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The potential application of hyaluronic acid coated chitosan nanoparticles in ocular delivery of dexamethasone

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ABSTRACT

This study investigates *in-vitro* transcorneal permeation on excised-rabbit cornea and its effect on corneal hydration-level, *in-vivo* ocular irritation, tear and aqueous humor dexamethasone-sodium-phosphate (DEX) concentration after topical administration of chitosan-nanoparticles (CS-NPs), hyaluronan-coated-CS-NPs (HA-CS-NPs) and DEX-aqueous-solution in rabbit eyes. The permeation parameters and irritation results indicated the ocular safety of NPs. The developed UPLC-method was successfully applied for DEX quantification in tears and aqueous-humors. Tear samples were collected and DEX-concentration was analyzed by UPLC. A statistically significantly ($p < 0.05$) high DEX-concentration in the inferior conjunctival-sulcus from NPs treated eyes was found as compared to DEX-solution treated eyes. Similarly, DEX-concentration in aqueous-humor was estimated. The drug was detected sufficiently high in aqueous-humor till 24 h following topical administration of NPs. The NPs have shown significantly ($p < 0.05$) higher bioavailability of DEX compared to DEX-solution. About 1.83- and 2.14-fold higher $AUC_{0\text{-}24h}$ was observed with the CS-NPs and HA-CS-NPs, respectively compared to DEX-solution. The reason for higher tear concentration and higher bioavailability of DEX from uncoated and HA-coated CS-NPs was assumed due to their prolonged precorneal-retention because of highly mucoadhesive characteristics of CS and HA. Moreover, presence of HA on CS-NPs, speed-up cellular-uptake by receptor-mediated-endocytosis could be another reason for enhanced bioavailability.

1. Introduction

Nanocarriers were found to overwhelm some shortcomings of the traditional eye preparations. Due to the strong defensive mechanism of eyes the transport of hydrophilic or hydrophobic drugs via topical instillation in to the eyes are limited, causing a low ocular availability of drugs [1] and hence only 2-7% of total topically applied drugs are available in to human eyes [2,3]. Hence, for the topical administration of DEX in to eyes, we developed chitosan (CS) based nanoparticles (NPs) and HA-coated CS-NPs to enhance the precorneal retention of the dosage form and to achieve therapeutically effective DEX concentration in to the eyes. CS is a hydrophilic mucoadhesive biodegradable polysaccharide which stabilizes tear fluids on the corneal surfaces, increasing the precorneal and corneal contact duration of NPs and reducing its nasolachrymal drainage due to increased viscosity [4–6].

In this study the ocular irritation potential of chitosan nanoparticles was performed, *in vitro* transcorneal permeation of DEX was determined, precorneal drug kinetics and aqueous humor or intraocular DEX availability was quantified. The NPs preparation, its characterization and *in vitro* release of DEX was done and reported previously [7], where the enough literature about CS and HA have been discussed, therefore discussion about CS and HA and their importance in the development of nanocarriers for ophthalmic application is not required here.

DEX is used as a 0.1%, w/v, topical steroid solution (Bausch & Lomb Inc, Tampa, FL, USA) in the treatment of ocular inflammatory conditions. Dexamethasone, 9α-fluoro-16α-methyl-11β, 17α, 21-trihydroxy-1, 4-pregnadiene-3, 20-dione, is a glucocorticoid, which is highly potent and long-acting glucocorticoid as compared to other glucocorticoids. Amongst the corticosteroids used in ophthalmics, DEX is having the highest potency and effectiveness [8], which acts by binding with the corticosteroid receptors present in the human trabecular meshwork cells and in rabbit iris ciliary body tissues. DEX inhibits phospholipase-A2, so, prostaglandins synthesis is inhibited which causes inflammation. In ophthalmics, DEX is used for the treatment of inflammations due to certain infections, injuries, surgery, or other conditions in the eyes. Though, DEX can have serious systemic side effects, so the controlled, continuous local delivery of DEX at infection site via nanoparticulate carrier system would be an efficient mean to bypass the side effects

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and achieve the goal. In some experiments, topically applied DEX-nanocarriers was found to have high concentrations on the corneal surfaces [9] and caused an effective delivery into the anterior and posterior segments of the eyes [10,11].

The ability of DEX to treat ocular inflammatory conditions at the anterior segment of eyes can be estimated by its concentrations in aqueous humor of eyes. Therefore, the determination of DEX concentration in aqueous humor is needed. For this, we developed and validated an ultra-performance liquid chromatography (UPLC) method with UV-detection by adopting and modifying the previously reported HPLC method for dexamethasone analysis [12,13], to determine the DEX concentration in rabbit aqueous humor with minimal sample pretreatment using direct injection into chromatographic column with retention time (R_t) of 1.169 min for DEX by using hydrocortisone (R_t, 3.121 min) as internal standard (IS). The rabbit aqueous humor DEX concentrations from DEX-aqueous solution and the two developed CS nanoparticles following topical administration into the rabbit eyes were estimated and compared by the developed UPLC method.

2. Materials and methods

2.1. Materials

Dexamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$; MW 516.40), hydrocortisone ($C_{21}H_{30}O_5$; MW 362.5), high purity deacetylated chitin (degree of acetylation is ≤ 40 mol.%, having viscosity average molecular weight (M_v) of around 140,000–22,0000) and the cross linker sodium tripolyphosphate (TPP) and sodium dihydrogen phosphate were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetic acid glacial was purchased from BDH Limited (Poole, England), hyaluronic acid (HA) 200 kDa was obtained from Medipol SA (Lausanne, Switzerland). Methanol and acetonitrile (HiPerSolv CHROMANORM for HPLC grade) were purchased from BDH, PROLABO®, LEUVEN, EC. Purified water was obtained by Milli-Q® water purifier (Millipore, France). All other solvents of HPLC grade and chemicals of analytical grade were used.

2.2. Methods

2.2.1. Transcorneal permeation study

Freshly excised rabbit cornea was fixed between donor and receptor compartments of double jacketed automated transdermal diffusion cells (sampling system-SFDC 6, LOGAN, New Jersy, USA) in such a way that the epithelial surface of the cornea faced the donor compartment. The receptor compartment was filled with simulated tear fluid (pH = 7.4), keeping 37 ± 1 °C temperature of water flowing in the outer jacket of the diffusion cell under 95% air and 5% CO_2 aeration, the air bubbles was expelled from the receptor compartment and was stirred with a magnetic bead throughout the experiment. Around 0.5 mL of each, the DEX solution and suspension of CS-NPs containing a finite dose of formulation (0.1%, w/v, DEX) was placed on the surface of cornea (donor compartment). The samples were withdrawn from the receptor at a predetermined time points up to 6 h and drug content was analyzed by UPLC. Permeation study was performed in triplicates ($n = 3$) and mean values were calculated with standard deviation (SD). The permeation parameters of DEX from the DEX-CS-NPs were calculated by plotting the amounts of drug permeated ($\mu g cm^{-2}$) through the cornea versus time (h) and the slope of the linear portion of the graph was estimated. The steady-state flux (J) values across the cornea were evaluated from the linear ascents of the permeation graphs by using following equation (Eq. 1),

$$J(?gcm^{-2}.s^{-1}) = dQ/dt. \ldots\ldots\ldots\ldots(1)$$

where 'Q' indicates the quantity of substance crossing cornea or dQ/dt is the linear portion of the slope, 'A' is the area of cornea exposed, and 't' is the time of exposure. The permeation coefficient (P) in each case was calculated by using the following equation (Eq. 2).

$$P(cm.s^{-1}) = J/C_0. \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

where C_0 represents the initial drug concentration ($\mu g mL^{-1}$) in the donor compartment [14]. Each treated cornea was weighed, soaked in methanol, dried overnight at 90 °C, and reweighed at the end of the permeation experiment. From the differences of weights, corneal hydration level of each treated cornea was calculated [15,16].

2.2.2. In vivo *animal study*

Male albino rabbits weighing 3.0 to 4.0 kg were obtained from College of Pharmacy, Animal Care and Use Center, King Saud University, Riyadh, Saudi Arabia, for the *in-vivo* studies. Animals were maintained according to the recommendations of the "Guide for the Care and Use of Laboratory Animals" approved by the center. The animals were given pellet diet with water ad libitum and fasted overnight prior to the experiments. On the basis of physical and physicochemical characteristics of the CS-NPs, *in vitro* drug release and transcorneal permeation studies, the developed DEX loaded CS-NPs coated with HA was subjected for eye irritation study. *In vivo* studies including precorneal drug kinetics and ocular absorption (ocular pharmacokinetics) of DEX was studied in male Albino rabbit's eye.

2.2.2.1. Ocular irritation study. Ocular irritation study was performed according to Draize's test [17,18] on rabbits. About 50 μL of nanosuspension of CS-NPs was instilled in to the lower conjunctival sac of left eyes of the rabbits and the right eyes was treated with 0.9% NaCl solution to serve as control [19]. The upper and lower eyelids were gently held together for few seconds to prevent the loss of nanosuspension due to immediate tear dilution. The suspension of CS-NPs was instilled thrice a day for up to 10 days, and the eyes of the rabbits were observed visually throughout the study period. The eye irritation level was evaluated by the animal discomfort, signs and symptoms in the conjunctiva, cornea, and eyelids, according to the scoring system of guidelines [18,20] for ocular irritation testing (Table 1).

2.2.2.2. In vivo *precorneal drug kinetics study.* For *In vivo* precorneal drug kinetics study three groups (A, B and C) of male albino New Zealand rabbits were taken, each group were containing three animals. 50 μL nanosuspension of CS-NPs (group-A), HA-coated CS-NPs (group-B) and DEX-aqueous solution (group-C) was administered into the lower conjunctival sac of left eyes of all rabbits of the mentioned groups. By using microcapillaries about 10 μL of tear fluids were collected without anesthesia at time points 30, 60, 90, 120, 150, 180 and 240 min. All the rabbits were placed in restraining boxes so that they could move their heads and eyes freely throughout the study period. The collected samples were transferred into centrifuge tubes and the microcapillaries was washed with 20 μL mobile phase repeatedly so that the final volume reached to 100 μL, the samples were centrifuged at 13,500 rpm by using ultracentrifuge (PRISM-R, Labnet International Inc. Edison, NJ, USA) for 20 min at 10 °C. The supernatant was collected and 10 μL of supernatant was injected into the UPLC system to determine the DEX concentration, the UPLC method was developed by adopting and modifying the previously reported HPLC method for the analysis of dexamethasone [12,13]. For each time points tear fluid samples was collected from three eyes ($n = 3$) of the three rabbits. Drug concentration in tear fluids was calculated from relative recovery