Journal of Drug Delivery Science and Technology 32 (2016) 21–30

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Research paper

Ocular inserts based on chitosan and brimonidine tartrate: Development, characterization and biocompatibility

Júlio Fernandes De Souza [a], Kamila Nunes Maia [a], Patrícia Santiago De Oliveira Patrício [b], Gabriella Maria Fernandes-Cunha [c], Marina Goulart Da Silva [d], Carlos Eduardo De Matos Jensen [d], Gisele Rodrigues Da Silva [a, *]

[a] Biotechnology, Federal University of São João del-Rei, Avenida Sebastião Gonçalves Coelho, 400, Chanadour, 35501-296, Divinópolis, Minas Gerais, Brazil
[b] Department of Chemistry, Federal Center of Technological Education, Avenida Amazonas, 5253, Nova Suíça, 30421-169, Belo Horizonte, Minas Gerais, Brazil
[c] School of Pharmacy, Federal University of Minas Gerais, Avenida Antônio Carlos, 6627, Pampulha, 31270-901, Belo Horizonte, Minas Gerais, Brazil
[d] School of Pharmacy, Federal University of São João del-Rei, Avenida Sebastião Gonçalves Coelho, 400, Chanadour, 35501-296, Divinópolis, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form
13 January 2016
Accepted 15 January 2016
Available online 27 January 2016

Keywords:
Glaucoma
Chitosan
Brimonidine tartrate
Ocular inserts
Inserts based on chitosan and brimonidine tartrate
Chorioallantoic membrane

ABSTRACT

The glaucoma is an ocular pathology characterized by the increase of intraocular pressure and possibility of retinal degeneration. The treatment is based on the administration of eye drops. However, they do not induce the permanence of drug in the eye, compromising its bioavailability. In this study, inserts based on chitosan and brimonidine tartrate were developed to treat the glaucoma by improving the drug bioavailability. Inserts were characterized using different analytical techniques FTIR, SEM, DSC and WAXS. The *in vitro* release profile of drug from inserts was evaluated. The *in vitro* biocompatibility against ARPE-19 and MIO-M1 cells was investigated. The *in vivo* biocompatibility using the chorioallantoic membrane was also demonstrated. Analytical techniques demonstrated that the amorphous drug was physically dispersed into the polymeric chains without chemical interactions. However, a portion of drug crystals was on the surface of systems. Inserts provided the controlled release of drug for 30 days without a burst effect. Inserts did not induce a deleterious effect to ocular cells, indicating their biocompatibility. They were also well tolerated *in vivo*, suggesting the absence of toxicity. These inserts could be potential delivery systems to reduce the intraocular pressure and to induce neuroprotective effects in glaucomatous patients.

1. Introduction

The glaucoma is a long-term ocular neuropathy defined by optic disc or retinal nerve fiber structural abnormalities, which induces visual field loss and/or irreversible blindness [1]. The glaucoma is the second cause of blindness in the United States and worldwide. It affects approximately 67 million people around the world and 10% of them are bilaterally blind [2]. The most important risk factor for progression of the glaucoma is the intraocular pressure (IOP) elevation, which leads to the optic nerve damage [3].

Eye drops are the pharmaceutical dosage forms most applied in the treatment of the glaucoma and other ocular diseases affecting the anterior segment of the eye. However, the ocular bioavailability of the drugs instilled topically is extremely poor, being lower than 5% of the total dose [4]. The inefficacy of this topical route is related to the precorneal factors such as tear turnover and drainage, dilution by tear flow, reflex blinking and lacrimation and highly selective corneal epithelial barrier [5]. The precorneal factors also lower the concentration gradient, which is the driving force for the passive absorption of drug across the cornea and conjunctiva [4,5]. These anatomical and physiological elements lead to the fluctuation of the IOP and the progression of the glaucoma, and consequently, lower the expectations of the patients to the daily therapy.

To overcome the drawbacks of the conventional eye drops,

* Corresponding author. Avenida Sebastião Gonçalves Coelho, 400, Chanadour, 35500-296, Divinópolis, Minas Gerais, Brazil.
E-mail addresses: juliofs77@gmail.com (J.F. De Souza), kamilamaia@yahoo.com.br (K.N. Maia), patriciapatricio@des.cefetmg.br (P.S. De Oliveira Patrício), gabriellafcunha@gmail.com (G.M. Fernandes-Cunha), marina@ufsj.edu.br (M.G. Da Silva), jensen@ufsj.edu.br (C.E. De Matos Jensen), giselersilva@ufsj.edu.br (G.R. Da Silva).

http://dx.doi.org/10.1016/j.jddst.2016.01.008
1773-2247/

ocular drug delivery systems, such as ocular inserts, could be applied as a therapeutic alternative to treat the glaucoma. Ocular inserts are solid or semi-solid devices, usually elaborated using natural or synthetic polymers, to be inserted in the conjunctival sac to deliver the drug in the anterior segment of the eye [6]. The polymeric matrix controls the delivery of therapeutic concentrations of the drug directly in the target tissues and provides its prolonged release, increasing its ocular residence time and bioavailability. As a consequence, the ocular inserts are capable of improving the glaucomatous patient compliance due to the efficacy of the therapy and the reduced frequency of administration. In this context, it is available, in the pharmaceutical market, the Ocusert®, an ocular device based on pilocarpine alginate enclosed by ethylene-vinyl acetate membrane. This non-biodegradable membrane delivers therapeutic concentrations of the pilocarpine for 7 consecutive days, reducing significantly the IOP in patients [7]. Additionally, many studies have been performed to demonstrate the development of ocular inserts containing different types of drugs to treat the glaucoma, including the bimatoprost [8], timolol maleate [9] and betaxolol hydrochloride [10].

In this study, ocular inserts based on chitosan and brimonidine tartrate were developed as alternative delivery systems to treat the glaucoma. The chitosan is a polycationic biopolymer obtained through the alkaline deacetylation of chitin, a natural polysaccharide found abundantly in marine crustaceans [11]. The chitosan contains a large number of hydroxy and amino groups, which provide different possibilities for derivatization or grafting of desirable substances [12]. Moreover, the chitosan is biocompatible, biodegradable, and non-toxic. It is able of interacting chemically with the mucus layer or the eye tissues, enhancing the residence time in the anterior segment of the eye, and consequently, increasing the bioavailability of the incorporated drug [13]. The brimonidine tartrate is an anti-glaucomatous drug highly selective for α-2 adrenergic receptors, which provides a potent hypotensive effect through increasing uveoscleral outflow along with decreasing aqueous humor production [6]. Moreover, it was evidenced that the brimonidine tartrate is capable of penetrating the posterior segment of the eye, leading to a neuroprotective function by promoting retinal ganglion cell survival [14,15].

The developed ocular inserts were characterized by applying different analytical techniques (FTIR, SEM, DSC and WAXS). The swelling study was performed. The *in vitro* release profile of the brimonidine tartrate from the chitosan inserts was also investigated for 30 consecutive days. The *in vitro* biocompatibility of these ocular inserts was analyzed using Müller glial cells (MIO-M1) and retinal pigment epithelial cells (ARPE-19) cultures, considering their viability and morphology; and the *in vivo* biocompatibility was described using the irritation test of the chorioallantoic membrane (CAM).

The brimonidine tartrate-loaded chitosan inserts represented a novelty in the field of the pharmaceutical technology due to their performance in the *in vitro* drug release study. This hydrophilic drug was leached from the inserts for a long period without inducing a burst release. The inexistence of the burst release demonstrated the capacity of the polymeric chains to control the delivery of the drug. It have been previously showed that inserts based on chitosan did not control the release of drugs presented at the surface of the systems, leading to a significant burst release [9,16]. The brimonidine tartrate-loaded chitosan inserts represented also an innovation in the field of the pharmaceutical technology, since they were designed without using preservatives in their formulation. The preservatives, such as the benzalkonium chloride, added in the multiple-dose conventional eye drops, may induce ocular inflammation and reduce tear secretion, and consequently, disrupt the homeostasis of the ocular surface [17]. Moreover, the inserts represented also originality in the field of the ophthalmology, since, nowadays, there are not in the pharmaceutical market mucoadhesive inserts capable of controlling and prolonging the ocular delivery of a hypotensive and neuroprotective drug in order to reduce the IOP and increase the patient compliance to the therapy.

2. Materials and methods

2.1. Preparation of the inserts based on chitosan and brimonidine tartrate

Inserts based on chitosan and brimonidine tartrate were produced by a casting/solvent evaporation technique [9]. Briefly, standard solution of chitosan and brimonidine tartrate was prepared by dissolving 500 mg and 56.25 mg, respectively, of these substances in 25 mL of 2% (v/v) acetic acid aqueous solution. The solution was dried at room temperature for 3 days. The dried film was cut into spheres of 4 mm diameter and 0.3 mm of thick to obtain inserts containing 1.0 mg of brimonidine tartrate. Inserts containing chitosan without drug were also produced.

2.2. Determination of the weight of inserts based on chitosan and brimonidine tartrate

For the determination of the weight of the inserts based on chitosan and brimonidine tartrate, the procedure stated in the United States Pharmacopeia was followed [18]. Briefly, 20 inserts were individually weighted. The average weight and the relative standard deviation were calculated.

2.3. Determination of the content of brimonidine tartrate incorporated into chitosan inserts

For the determination of content uniformity of brimonidine in the chitosan inserts, the procedure stated in the United States Pharmacopeia was followed [18]. The determination of brimonidine tartrate was performed as following: ten inserts were selected and weighted. Each insert was dissolved in 50 mL of 2% (v/v) acetic acid aqueous solution. The standard solution of the brimonidine tartrate was also prepared as described above. The absorbance of the resultant solutions was measured at 258 nm using a chitosan solution as blank. The spectrophotometric method of quantitation of brimonidine tartrate was previously validated [19]. The uniformity content of brimonidine tartrate in the inserts was expressed as the percent of the pre-indicated value (approximately 1.0 mg). The relative standard deviation was also calculated.

2.4. Characterization

2.4.1. Fourier transform infrared spectroscopy

Infrared spectra were collected in a Fourier transform infrared spectrophotometer (FTIR; Perkin Elmer, model Spectrum 1000). Measurements were carried out using the attenuated total reflectance (ATR) technique. Each spectrum was a result of 32 scans with a resolution of 4 cm^{-1} [20].

2.4.2. Differential scanning calorimetry

Differential scanning calorimetric (DSC) thermograms were obtained on a Mettler Toledo DSC (Switzerland). Samples were put into aluminum pans. The calorimeter was calibrated for temperature and heat flow accuracy using pure indium melting (m.p. 156.6 °C and $\Delta H = 25.45$ J g^{-1}). The temperature ranged from 0 to 700 °C with a heating rate of 25 °C min^{-1} under nitrogen atmosphere [20].