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Pharmacokinetics of topically applied recombinant human keratinocyte growth factor-2 in alkali-burned and intact rabbit eye

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ABSTRACT

Keratinocyte growth factor-2 (KGF-2), an effective agent in the development of epithelial tissue and regeneration during corneal wound healing, is a potential therapeutic option to treat the corneal diseases with corneal epithelial defects. However the tissue distribution and pharmacokinetics of KGF-2 have not been explored yet in eye upon topical application. Using ^{125}I-labeled recombinant human KGF-2 (^{125}I-rhKGF-2), tissue distribution of rhKGF-2 in alkali-burned and control rabbit eyes was studied. Our results revealed that ^{125}I-rhKGF-2 was distributed to all eye tissues examined. The highest radioactivity level was found in the cornea, followed by iris, sclera, ciliary body, lens, aqueous humor, vitreous body, and serum in a greatest to least order. The levels of ^{125}I-rhKGF-2 were higher in corneas of alkali-burned eyes than those in control eyes though without statistical significance. Calculated pharmacokinetic parameters of $t_{1/2}$, C_{max}, and T_{max} of rhKGF-2 in the rabbit corneas were 3.4 h, 135.2 ng/ml, and 0.5 h, respectively. In iris, lens, aqueous humor, and tear, $t_{1/2}$, C_{max}, and T_{max} values were 6.2, 6.5, 5.2, and 2.5 h; 23.2, 4.5, 24.1, and 29,498.9 ng/ml; and 1.0, 0.5, 0.5, and 1.0 h, respectively. Predominant and rapid accumulation of rhKGF-2 in corneas suggests that therapeutic doses of rhKGF-2 could be delivered by topical application for treatment of corneal diseases.

1. Introduction

Keratinocyte growth factor-2 (KGF-2), also called fibroblast growth factor-10 (FGF-10), is a soluble 170-amino-acid polypeptide secreted by fibroblasts and endothelial cells that acts primarily on epithelial cells (Saksena et al., 2013). Its diverse effects are mainly mediated by FGF-2 IIIb receptor, a transmembrane receptor, expressed exclusively by epithelial cells (Wang et al., 2009). KGF-2 promotes the growth, proliferation, and differentiation of epithelial cells (Kruse and Tseng, 1993; Marchese et al., 2001). It has been extensively investigated for its promising effects in treating epithelial damage (Han et al., 2000; Jimenez and Rampy, 1999; Miceli et al., 1999; She et al., 2012; Smith et al., 2000; Xia et al., 1999).

The surface of the cornea consists of a stratified squamous epithelium that must be continuously renewed (Pajoohesh-Ganji and Stepp, 2005). The corneal epithelium plays important roles in the maintenance of corneal function and integrity. Corneal surface injuries are among the most frequent traumas of the eye, however, no commercial eye drops have been approved for clinical treatment (Martin et al., 2013). Corneal epithelium is very vulnerable to chemical, thermal, and mechanic injury. Chemical and thermal injuries are often used in animal model study. Chemical injury accounts for up to one-fifth of ocular traumas. Alkali injury happens

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more frequently than acid injury as alkali materials are more commonly used in building materials and cleaning agents (Wagoner, 1997). Since it can promote corneal epithelial cell growth and inhibit injury-induced neovascularization, KGF-2 may be a therapeutic option to treat corneal diseases with corneal epithelial defects. Topical application of KGF-2 enhanced corneal wound healing in a rabbit model of carbon dioxide laser-induced corneal Injury (Wang et al., 2010). In consistent with our report, topical administration of KGF-2 has been shown to significantly enhance corneal wound healing in rabbit alkali-burned corneas (Liu et al., 2007). The promising wound healing effect of KGF-2 on injured corneas encourages us to perform pharmacokinetic study, a critical component in drug development (Chen et al., 2012). Tissue distribution and pharmacokinetics of KGF-2 upon topical application have not been explored for in eye and are paramount in the characterization of both physiological and pathological conditions, particularly those of the cornea.

We have successfully developed a rapid and efficient expression and purification system for large-scale production of biologically active KGF-2 (Wu et al., 2009), which has been modified to improve biostability and reduce immunogenicity (Huang et al., 2009). KGF-2 produced by this system significantly promoted the proliferation of conjunctival epithelial cells in vitro and stimulated the expression and synthesis of mucins (Ma et al., 2011). By topical application of this recombinant KGF-2, we studied tissue distribution of rhKGF-2 in alkali-burned and control rabbit eyes and determined pharmacokinetic parameters of rhKGF-2 in cornea, iris, aqueous humor, and lens. Our results showed that KGF-2 predominantly distributes in corneas and alkali injury leads to further accumulation of KGF-2 in the corneas.

2. Materials and methods

2.1. Preparation and biological activity assay of ^{125}I-rhKGF-2

RhKGF-2 protein was produced as previously described (Wang et al., 2010). 1 mg iodogen was dissolved in 0.5 ml chloroform. Iodogen solution was aliquoted into the tubes (50 μL, each) and dried with nitrogen gas. 6 mg rhKGF-2 and 4 mCi Na ^{125}I (PerkinElmer™) were pipetted into the tube, mixed, and kept at 15 °C for 30 min for iodination reaction. The reaction mixture was chromatographed on Sephacryl S-300 HR (1 × 50 cm, GE Health, Connecticut, USA). The purity of ^{125}I-rhKGF-2 was confirmed by high-performance liquid chromatography (HPLC).

The biological activity of ^{125}I-rhKGF-2 was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) proliferation assay of NIH3T3 cells. Briefly, NIH3T3 cells at 7×10^3 cells per well were seeded in 96-well plates for 24 h in DMEM with 10% fetal bovine serum and then starved for overnight in DMEM with 0.5% fetal bovine serum. ^{125}I-rhKGF-2 or rhKGF-2 was diluted in DMEM supplemented with 0.5% fetal bovine serum to prepare samples of different concentrations. Starved NIH3T3 cells were incubated for 48 h with 0, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 μg/ml of ^{125}I-rhKGF-2 or rhKGF-2 (final volume, 100 μl/well) in 6 replicates and 4 replicates respectively in each concentration. 0.5% fetal bovine serum was used as blank control. At the end of the incubation, the medium was replaced with 50 μl of MTT solution (0.5 mg/ml in PBS buffer; Sigma–Aldrich Corp., St. Louis, MO, USA) and the plates were incubated at 37 °C for 4 h followed by the addition of 200 μl of dimethyl sulfoxide to each well and incubation with shaking at 37 °C for 20 min to ensure complete dissolution of the formazan crystals. The plates were read at 570 nm with SpectraMax M2 microplate reader (Molecular Devices, USA). Cell proliferation rates were presented as percentage of control.

2.2. Animals protocols

Japanese white rabbits, obtained from Laboratory Animal Center of Academy of Military Medical Science, Beijing, China, were handled in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All animal protocols in this study (license No. SYXK (jing) 2013-0007) were approved by the Institutional Animal Care and Use Committee (IACUC) of the Academy of Military Medical Science. All procedures were performed under deep anesthesia using 30 mg/kg of ketamine and proparacaine hydrochloride (0.5%) was used for topical anesthesia. A round filter paper with 6 mm diameter was soaked for 3 s in 0.5 N NaOH and then applied to the cornea of one eye for 30 s. The eyes were then washed with 10 mL of 0.9% NaCl. Three animals were sacrificed by air embolism at the end of each time point after topical application of ^{125}I-rhKGF-2.

2.3. Analysis of ^{125}I-rhKGF-2 with TCA-RA method

Trichloroacetic acid precipitation-radiometric assay (TCA-RA) method was used to determine the concentration of iodinated rhKGF-2 in eye tissue samples. The rabbit eye tissue samples were isolated and weighed. Approximately 0.5 g of the each tissue sample was individually mixed with 5 volumes (w/w) of deionized water in a clean tube and homogenized. Equal amount of each tissue homogenate was mixed with various amount of ^{125}I-rhKGF-2 to prepare a serial calibration standard of ^{125}I-rhKGF-2 at concentrations of 0.51 ng/ml, 1.49 ng/ml, 19.6 ng/ml, 463.1 ng/ml. Proteins were precipitated by addition of equal volume of 20% TCA and centrifuged at 2000 × *g* at 4 °C for 5 min. The radioactivity in the pellets was counted with a scintillation counter. The standard recovery curves of ^{125}I-rhKGF-2 from different rabbit eye tissues were generated by graphing experimental data against added concentrations of ^{125}I-rhKGF-2. The limit of quantitation (LoQ), linearity and accuracy of the method were determined and validated.

2.4. Tissue distribution and pharmacokinetics of ^{125}I-rhKGF-2 in rabbit eye tissues

Twenty-four Japanese white rabbits, approximately 2.5–3.0 kg each, were randomly assigned into two groups, 12 animals each. In treated group, rabbit eyes were burned with NaOH. Rabbit eyes in control group received a vehicle treatment. Three animals in each group were sacrificed at 1, 2, 4, and 24 h after topical application of 50 μg/mL ^{125}I-rhKGF-2. Cornea, iris, ciliary body, sclera, lens, aqueous humor, vitreous body, and serum samples were isolated and weighed. Another 33 Japanese white rabbits were given topical application of ^{125}I-rhKGF-2 50 μL (2032.27 Bq, 25 μg/ml). Three animals were sacrificed by air embolism at time points 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 48 h after topical application of ^{125}I-rhKGF-2. Cornea, aqueous humor, iris and lens tissue samples were isolated and weighed. Each aliquot of tissue homogenate was vortex-mixed with the same volume of 20% TCA to precipitate proteins, and then was centrifuged at 2000 × *g* at 4 °C for 5 min. The total radioactivity assay and the radioactivity assay after precipitation with trichloroacetic acid (TCA-RA method) were both used to determine the tissue distribution and pharmacokinetics of ^{125}I-rhKGF-2.

2.5. Data and statistical analysis

Pharmacokinetic parameters were calculated using the software package DAS (version 3.0, Bontz Inc., Beijing, China). The terminal elimination half-life ($t_{1/2}$) was obtained by non-compartmental analysis of ^{125}I-rhKGF-2 concentrations in the eye tissues. Maximum concentration (C_{max}) and the time to reach the