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Thermo-sensitive gel in glaucoma therapy for enhanced bioavailability: *In vitro* characterization, *in vivo* pharmacokinetics and pharmacodynamics study

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ABSTRACT

Aims: Glaucoma is a chronic ophthalmic disease, which has become one of the leading causes to progressive and irreversible blindness. Current ophthalmic drug delivery to treat glaucoma is mostly eyedrop, whose rapid elimination on corneal surface can lead to poor bioavailability. The present study was aimed to develop a timolol maleate loaded thermo-sensitive gel (TM-TSG) with improved bioavailability to treat glaucoma.

Main methods: TM-TSG was prepared by homogeneously dispersing 0.3% (*w/v*) timolol maleate, 24.25% (w/v) poloxamer 407 (P407) and 1.56% (w/v) poloxamer 188 (P188) into phosphate buffer solution (pH = 7.4) and the formulated TM-TSG was characterized.

Key findings: TM-TSG was stored in liquid form at room temperature (25 °C) and transited to semisolid gel at physiological temperature (32 °C). The rheological property of TM-TSG was in favor of uniform distribution of drug. TM-TSG showed good stability at different conditions including centrifugation, autoclaving and different temperature. *In vivo* pharmacokinetic studies indicated that TM-TSG could enhance absorption of TM in aqueous humor and improve the ocular bioavailability in comparison of commercial TM eyedrops. *In vivo* experiment result showed that TM-TSG had greater effect in treating glaucoma than TM eyedrops by sustainably lowering intraocular pressure (IOP) for a week. Moreover, slit lamp test and histopathological analysis demonstrated that TM-TSG had excellent biocompatibility.

Significance: TM-TSG could be a promising ophthalmic delivery system for glaucoma therapy.

1. Introduction

Glaucoma is a chronic ophthalmic disease with degeneration of retinal ganglion cells, resulting in progressive and irreversible blindness [1–3]. There are > 67 million people suffering with glaucoma all over the world, and glaucoma is becoming a leading cause of blindness [4].

Although the pathogenesis of glaucoma is complicated and not fully understood, it is widely believed that the elevated level of intraocular pressure (IOP) is related to retinal ganglion cell death [3]. IOP can induce mechanical stress and strain on the lamina cribrosa and adjacent tissues of eye, causing compression and deformation of the lamina cribrosa with subsequent mechanical axonal damage [5]. Therefore, lowering IOP by pharmaceutical therapy or surgical procedures to ameliorate damage to the eye is the main treatment during therapy of glaucoma [6,7].

β-Receptor inhibitor is a commonly used drug during the therapy of glaucoma due to the inexpensive price and notable efficacy [8]. Timolol maleate (TM) is a potent β-receptor inhibitor, which is widely used to ameliorate glaucomatous symptom by lowering the production of the aqueous humor *via* inhibiting the sympathetic nerve endings in the ciliary epithelium [9]. The common commercial formulation for TM is aqueous eyedrops, which is low-cost and widely used for the patients of

Abbreviation: CCD-RSM, central composite design-response surface methodology; HE, hematoxylin and eosin; IOP, intraocular pressure; LCST, lower critical solution temperature; LSD, least significant difference test; P188, poloxamer 188; P407, poloxamer 407; PEO, polyoxyethylene; PPO, polyoxypropylene; TM, timolol maleate

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open-angle glaucoma [10–12].

However, the efficacy of TM is limited since eyedrops is rapidly eliminated by the turnover and scour of lachrymal fluid and drainage system of eye, resulting in decreasing the ocular residence time of TM [10,13,14]. Specific anatomic structure and protective mechanisms of eye as described lead to poor corneal permeability and low ocular bioavailability of TM causing limitation of therapeutic effect [15]. Ophthalmic gel can elongate the drug retention time but the application incompliance of bulk gel remained an issue. To overcome above-mentioned drawbacks, various ophthalmic drug delivery systems have been developed, such as liposomes, cyclodextrin compounds, nano-sized carriers and *in situ* gelling system [16]. However, disadvantages like difficulty in particle size control for nano-sized carriers, poor stability for liposomes and narrow application on solubilizing for cyclodextrin compounds still need to be solved.

Among all the drug delivery approaches mentioned above, *in situ* gelling system is the most promising drug delivery system due to the phase transition triggered by environmental factors, such as water, pH or temperature [17]. Thermo-sensitive gel is an *in situ* gelling systems remaining liquid at room temperature and making a phase transition to form semisolid gel at the temperature of ocular surface [18–21]. Thermo-responsive phase transition was attributed to the fact that water-soluble thermo-sensitive polymers become insoluble owing to destruction of hydrogen bond between polymers and water when the temperature reaches its lower critical solution temperature (LCST) [22]. Thermo-responsive polymers can be broadly categorized including poly (N-isopropylacrylamide), cellulose derivatives and so on. And poloxamers are extensively used in ophthalmic formulations.

In the present study, poloxamer 407 (P407, or Pluronics® F127) and poloxamer 188 (P188) are chosen as constituents of gel system loaded with TM for ophthalmic delivery, owing to its nontoxicity, biodegradability, biocompatibility, and bioadhesiveness. P407 has been reported to effectively enhance ocular drug permeation and improve bioavailability of drugs while P188 was often used to adjust thermal sensitive phase transition temperature [23–26]. Moreover, excellent rheological properties caused by the phase transition are also in favor of drug administration to the ocular surface and storage. Besides, the matrix material for the ophthalmic thermo-sensitive gel, many excipients were added to the formulation, such as gellan gum, chitosan or cellulose and derivative, mainly as bio-adhesive agents to enhance the viscosity and retention time [24,26,27]. Nevertheless, these formulations still have some shortages such as complex composition of formulation and complicated preparation method. The compatibility of matrix and bio-adhesive agents in one formulation remained to study while the irritation and biodegradation required further evaluation.

The present work was aimed to prepare a thermo-sensitive gel system containing only poloxamers (P407 and P188) for ophthalmic delivery of TM (TM-TSG), which could avoid complicated formulation composition and relevant issues. The thermo-sensitive gel remained liquid phase for ophthalmic administration while underwent phase transition at physiological temperature to form bulk gel and achieve prolonged drug release. TM-loaded thermo-sensitive gel could be simply prepared *via* modified cold method. Our previous study has optimized the formulation of TM-TSG with appropriate gelation temperature. TM-TSG precursor was liquid at room temperature and transited to gel at the physiological temperature, which is in favor of administration and retention. In this paper, rheological properties and stability of TM-TSG were evaluated *in vitro*. *In vivo* pharmacokinetics and pharmacodynamics of TM in rabbit were comparatively studied between TM-TSG and commercial TM eyedrops to prove that the formulation of thermo-sensitive gel composed of poloxamers was able to elongate drug retention time and enhance therapeutic effect.

2. Materials and methods

2.1. Materials

Timolol maleate (TM) was obtained from Suzhou Yacoo Science Co., LTD (Suzhou, China). Poloxamer 407 (P407) and Poloxamer 188 (P188) were purchased from BASF (Shanghai, China). Commercial TM eyedrops was obtained from Zhongshan ophthalmic center, Sun Yat-sen University (Guangzhou, China). Proparacaine hydrochloride eyedrops (Alcaine®) and tobramycin and dexamethasone eyedrops (TobraDex®) were purchased from Alcon Laboratories, Inc. (Beijing, China). Other reagents were of analytical grade.

New Zealand white rabbits (female, 2.0–2.5 kg) were obtained from Guangdong Experimental Animal Center (Guangzhou, China). Animals were maintained in an animal room with constant levels of humidity and temperature on 12-h light/dark cycles and provided with standard pellet diet and water *ad libitum*. All animal protocols were in line with the Principles of Laboratory Animal Care and Use in Research published by the Chinese Ministry of Health.

2.2. Preparation of TM-TSG

TM-TSG was prepared using a modified cold method and optimized by central composite design-response surface methodology (CCD-RSM) method as described in our previous study, which presented instant phase transition and prolonged drug release in favor of ocular administration with convenience and effectiveness [28,29]. In brief, 24.25% (w/v) P407 and 1.56% (w/v) P188 were dispersed into phosphate buffer (pH = 7.4) and refrigerated at 4 °C overnight to obtain the precursor solution. 0.3% (w/v) TM was added into the abovementioned precursor solution which was stirred until completely dissolved to obtain TM-TSG precursor solution.

2.3. Characterization of TM-TSG

2.3.1. Determination of gelation temperature and pH value

Gelation temperature at which phase transition from precursor solution to gel was triggered by rising temperature, was performed using the tube inversion method [30]. Briefly, 4 mL TM-TSG precursor solution was transferred to vial with a mercury thermometer submerged throughout the experiment. Then, the vials were placed in a water bath for fluidity observation of precursor solution after tube inversion with rising temperature of 1 °C/min. For the gelation temperature with artificial tears, TM-TSG precursor solution was mixed with artificial tears at volume ratio of 40:7 before test and the rest steps were the same [31]. The artificial tears was prepared by dispersing 6.78 g NaCl, 1.38 g KCl, 2.18 g NaHCO3 and 0.084 g $CaCl_2{\cdot}2H_2O$ into 1 L distilled water. The sol-gel phase transition temperature at which the precursor solution stopped flowing after tube inversion was recorded as the gelation temperature. The pH value of precursor was measured using pH detector (Sartorius, Germany). All measurements were made in triplicate.

2.3.2. Rheological studies

Rheological properties of gel were studied using HAAKE MARS III Advanced Rheometer System (Thermo Scientific, USA). The samples were equilibrated at 25 °C prior to each measurement. The viscosity of precursor solution at 25 °C was determined from the viscosity curve obtained at shear rate ranging from 0.1 s^{-1} to 100.0 s^{-1}. Afterwards, another test was conducted to investigate the effect of temperature and artificial tears on phase transition. Specifically, the elastic modulus and viscous modulus of gel with or without artificial tears was determined with the increase of temperature at a rate of 1 °C/min, respectively.

2.3.3. Stability studies

TM-TSG was subjected to stability studies at different conditions including centrifugation, autoclaving and different temperatures in