Biocompatibility and drug release behavior of chitosan/poly (vinyl alcohol) corneal shield in vivo

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Abstract: Background: Chitosan/polyvinyl alcohol corneal cap has good biocompatibility and drug slow release characteristics, which provided new treatment method for anterior segment disease. Our study was to evaluate biocompatibility of poly (vinyl alcohol)/chitosan corneal shield’s intraocular and investigate its feasibility to treat ocular surface disorders. Methods: Thirty-six white rabbits were randomly divided into four groups. Slit lamp observation were conducted at 1, 3, 7 and 10 days after operation. Concomitant and conjunctiva tissue harvested from the experimental groups was observed by HE staining 10 days after operation. The aqueous humor was aspirated from the anterior chamber at each designated time point (1, 3, 7 and 10 days). The cornea and conjunctive were collected at 10 days. The concentration of each tissue was analyzed by ultra-performance liquid chromatography and microscope observation. Results: In all groups, mild hyperemia was observed 1 day after operation, and there was no obvious inflammatory reaction occurring on the seventh and tenth day. No corneal edema and inflammatory reaction of anterior chamber occurred till the tenth day. For histopathology, there was no obviously mild chronic and inflammatory reaction occurred, and no significant difference between the corneal shield with-in groups and with-out groups. The drug concentrations in corneal and conjunctival in group (A, B) were significantly lower than eye drops in the control group (C, D), and blank corneal cover in group C was significantly sham operation in group D. Conclusion: The results indicated that the proposed membrane combined with ophthalmic solution has substantial potential as ocular delivery system.

Keywords: Chitosan, poly (vinyl alcohol), corneal shield, drug delivery, ocular tolerance, biocompatibility

Introduction

Eye drops are the most commonly treatment methods used for anterior segment diseases such as infectious conjunctivitis, keratitis and the others. However, eye drops are constantly diluted by tears, which results in the shorty contact time between eye drops and eye tissue. Moreover, reflex tears increase and further exacerbate the dilution and loss due to the stimulation of eye drops. It has been reported that the bioavailability of eye drops was only about 1% [1]. To address the inadequacy of the traditional local administration, many scholars have committed to the new ophthalmic drug delivery ways recently, and sustained-release carrier has become a hot topic. Although some new materials used for delivering drugs are emerging, they still have some common shortcomings, such as organization of irritation, local granuloma formation, repeated administration difficult and expensive [2]. To overcome some shortcomings of the traditional administration, many drug delivery carrier have been prepared which contains chitosan [3-5] or polyvinyl alcohol [6], and the toxicity or stimulation on eyes was evaluated by applied on the ocular surface or intraocular of experimental animal. However, the side effect of chitosan or PVA sustained-release carrier has not been evaluated in respect to histopathology.

Corneal cap was a device similar to corneal contact lens. In this study, a new drug-loaded corneal cap which was applied to the rabbit eye surface was prepared by using chitosan/poly (vinyl alcohol) as the carrier material and levofloxacin as a model drug. Biopsy under the slit-lamp biomicroscopy, HE staining, observing the conjunctiva and corneal histopathological
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change with transmission electron microscopy were performed to verify the histocompatibility. The drug concentration in the tissues such as aqueous, cornea, conjunctiva was analyzed by tandem mass spectrometry to observe the drug sustained-release effect.

Materials and methods

Animals

Thirty-six healthy New Zealand white rabbits of both sexes, weighing 2.0~2.5 kg, were used in this study. The animals were provided by Shanghai Sheng Wang Experimental Animal Breeding Ltd. All of the methods used in this study were approved by the Animal Ethics Committee, Wenzhou Medical College, and conformed to the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983). No abnormal eyes were observed by slit lamp or ophthalmoscopy before the experiment. The right eye was experimental, the other was control. The animals were wore corneal cap and randomly divided into four groups: A) group wore 2 mg/g Chitosan/Poly drug-loaded of corneal cap, B) group wore 4 mg/g Chitosan/Poly drug-loaded corneal cap, C) group wore blank corneal cap, levofloxacin eye drops was dropped (two times/day), D) group not wore any corneal cap, levofloxacin eye drops was dropped (four times/day).

Reagents

Levofloxacin ophthalmic solution (Santen Pharmaceutical Co., Ltd.); ketamine hydrochloride injection (Fujian Kutian Pharmaceutical Co., Ltd.); hydrochloric acid toluene thiazide (Nanjing Pharma Chemical Plant); sodium pentobarbital (Beijing Chemical Reagent Company); povidone iodine solution (the Zhejiang Apeloa Pharmaceutical Co., Ltd.).

Surgical procedure

The corneal shield (14 mm) was dipped in 1 ml care solution for 3 seconds to clean the attachment drug on the surface, and then was sutured 8 pins at the corneoscleral limbus with 10-0 nylon thread during the preoperative period. After surgery, the rats in group C and group D were treated with levofloxacin eye drops 2 times/day and 4 times/day, respectively. The eye surgical situation, such as conjunctival hyperemia, corneal edema, hyperemia, anterior chamber cells, flare and corneal neovascularization, was observed by slit lamp microscope and photographed at the postoperative 1 day, 3 days, 7 days and 10 days. The anterior chamber liquid was extracted 50 μL with a 29 G needle insulin syringe at each time point and tested drug concentration, then and then the animals were sacrificed. The right corneas of rabbits was drilled in the ring of 10 mm diameter under a surgical microscope with the clipping of the cornea, half of the animals in each group were measured concentration, and the others used for tissue sections. The bulbar conjunctiva and the cornea of rabbits which were used for detecting concentration were removed immediately into 4°C environment; The cornea of rabbit for tissue sections were removed and placed in sterile saline rinse clean. The animals were anesthetized by a mixture of intramuscular ketamine (36 mg/kg) and xylazine (5.2 mg/kg) 7:1. before surgery, and the rabbits were sacrificed by intravenous injection with pentobarbital sodium solution at tenth day after surgery.

Draize eye irritation test

Draize eye irritation test was induced following the previously procedure [7]. OECD guideline for the eye irritation test was the most valuable and reliable method to evaluate the danger and safety of the eye or eye alternatives, the stimulation of which was evaluated by the damage to the tissues such as cornea, iris and conjunctiva. The security was evaluated by the observation of the tissues under the slit lamp.

Histopathological processing

The pathology of the corneal tissue was observed after 10 days after operation. The corneal specimens were fixed in 4% paraformaldehyde, then the samples were embedded in paraffin, sectioned with microtome at a thickness of 4 μm, stained with hematoxylin and eosin and finally observed under light microscope [7].

Aqueous sample preparation

The aqueous (10 μl) was mixed with the gatifloxacin solution (10 ng/ml, 100 μl), then added with the phosphate buffer (40 μl, pH=7.4) and acetonitrile (1 ml), swirled for one minute after centrifugated (2500 rpm, 10 min), drawn the supernatant (2.7 ml) to the bottom of the centrifuge tube, then filtered and injected. The sam-
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Samples could be dried by nitrogen and stored at -20°C after bathing in 50°C water, then remelted in mobile phase (200 μL) when ready to used.

**Figure 1.** Slit lamp photograph after operation. A: 1st after operation, bulbar conjunctiva is mild hyperemia. B: The 3rd after operation, hyperemia faded.

**Figure 2.** Histopathological changes of corneal tissue in the (A) group (A), (B) group (B), (C) group (C), (D) group (D). No abnormal changes in joint tissue were observed.

**Chromatographic conditions**

Chromatographic column: acquity UPLC BEH C18 ultra-high performance liquid column (2.1 mm x 100 mm, 1.7 μm). The mobile phase consisted of water and methanol (90:10, v/v). Gradient elution was performed with a 10-min linear gradient from 100% water to 95% methanol. The flow rate was 0.4 mL/min, and the column temperature was 50°C. The injection volume was 3 μL. The detection wavelength was set at 254 nm. The run time was 10 min.

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Results

Draize eye irritation test

No irritation of eyelid edema and tears was appeared during post-operative period. Mild hyperemia of the bulbar conjunctiva was observed on the first day after surgery, and then subsided and returned to normal on the third day (Figure 1). A small amount of folds, transparent, slow degradation, no loose and suture reign were observed on the edge of drug-loaded corneal shield. These findings indicated that the delivery vector used in this experiment did not cause toxicity or stimulation of rabbit ocular.

Pathological observation of the corneal tissue

According to the results of HE-staining, we found that the corneal tissue was normal and the epithelial cells were integrated and tightly packed. In addition, the structure of matrix layer fiber was consistent, and the elastic membrane was continuous and completed. The shape of the endothelial cell was rule. The results indicated that the corneal cap has good histocompatibility (Figure 2).

Concentration of drug concentration in aqueous humor

The drug concentration in aqueous humor which maintained a relatively constant level for a week suggested that the corneal shield was not degraded and had a certain sustained-released effect (Figure 3).

Drug concentration in cornea and conjunctiva

The levofloxacin concentration of corneal shield groups loaded chitosan/poly (group A and B) was lower than levofloxacin eye drops groups (group C and D). Moreover, the levofloxacin concentration of blank corneal shield (group C) was higher than the sham group (group D), which indicated that chitosan/poly (vinyl alcohol) corneal shield could reserve the drug and extend the retention time of drugs in ocular surface, improving drug bioavailability and reducing the frequency of medication to a certain extent (Figure 4).

Discussion

The critical question is how to improve drug bioavailability and reduce the toxicity of pharmaceutical preparations for local organizations in the process of design and development of effective, safe, topical application of pharmaceutical preparations [8]. The main toxicity of ocular administration include local irritation, tissue damage, subcutaneous toxicity and ciliotoxicity [9]. Of all local mucoadhesive agent used for eye, there is no one used for the study of mucosa and ciliotoxicity, such as chitosan [10, 11]. After contacting with biological materials, cell will occur morphological changes to stabilize the both of the contact interface. Therefore, the behavior of cells attached to the biological material is an important factor to...
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Figure 4. Levofloxacin concentration in the cornea and conjunctiva of rabbits with or without corneal shield treatment. Data are mean ± SD. *P<0.05, **P<0.01, ***P<0.001 vs. treatment; #P<0.05, ###P<0.01, ####P<0.001 vs. blank cornea levofloxacin + cap + levofloxacin treatment (evaluated with student’s t-test).

In our study, aqueous humor drug concentrations in group (A, B) are maintained at a relatively constant level on four time points after surgery, with a sustained release effect. In the end of the trial, the shape of cornea cover is still good and no degradation signs. There was no significant difference between the two groups at any time point. The drug concentra-
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The aqueous humor drug concentrations in Group C (Blank corneal cap + eye drops, 2 times/day) and Group D (Eye drops, 4 times/day) were maintained at a relatively constant level in the four time points after surgery. Between the two groups there were no statistical differences. The drug concentrations of cornea and conjunctiva in group C were higher than those in group D at tenth day after surgery, and statistically significant. This suggested Chitosan/PVA corneal cover played a role of drug reservoir, could prolong the drug retention time of the ocular surface, improving drug bioavailability and reducing the frequency of use to some extent.

In our experiment, the drug concentrations of aqueous humor, cornea and conjunctiva in groups (A, B) were lower than in groups (C, D) at each time point. There maybe are two possible reasons: one is the low drug loading of chitosan/PVA corneal cover, the other is the initial burst release phenomenon of chitosan/PVA cornea cover. Consequently, in order to achieve effective drug concentrations, we need to further improve the drug loading of corneal cap. Through wearing different drug-loaded caps for rabbit eyes and detecting of drug released after the burst effect, we determined a drug delivery manner of corneal cap after burst release effect.

Although the drug concentration in A and B groups of different corneal cap drug-loaded was lower after the burst effect of drug, it has been released in our monitoring process. This suggested the corneal cap used has a certain degree of sustained-release effect. It supplied medication way for us to treat ocular infectious diseases with combination drug-loaded corneal cover with eye drops.

Through this study, we preliminarily confirmed that chitosan/PVA corneal cover which applied to the surface of rabbit eyes has good biocompatibility, drug delivery and drug “reservoir” effect.

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Disclosure of conflict of interest

None.

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