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Topical treatment of corneal alkali burns with Gly-thymosin β_4 solutions and *in situ* hydrogels *via* inhibiting corneal neovascularization and improving corneal epidermal recovery in experimental rabbits

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ABSTRACT

Aim: Corneal alkali burns are a severe disease and commonly encountered in the emergent clinic. A rapid medical treatment for the burn is very important. Gly-thymosin β_4 (Gly-Tβ_4) is a biomimic derivative of natural thymosin β_4. The aim of this study is to evaluate the corneal recovery effects of Gly-Tβ_4 topical therapy on alkali burns in rabbit corneas.
Methods: Rabbit alkali burns were induced with NaOH-contained filter paper. Phosphate-buffered solutions at pH 7.0, Gly-Tβ_4 solutions, blank *in situ* hydrogels, and Gly-Tβ_4 *in situ* hydrogels were dropped on the burned corneas. The treatments were continued for 14 days. Conjunctiva hyperemia, corneal edema, intraeye extravasation, hemorrhaging, corneal neovascularization (CNV), and corneal opacity were observed. Corneal immunohistochemistry and histopathology were performed.
Results: Gly-Tβ_4 solutions led to a lower corneal burn index than the other regimens. Hydrogels may stimulate the burned corneas due to the direct contact of them, and prevent the rapid release of Gly-Tβ_4. Gly-Tβ_4 significantly inhibited CNV according to the images of the corneas, CNV areas, and CD31 expression. Furthermore, Gly-Tβ_4 improved corneal epidermal recovery according to the histopathological result.
Conclusion: Gly-Tβ_4 solutions are a promising formulation for topical treatment of corneal alkali burns.

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1. Introduction

Cornea is the outer most tissue of the eye, also as one of most readily burned tissues. Corneal burns may result from many factors, *e.g.* mechanical damage, chemical damage, and infections. Alkali-induced corneal burns are commonly encountered in the emergent clinic. A rapid medical treatment of alkali-induced corneal burns is very important for the future corneal functional recovery [1]. Irrigation with saline or buffered solutions must be immediately applied when alkali-induced corneal burns occur [2]. However, simple irrigation only eliminates the residual alkali agents on the surface of cornea, but not is helpful for the corneal recovery. Delays of corneal recovery would lead to the formation of corneal scars, corneal neovascularization (CNV), and potentially permanent blindness [3]. Therefore, a subsequent sustained medical treatment is necessary to improve corneal recovery besides irrigation.

Thymosin β_4 ($T\beta_4$) is a natural small 43-amino acid intracellular peptide that is first isolated from bovine thymus tissues [4]. $T\beta_4$ has been used successfully in several clinical trials involving tissue repair and regeneration [5]. A biomimic derivative of $T\beta_4$, Gly-thymosin β_4 (Gly-$T\beta_4$), was developed in our lab [6]. Gly-$T\beta_4$ consists of 44-amino acids with a single glycerin terminal residue addition to $T\beta_4$. The Gly-$T\beta_4$ amino acid sequence is listed as the following: GSDKP DMAEI EKFDK SKLKK TETQE KNPLP SKETI EQEKQ AGES.

Here we report that Gly-$T\beta_4$ exhibits effective topical therapy of corneal alkali burns. Moreover, the mechanism of Gly-$T\beta_4$ improving corneal recovery was deeply explored in this study. Two formulations of Gly-$T\beta_4$ were applied and compared, including solutions and *in situ* hydrogels.

2. Materials and methods

2.1. Materials

Gly-$T\beta_4$ was prepared by Beijing Northland Biotech Co., Ltd. (Beijing, China). Their concentrated solutions in the pH 7.0 phosphate buffered solutions were preserved at −20°C before dilution and application. Two *in situ* hydrogel-forming materials, *i.e.* poloxamers 188 and 407, were purchased from BASF (Ludwigshafen, Germany). Purified water was prepared with Heal Force® Super NW Water System (Shanghai Canrex Analytic Instrument Co., Ltd., China), and always used unless otherwise indicated.

2.2. Animals

New Zealand albino rabbits (8–12 weeks, 2.0–2.5 kg) from the Laboratory Animal Center of the Beijing Institute of Radiation Medicine (BIRM) were used. The handling and surgical procedures of animals were conducted strictly according to the Guiding Principles for the Use of Laboratory Animals. All of the studies were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals. The animal experiments were approved by the Animal Care Committee of BIRM. The research followed the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.3. Preparation of eye drops

Two formulations of Gly-$T\beta_4$ eye drops were prepared. One formulation included a buffered solution containing Gly-$T\beta_4$ (50 μg/ml) that was created by diluting the concentrated Gly-$T\beta_4$ in the Buffer A (10 mM KH_2PO_4 and 0.15 M NaCl adjusted with 0.1 M NaOH to a pH of 7.0). The other formulation was the *in situ* hydrogel containing Gly-$T\beta_4$ (50 μg/ml). The preparation of hydrogels was performed according to our previously reported method [7]. The procedure was briefly described as follows. Poloxamer 188 (0.43 g) and poloxamer 407 (2.41 g) were dissolved in water (6 ml) followed by addition of the Gly-$T\beta_4$ concentrated solution and some water to obtain Gly-$T\beta_4$ *in situ* hydrogels (50 μg/ml). This temperature-sensitive hydrogel had a gelation temperature of 27.5°C.

2.4. Corneal alkali burn

A slit lamp microscope (Chongqing Sun Kingdom Medical Instrument Co., Ltd., China) was used to observe the anterior segments and accessory organs of rabbits. A chloral hydrate solution (10%) was intraperitoneally (i.p.) injected to anesthetize the rabbits. Oxybuprocaine hydrochloride eye drops were dropped onto the eyes three times with 5 min intervals. An eye speculum was used to open the eyes and the liquids were subsequently removed with medical cotton. Filter paper (Type 3, Whatman Co., USA) was cut into the circular discs of 8 mm in diameter. An aliquot (20 μl) of 1M NaOH solution was dropped onto the discs that maintained for 30 s. The wet disc was transferred to the center of the eye surface and maintained for 60 s before the subsequent removal of the paper. Saline (60 ml) was immediately used to irrigate the burned eye for more than 2 min. The corneal alkali burn models were considered to be successfully constructed if corneal matrix edema and corneal opacity were observed, and the irises were minimally visible. If either infections or the formation of holes appeared in the cornea, the eyes were withdrawn.

2.5. Treatment of corneal alkali burns

The burned eyes were separately treated with the Buffer A (pH 7.0, 40 μl), the Gly-$T\beta_4$ solution (50 μg/ml, 40 μl), the blank *in situ* gel, and the Gly-$T\beta_4$ *in situ* hydrogel (50 μg/ml, 40 μl). The treatments were repeated four times each day and continued for 14 days. The anterior eye segments and accessory organs of the rabbits were observed with the slit lamp microscope on Days 1, 3, 7, and 14. One hour post-treatment, 0.3% norfloxacin eye drops were topically applied to the eye for prevention of bacterial infection for three times per day within the first three days [8,9]. Additionally, 0.5% erythromycin eye cream was topically applied to the eye surfaces every night for the first three days.

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