Journal of Pharmaceutical Sciences xxx (2016) 1-8

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Nanotechnology

Novel Polymeric Nanoparticles Intended for Ophthalmic Administration of Acetazolamide

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ARTICLE INFO

Article history:
Received 27 January 2016
Revised 19 June 2016
Accepted 24 June 2016
Available online xx

Keywords:
nanotechnology
ophthalmic drug delivery
polymeric drug delivery systems
drug delivery systems
nanocapsules

ABSTRACT

Glaucoma is characterized by increased intraocular pressure (IOP) that results in blindness if it remains untreated. Acetazolamide (AZM) is a carbonic anhydrase inhibitor, mainly used to reduce IOP in the treatment of glaucoma. However, the potential of topical treatment is limited, due to its low permeability across the ocular epithelium. An alternative to overcome this limitation is the incorporation of AZM in nanoparticulate systems, such as polymeric nanocapsules (NCs). In this way, the aim of this work was to prepare and characterize NC formulations containing AZM, using ethylcellulose (EC) and Eudragit® RS100 (EUD) as encapsulating polymers. The formulations showed high encapsulation efficiency. Particle size measurements showed that NCs are in the nanometric range. Comparing both groups of formulations, the NCEC proved to be smaller than those prepared with EUD. The formulations prepared with EC showed negative zeta potentials, while NCs of EUD were positively charged. For both groups of formulations, no more than 30% of drug was released in 120 min. *Ex vivo* and *in vivo* studies evidenced that the NCEC formulations were the most efficient, because an increased amount of permeated drug was observed, along with a greater IOP decrease and longer duration of the effect in normotensive rabbits.

Introduction

It is estimated that glaucoma, the leading cause of irreversible blindness in the world, is affecting about 67 million people. Glaucoma is the term used for a group of ophthalmic disorders characterized by an increase in intraocular pressure (IOP), which results in damage of the optic disc and visual field disturbances. IOP increases through an imbalance between the production and drainage of aqueous humor. The main strategy to treat glaucoma is the administration of drugs aiming to decrease IOP. These drugs limit aqueous humor production in the ciliary body and/or enhance aqueous outflow through the trabecular meshwork or the uveoscleral pathway. The drugs used in the long-term management of glaucoma include β-adrenergic blockers, miotics, α-adrenergic agonists, carbonic anhydrase inhibitors (CAIs), prostaglandin analogs, and hyperosmotics. Among them, acetazolamide (AZM), a CAI which has been used in the management of glaucoma for more than 40 years, inhibits the production of aqueous humor without interfering its outflow.[1,2] Although AZM showed to be very effective, systemic side effects, such as diuresis and metabolic acidosis, are the major problems associated with the oral therapy. In this sense, topical administration of AZM could overcome the side effects. However, the potential of topical treatment of glaucoma with AZM is quite limited, mainly due to its poor penetration coefficient (4.11×10^{-6} cm/s) and low aqueous solubility (0.7 mg/mL).[3] An alternative to overcome these limitations is the administration of AZM incorporated in nanostructured systems.

Polymeric nanoparticles are one of the strategies currently used to improve drug absorption across biological membranes. This type of nanocarrier generally presents sizes ranging from 100 to 500 nm and it is subdivided into 2 types of nanostructures, named nanospheres and nanocapsules (NCs).[4,5] Nanospheres are matricial systems, while NCs possess a vesicular organization in which the polymer surrounds a liquid (lipophilic or hydrophilic) core. In such systems, the drug can be entrapped or dissolved inside or adsorbed on to a particle surface.[6] In the case of AZM, its high lipophilicity favors the drug confinement in oily core NCs.

Biocompatible polymers play an important role in safety, stability, and efficiency of nanoparticles for drug delivery. Ethylcellulose (EC) is a hydrophobic cellulose derivative commonly used

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http://dx.doi.org/10.1016/j.xphs.2016.06.023
0022-3549/

for its mucoadhesiveness and controlled-release properties. Due to the negative surface density of charge, nanoparticles prepared from EC usually present negative zeta potential (ZP).[7] On the other hand, Eudragit® RS100 (EUD), a cationic methacrylate copolymer, produces positively charged nanoparticles that favor mucoadhesion. It is well known that in the case of ophthalmic drug delivery, an appropriate particle size and a narrow size range are highly recommended, in order to ensure low irritation, adequate bioavailability, and compatibility with ocular tissues.[8]

In this work, we report the preparation and some relevant properties of AZM-loaded polymeric nanoparticles made from EC or EUD. The formulations were evaluated in terms of physicochemical characteristics, stability, *in vitro* drug release, transcorneal permeation studies, and *in vivo* IOP reduction, after topical ophthalmic application in nonsedated normotensive rabbits.

Materials and Methods

Materials

AZM was obtained from Parafarm. EC (megawatt 170 KDa) was kindly donated by Colorcon (Cotia, Brazil) and EUD was obtained from Degussa (São Paulo, Brazil). Span 60® (sorbitan monostearate) was purchased from Sigma-Aldrich (São Paulo, Brazil) and Tween 80 (polysorbate 80) was supplied by Henrifarma (São Paulo, Brazil). Medium chain triglycerides (MCT) were purchased from Delaware (Porto Alegre, Brazil). Phosphate buffered saline solution was prepared according to Zimmer et al.[9] All other chemicals and solvents were pharmaceutical grade and used as received.

Preparation of Nanocapsule Suspensions

Nanoparticles were prepared by interfacial deposition of preformed polymers.[10] A solution of polymer (EC or EUD), AZM, lipophilic surfactant (Span 60), and oil (MCT) in acetone was submitted to magnetic stirring for 60 min at 40°C. Then, the organic phase was added into an aqueous dispersion of Tween 80 (hydrophilic surfactant). The mixture was kept under magnetic stirring for 10 min. Then, acetone was removed and the aqueous phase was concentrated by evaporation under reduced pressure. The final volume was adjusted to 10 mL. For comparison, formulations without AZM were prepared (blank formulations). The quantitative composition of formulations is shown in Table 1. Each sample was assayed in triplicate ($n = 3$).

Acetazolamide Content and Encapsulation Efficiency

AZM content was determined after dissolution of an aliquot of NC suspension in methanol, under magnetic stirring. The samples were centrifuged, filtered, and assayed by ultraviolet visible (UV) spectrophotometry at 264 nm.

Encapsulation efficiency (%) was determined by adding an aliquot of the samples in a 10,000 megawatt device (Amicon® Ultra; Millipore). Free drug was separated from the nanostructures by an ultrafiltration and centrifugation technique at 2200 × *g* for 10 min. The difference between the total and the free concentrations of AZM, determined in the nanostructures and in the ultrafiltrate, respectively, was calculated as the encapsulation efficiency (EE%) of the nanoparticles according to the following equation: EE = [(total content − free content)/total content] × 100.

In this experiment, the drug was quantified by high-performance liquid chromatography according to the following conditions: Gemini RP-18 column (150 mm × 4.60 mm, 5 μm; Phenomenex) coupled to a Shimadzu instrument (LC-10AVP Pump, UV-VIS SPD-10AVP Module, Class-VP Software, Shimadzu) at room temperature. The mobile phase was compounded by acetonitrile/sodium acetate 0.1 M (80:20%, vol/vol) adjusted to pH 4.5 ± 0.5 with glacial acetic acid and the flow rate was set at 1.0 mL/min. The volume injected was 20 μL and AZM was detected at 276 nm. Each sample was assayed in triplicate ($n = 3$).

pH Measurements

After preparation, the pH values of nanoparticle suspensions were determined using a potentiometer (Micronal B-474). Each sample was assayed in triplicate ($n = 3$).

Particle Sizes and Polydispersity Index

The particle sizes and polydispersity index of the formulations were determined by photon correlation spectroscopy after dilution of the samples with ultrapure water (1:500) (Zetasizer Nanoseries; Malvern Instruments). Each sample was assayed in triplicate ($n = 3$).

Evaluation of Zeta Potential

The ZP of NC suspensions was measured by the nanoparticle velocity while they were moving due to electrophoresis, after dilution of samples in 10 mM NaCl (1:500) using a Zetasizer Nanoseries Malvern Instrument. Each sample was assayed in triplicate ($n = 3$).

Stability Studies of the Formulations

Drug content, encapsulation efficiency, pH, particle size, polydispersity index, and ZP of all formulations were monitored during 60 days at room temperature and protected from light. Each sample was assayed in triplicate ($n = 3$).

In Vitro *Drug Release From Nanoparticles*

Experiments were performed in a modified Franz diffusion assembly at 35.0 ± 0.5°C. Semipermeable acetate cellulose

Table 1
Composition of Nanocapsule Suspensions

Variable	NCEUDB	NCEUD1	NCEUD1.5	NCEUD2	NCECB	NCEC1	NCEC1.5	NCEC2
Aqueous phase								
Tween 80 (g)	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077
Water (mL)	53	53	53	53	53	53	53	53
Organic phase								
EUD (g)	0.100	0.100	0.100	0.100	–	–	–	–
EC (g)	–	–	–	–	0.100	0.100	0.100	0.100
AZM (g)	–	0.010	0.015	0.020	–	0.010	0.015	0.020
MCT (mL)	0.330	0.330	0.330	0.330	0.330	0.330	0.330	0.330
Span 60 (g)	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077
Acetone (mL)	27	27	27	27	27	27	27	27