European Journal of Pharmaceutics and Biopharmaceutics 114 (2017) 119–134

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Poloxamer-based thermoresponsive ketorolac tromethamine in situ gel preparations: Design, characterisation, toxicity and transcorneal permeation studies

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ARTICLE INFO

Article history:
Received 25 April 2016
Revised 15 January 2017
Accepted in revised form 20 January 2017
Available online 24 January 2017

Keywords:
In situ gel
Poloxamer
Mucoadhesion
Ocular delivery
Ketorolac
Physico-chemical characterisation
Toxicity studies
In vitro release
Ex vivo permeation
HET-CAM test
BCOP test

ABSTRACT

This study was aimed at preparing, characterising and evaluating in situ gel formulations based on a blend of two hydrophilic polymers i.e. poloxamer 407 (P407) and poloxamer 188 (P188) for a sustained ocular delivery of ketorolac tromethamine (KT). Drug-polymer interaction studies were performed using DSC and FT-IR. The gelation temperature ($T_{sol-gel}$), gelation time, rheological behaviour, mucoadhesive characteristics of these gels, transcorneal permeation and ocular irritation as well as toxicity was investigated. DSC and FT-IR studies revealed that there may be electrostatic interactions between the drug and the polymers used. P188 modified the $T_{sol/gel}$ of P407 bringing it close to eye temperature (35 °C) compared with the formulation containing P407 alone. Moreover, gels that comprised P407 and P188 exhibited a pseudoplastic behaviour at different concentrations. Furthermore, mucoadhesion study using mucin discs showed that in situ gel formulations have good mucoadhesive characteristics upon increasing the concentration of P407. When comparing formulations PP11 and PP12, the work of adhesion decreased significantly ($P < 0.001$) from 377.9 ± 7.79 mN mm to 272.3 ± 6.11 mN mm. *In vitro* release and *ex vivo* permeation experiments indicated that the in situ gels were able to prolong and control KT release as only 48% of the KT released within 12 h. In addition, the HET-CAM and BCOP tests confirmed the non-irritancy of KT loaded in situ gels, and HET-CAM test demonstrated the ability of ocular protection against strongly irritant substances. MTT assay on primary corneal epithelial cells revealed that in situ gel formulations loaded with KT showed reasonable and acceptable percent cell viability compared with control samples.

1. Introduction

Conventional ophthalmic dosage forms, such as solutions and suspensions have many drawbacks, including - rapid precorneal elimination of the drug mainly due to nasolacrimal drainage [3,5], the need for frequent application, and pulse release from solutions in particular [13]. On the other hand, ophthalmic ointments, although provide a prolonged contact with the eye, they may trigger foreign body sensation, blurred vision and cause inconvenience to the patient [2,54].

A relatively novel strategy in increasing the contact time of ocular formulations is through the formation of in situ gels using environment responsive polymers [50,56]. These polymer-based systems are liquid at room temperature, but undergo sol-gel transition on the ocular surface hence prolonging ocular residence time [13]. Stimuli that may trigger sol-gel phase transition of the polymer network on the ocular surface could be owing to physical (temperature, light) or chemical (ions, pH). Amongst the natural polysaccharides that are considered as ion-activated polymers, the popular ones include gellan gum, kappa-carrageenan, alginates and xanthan gum. These polymers are able to interact with different cations that can be used to form ion-activated in situ gelling systems [27].

Gellan gum and xanthan gum are the constituents of the commercially available products Timoptic® XE (Merck) and Timoptic®

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http://dx.doi.org/10.1016/j.ejpb.2017.01.008
0939-6411/

GFS (Alcon) respectively. These exhibit phase transition with increased ionic strength [6]. Also, it has been found that the extent of gel-formation of gellan gum increases proportionally with the amount of mono- or divalent cations present in the tear fluid. Hence, the main triggering effect inducing phase transition is the adequate availability of mono- and divalent cations associated with reflex tearing [50,58].

On the other hand, carbopol is a polyacrylic acid (PAA) polymer, which exhibits sol-gel transition in an aqueous solution upon raising the pH above its pKa of 5.5. However, with the increase in the concentration of carbopol in the vehicle, in order to improve its rheological properties, the acidity of the vehicle increases. This increase in the acidity of the vehicle could induce ocular tissue irritation and induce lacrimation [43].

Also among commonly used in situ gel polymers is poloxamer 407 (P407), a thermoresponsive polymer that exists in a liquid state at low temperature i.e. between 4 and 5 °C at a concentration range of 20–30% w/w, while converting into a gel upon increasing the temperature of the medium. P407 is a non-ionic polymer (poly oxyethylene-polyoxypropylene-polyoxyethylene; PEOn–PPOn–PEOn), consisting of a central hydrophobic block of polypropylene glycol in addition to hydrophilic blocks of polyoxyethylene [18]. P407 has been widely used in nasal [36], ophthalmic [30], vaginal [49], and topical [31] formulations. However, one of the limitations of P407 is its weak mechanical strength leading to a rapid erosion of the polymer. Furthermore, it was previously reported that P407 at a concentration of 18% (w/v) or higher, has the ability to transform from a low viscosity solution into a gel under the ambient temperature [33,46]. However, at this concentration the solution will lose its gelation ability after being diluted by lacrimal fluid upon instillation into the eye. Hence, 25% (w/w) P407 can be used in order to ensure the completion of the phase transition process of the polymer under ocular physiological condition. But, under these circumstances, the gelation temperature will be lower than room temperature and P407 solution have to be stored in the fridge, which makes it inconvenient for use [62]. Therefore, P188, which is an analog of P407, may be added to P407 solution as a gelation temperature modifying substance [33,46].

Moreover, previous reports have revealed that higher concentrations of P407 are required in a formulation when used on its own; such concentrations were found to be irritant to the eye. In order to overcome this challenge, researchers adopted the approach of blending P407 with other polymers like methyl cellulose, chitosan, and P188 in order to decrease the total concentration of P407 used, improve its gelling characteristics as well as mechanical properties of P407 and reduce its ocular irritation potential [13].

Ketorolac tromethamine (KT) is a non-steroidal anti-inflammatory drug that has potent analgesic activity; it is used for treating post-operative eye inflammation and reducing conjunctivitis [29,55]. There are other potent NSAIDs that are currently used for ophthalmic conditions e.g. nepafenac 0.1% w/v (Nevanac®) which is a prodrug that is rapidly converted into amfenac after passing through the cornea. Also, bromfenac 0.09% w/v (or 0.1% w/v when labelled as the salt form) (Xibrom® and Bromday®) is available as ophthalmic solution. A previous animal study demonstrated that bromfenac 0.09% w/v, pH 8.3 readily penetrates ocular tissues. Both drugs are used for the treatment of pain and inflammation associated with cataract surgery [19,34].

It has been reported that the ocular bioavailability was up to 4% for the KT ophthalmic solution (0.5% w/v) following topical ocular administration in the form of conventional eye drops in anesthetized rabbits. The drug concentrations in the aqueous humour were compared after topical application with those obtained after intracameral injection of an equivalent dose of 0.25 mg of ketorolac tromethamine per eye using 14C [37,61]. Such factors have prompted research for the development of a more safe and effective KT formulations [52,53]. Various in situ ophthalmic preparations have been formulated incorporating KT in order to sustain its release, thus improving its ocular bioavailability as well as reducing the frequency of administration. Nanjwade et al. prepared pH-triggered in situ gel for sustained ophthalmic delivery of ketorolac tromethamine, using carbopol 934 and hydroxypropylmethylcellulose [44]. Also, KT has been used in form of hydrogel for nasal delivery using poloxamer 407 and carrageenan [36].

More recently, ofloxacin loaded Pluronic F127 and Pluronic F68 (20% w/v) in situ gelling formulation have been prepared and characterised. These deemed promising ocular formulation due to prolonged pre-corneal retention and good ocular tolerability using slug mucosal irritation assay and bovine corneal erosion study [11].

The present study focuses on investigating into different polymer combination of P407 and P188 for ocular delivery of the non-steroidal anti-inflammatory drug KT. Physicochemical characterisation of drug/polymer interactions were investigated with DSC and FT-IR studies. The gelling properties and rheological characteristics for these formulations were studied. The mucoadhesive characteristics, *in vitro* release as well as *ex vivo* corneal permeation of KT were investigated. Furthermore, the ocular irritation potential of the KT-loaded in situ gel formulations was determined using a combination of the HET-CAM and BCOP tests. Finally the MTT cytotoxicity assay was carried out using a corneal epithelial cell line in order to elucidate the toxicity of the developed KT-loaded in situ gel formulations.

2. Materials and methods

2.1. Materials

Ketorolac tromethamine (KT), poloxamer 407 (P407, culture tested), and poloxamer 188 (F-168) were purchased from Sigma Aldrich chemical Co., Gillingham, UK. Whole fresh porcine eyes were purchased from C.D Jennings & Sons abattoir, Surrey, UK. All other chemicals and solvents were of analytical grade and used as received from the supplier. Fertilised white Leghorn eggs were purchased from Henry Stewart & Co. Ltd. Fakenham, Norfolk, UK. Bovine eyes were purchased from ABP Guildford Slyfield Industrial Estate, Guilford, UK. Primary Corneal Epithelial Cells; Normal, Human (ATCC® PCS-700-010™) were acquired from ATCC company, Manassas, VA, USA.

2.2. Determination of drug – polymer interactions

2.2.1. Differential scanning calorimetry (DSC) study

DSC study was carried out on Mettler Toledo DSC 822e0, Switzerland. The drug, the polymers (P407 and P188) as well as their physical mixtures (PM) with KT was weighed separately in aluminium pans, covered with aluminium lids and hermitically sealed using a pan press (Thermal Science, USA). Once in the calorimeter, the temperature of the pan was gradually increased from 25 °C to 300 °C at a rate of 10 °C/min. Nitrogen was purged at a flow rate of 45 mL/min. The data generated was consolidated using Mettler STARe software version 8.10.

2.2.2. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectrometer (Thermo Scientific Nicolet iS5, Thermo fisher, USA) was used to record the FT-IR spectra of KT, P407, and P188 and their PM. Sufficient amount (2–4 mg) of the sample was placed to form a thin film covering the diamond window. The FT-IR spectra were recorded at a resolution of 2 cm^{-1} with