pharmacokinetic analysis

Noncompartmental analysis was performed to calculate the PK parameters of serum and tear EGF using Phoenix®WinNonlin® software version 8.0 (Certara, St. Louis, MO, USA). The observed serum concentrations and times were used to estimate the maximum concentrations (C_{max}) and the observed serum concentration after 12 hours of the administration (C_{12h}) of serum and tear EGF and the time to reach C_{max} (T_{max}). The area under the concentration-time curve from the pre-dose to 12 hours ($AUC_{0\text{-}12h}$) was calculated using the linear up/log down trapezoidal method.

Immunogenicity evaluations

Anti-EGF antibodies were determined for assessment of immunogenicity from serum samples at the scheduled times; prior to following the rhEGF eyedrops administration at week 4 in the SAD study and prior to following the rhEGF eyedrops administration at week 1, 2, and 6 in the MAD study. The anti-EGF antibodies were determined using validated ELISA. The confirmation of specificity used a floating cut point.

Safety/tolerability evaluations

Safety/tolerability evaluations including adverse events (AEs), physical examinations, clinical laboratory tests, vital sign measurements, and ECG were conducted In addition, ophthalmic examinations including best corrected visual acuity, intraocular pressure, refractive error, slit-lamp examination, fundus examination, tear break-up time test, Schirmer's test, and ocular surface disease index (OSDI) were conducted.

RESULTS

Demography

Fifty subjects (twenty-five each in SAD and in MAD study) were enrolled. A total of 48 subjects completed this study as planned. Two subjects (one each in SAD and MAD study) dropped out due to withdrawal of their own consent. There were no significant differences in demographic characteristics among the dose group in SAD and MAD study, respectively (Supplementary Table 1).

Safety

rhEGF eyedrops were well tolerated in all dose groups in SAD and MAD study. There were 6 adverse drug reactions (ADRs) by 5 subjects treated with rhEGF eyedrops and 2 ADRs by 2 subjects treated with placebo in SAD study, and 6 ADRs by 5 subjects treated with rhEGF eyedrops and 5 ADRs by 4 subjects with placebo in MAD study, respectively (Table 1). All ADRs were mild and transient, and there were no serious adverse events. The most common ADR was corneal erosion probably resulted from tear sampling procedure, which was reported 6 times by 6 subjects and 8 times by 7 subjects in SAD and MAD study, respectively. There were no significant differences in the incidences of ADRs in SAD (p -value 0.7660. Fisher's exact test) and MAD (p -value 0.4631. Fisher's exact test) studies, respectively.

There were no clinically significant findings for vital signs, physical examinations, and ECG. In addition, there were no changes in ophthalmic examinations after the administrations of rhEGF eyedrops (Supplementary Table 2).

Pharmacokinetics

After administration of rhEGF eyedrops, the mean serum EGF concentration showed numerous peaks, but it did not change compared to those of baseline and placebo, while the mean tear EGF concentrations increased compared to those of baseline and placebo in SAD and MAD studies (Figure 1, Figure 2). rhEGF eyedrops were rapidly absorbed with tear T_{max} at ranges of 0.18-0.2 and 0.18–0.27 hours in SAD and MAD studies, respectively (Table 3). Then, the mean tear EGF concentrations became similar compared to those of baseline and placebo after 4 hours of administration in SAD and MAD studies. Tear C_{max} and $AUC_{0\text{-}12h}$ increased by dose in SAD and MAD studies. However, Tear C_{12h} did not change by rhEGF eyedrops in SAD and MAD studies.

Immunogenicity

After administration of rhEGF eyedrops, no anti-EGF antibodies were detected in SAD and MAD studies. Although anti-EGF antibodies were detected in a subject in the placebo group before the administration of rhEGF eyedrops, it was not detected after administration of the placebo. (Supplementary Table 3).

DISCUSSIONS

This study was the first-in-human study to evaluate the safety/tolerability, and PK of rhEGF eyedrops after single and multiple administrations in healthy male subjects. There were some published EGF eyedrops in corneal injury including dry eye syndrome, however, there has been no PK studies of EGF eyedrops. [14] Therefore, this study was the first published PK study for rhEGF eyedrops.

The one of the primary goals of first-in-human study were to determine safety/tolerability. Considering the safety profiles, rhEGF eyedrops at doses ranging from 10 μg/mL to 100 μg/mL in SAD and MAD studies were well tolerated. No subjects experienced serious adverse events. Among subjects who received rhEGF, corneal erosion was the most common ADR. The incidences of corneal erosion showed similar among dose groups including placebo. In addition, corneal erosions occurred in medioinferior side in which tear sampling was commonly conducted. Therefore, corneal erosion may be caused by tear sampling procedures rather than rhEGF eyedrops.

After administrations of rhEGF eyedrops, there were numerous peaks in time-serum EGF concentration profiles. Serum EGF levels show high variability through numerous causes including circadian rhythm, ultradian rhythm, and venous puncture. [15, 16] In addition, normal serum EGF levels are 0.1-1.281 μg/L. [16, 17] In this study, all of the serum EGF levels were within the normal serum EGF levels. Therefore, the numerous peaks are thought to be due to ultradian rhythm rather than rhEGF eyedrops, and rhEGF eyedrops did not affect serum EGF levels. Given that high serum EGF level is associated with numerous toxicities including esophageal adenocarcinoma and non-small lung cancer, rhEGF eyedrops can be administered without systemic toxicity. [17-20]

In this study, tear EGF was rapidly absorbed, then, tear EGF concentrations became similar compared to those of baseline and placebo after 4 hours without accumulation. The rapid decline of tear EGF concentrations may result from precorneal fluid drainage including nasolacrimal drainage and blinking despite the lack of clear mechanisms of rhEGF binding to ocular tissue.[21] Further study may be needed to find the mechanism of corneal binding of rhEGF after administration of rhEGF eyedrops. The absence of tear EGF accumulation means that the possibility of toxicity due to the accumulation of rhEGF eyedrops is low during long-term administration.

There has been no published data of therapeutic tear EGF concentrations for corneal injury in human. Some *in vitro* studies reported that the therapeutic EGF concentrations was 1–10 μg/L.[22, 23] In our study, the average tear EGF concentrations among dose groups were 0.74-18.8 μg/L and 2.6-12.4 μg/L in SAD and MAD studies, respectively. Therefore, 50 μg/mL of rhEGF eyedrop twice a day may be considered a therapeutic dose because the average tear EGF concentrations in the dose group of 50 μg/mL were 5.8 μg/L and 4.1 μg/L in SAD and MAD studies, respectively.

In this study, tear EGF exposure did not show dose-linearity although tear EGF exposure increased by dose. The amount of tear may affect the tear EGF concentration, which is thought to have influenced the PK variability of EGF after administration of rhEGF eyedrops. Considering the PK variability and lack of accumulation of rhEGF eyedrops, trough tear EGF concentration is not thought a suitable biomarker for the evaluation of the efficacy of rhEGF eyedrops.

Although rhEGF eyedrops have been emerging as a good treatment option for corneal injury, no appropriate dose of rhEGF eyedrops has been known. There are some studies showing that higher dose of EGF eyedrops might be inappropriate for the treatment of the corneal injury because auto-inhibition may occur by high dose of EGF, however, these studies did now show any dose-tear EGF concentration relathionships. [22, 24, 25] Therefore, further study for corneal injury patients may be needed considering tear PK variability and safety profile of rhEGF eyedrops.

In this study, no anti-EGF antibodies were detected except for a subject in placebo before rhEGF administration. Generally, there are numerous soluble proteins which bind with EGF including human EGF receptor 1, human EGF receptor 2, and arginine esterase. [26-28] Therefore, rhEGF eyedrops might be administered without decrease in the efficacy due to anti-EGF antibody formation even if administered for a long time.

There are some limitations in this study. This study was performed in healthy subjects to minimize confounding factors that could influence the study results. Further study for corneal injury patients is needed to evaluate the efficacy of the drug. There were missing values of tear PK sampling due to the difficulty of the sample collection. Nevertheless, the PK profile could be observed in all dose groups.

In conclusion, dose range from 10-100 μg/mL solution of rhEGF eyedrops in single and multiple administration was safe and well tolerated in healthy male subjects. All ADRs were mild and transient. rhEGF eyedrops increased tear EGF level without effect on systemic exposure. The results of this study justifies further evaluation of the efficacy and safety of rhEGF eyedrops for corneal injury patients.

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