International Journal of Pharmaceutics 471 (2014) 297–302

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Personalised medicine

Preclinical evaluation of thermoreversible triamcinolone acetonide hydrogels for drug delivery to the inner ear

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ARTICLE INFO

Article history:
Received 12 March 2014
Received in revised form 28 May 2014
Accepted 29 May 2014
Available online 2 June 2014

Keywords:
Triamcinolone acetonide
Thermoreversible hydrogel
Poloxamer 407
Intratympanic administration
In-vitro release

ABSTRACT

Intratympanic glucocorticoid therapy aims to reduce the side effects associated with systemic long-time therapy of inner ear diseases or traumata after cochlear implantation. For that purpose, thermoreversible hydrogels being fluid at room temperature but solid at body temperature are known to be appropriate drug delivery systems. In this work, the two key parameters sol–gel transition time and temperature of Poloxamer 407 (POX 407) based hydrogels containing oto-compatible micronized triamcinolone acetonide (TAAc) were evaluated by rheological experiments varying the concentrations of the different compounds. A 20% POX 407 hydrogel in PBS containing 30% TAAc emerged as the most appropriate formulation. Oscillation–rotation–oscillation studies at two temperature levels were found to be an useful in-vitro test system for the hydrogel which revealed sufficient storage stability at 4 °C, injectability of the sol, solidification within 20 s at body temperature and persistent stiffness indicating prolonged adhesion at the round window membrane. According to the in-vitro release studies using the Transwell™ system, absorption of the poor water soluble TAAc is partly due to the low amount of dissolved drug but predominantly due to micellar transport resulting in a cumulative release of 262.6 ± 13.4 μg TAAc within one week followed by a sustained release of 193.1 ± 8.3 μg TAAc within the next three weeks. Thus, the formation of POX 407 micelles is the basis not only for gel formation but also absorptivity of TAAc. All in all, fine tuned rheological experiments and absorption studies emerged as useful tools for preclinical evaluation of intratympanally administered hydrogels.

1. Introduction

As opposed to conventional systemic therapy, which suffers from pronounced side effects in case of long-term treatment, local glucocorticoid therapy of inner ear diseases or traumata after cochlear implantation is a new and patient-friendly approach (Bird et al., 2007). Unfortunately, unfavorable physiological conditions such as low blood flow in the cochlea as well as the blood–perilymph barrier limit therapeutic efficacy of drugs in cochlear fluids. In an effort to reach therapeutically relevant drug levels in the inner ear aqueous solutions of dexamethasone or betamethasone were administered intratympanally. These formulations, however, suffered from rapid drainage through the Eustachian tube and thus low drug levels in the inner ear due to short contact time with the round window membrane (Bird et al., 2007, 2011; Ye et al., 2007). To counteract the latter, semi-solid formulations were administered directly on the round window membrane and led to improved drug delivery into the inner ear due to prolonged contact with the membrane (Wang et al., 2011). According to Plontke et al. (2002), the amount of drug permeating into the cochlea depends mainly on the residence time of the drug on the round window membrane.

Out of these reasons, the thermoreversible characteristics of the oto-compatible (Piu et al., 2010; Wang et al., 2009), FDA-approved and autoclavable Poloxamer 407 (POX 407) (Dumortier et al., 2006; Guzman et al., 2006), being fluid at room temperature and semi-solid at 37 °C, seems to be most convenient for intratympanic administration. POX 407 is a triblock copolymer consisting of two hydrophilic polyethylene glycol end chains and a hydrophobic

Abbreviations: POX 407, Poloxamer 407; TAAc, Triamcinolone acetonide; DEX, Dexamethasone; POX 188, Poloxamer 188; ORO-test, Oscillation–rotation–oscillation test.

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http://dx.doi.org/10.1016/j.ijpharm.2014.05.057
0378-5173/

polypropylene glycol core block. Upon increasing the temperature, POX 407 molecules arrange to form micelles which subsequently aggregate to become a semi-solid hydrogel (Dumortier et al., 2006).

In general, the anti-inflammatory and immunosuppressive effects of glucocorticoids are best known, and they exert long-term effects on the tissue reaction against foreign objects like cochlear implants (Enticott et al., 2011). POX 407 gels containing dexamethasone (DEX) have been successfully used in several studies (Wang et al., 2011; Piu et al., 2010; Wang et al., 2009; Salt et al., 2011), and proved oto-compatibility with preservation of hearing thresholds. However, to the best of our knowledge there is a lack of studies about POX 407 gels containing triamcinolone acetonide (TAAc) for intratympanal administration although it is well established in clinical use, considered safe and preserves residual hearing together with a protective effect on hair cells (Ye et al., 2007; Guzman et al., 2006). The concentrations of TAAc applied in this study were related to the relative glucocorticoid potential of DEX as well as the release of DEX in therapeutically relevant levels as demonstrated by Piu et al. (2010) so that the efficacy of formulations containing 6% DEX and 30% TAAc might be comparable.

The aim of this work was to develop and characterize POX 407 based hydrogels with emphasis on the sol–gel transition temperature and sol–gel transition time upon varying the POX 407- and the TAAc-content. For that purpose, different rheological experiments as well as an adopted release model were applied. Additionally, the utility of additives such as POX 188, Miglyol, inorganic salts and ions (Dumortier et al., 2006; Müller Goymann and Luisana, 2011) to tune the sol–gel transition temperature is elucidated. All in all, this in-vitro study should offer a deeper insight into the structural characteristics as well as the release mechanisms of micelle-based hydrogels.

2. Materials and methods

2.1. Materials

Micronized triamcinolone acetonide (TAAc) was purchased from Fagron (Barsbüttel, Germany). Micronized dexamethasone (DEX) was obtained from Gatt-Koller (Absam, Austria). Pluronic® F-127 Prill (Poloxamer 407, POX 407) was bought from BASF (Lampertheim, Germany). Pluronic® F-68 (Poloxamer 188, POX 188) was acquired from Sigma–Aldrich (Vienna, Austria). 24-well-plates and filter inserts (ThinCert™ - 24 Well, Pore ø 0.4 μm, translucent, PET Membrane Ro Trac®) were from Greiner (Kremsmünster, Austria). All other chemicals were purchased from Sigma–Aldrich and were of analytical grade.

2.2. Methods

2.2.1. Hydrogel preparation

The hydrogel was manufactured according to Schmolka (1972) by the "cold technique" using 10 mM phosphate buffered saline (PBS) pH 7.4 instead of water, and the POX 407 solution has been stored overnight at 4 °C prior use. Drug-loaded gels were prepared by homogeneously dispersing micronized particles of either TAAc or DEX (180 or 20 μm) in the fluid gel matrix by vortexing and storing at 4 °C overnight prior use.

2.2.2. Sol–gel transition time

Thermosensitivity of the POX 407 hydrogel was determined by incubating 15–25% POX 407 solutions in PBS at 37 °C. In brief, 100 μl POX 407 solution at 4 °C was transferred into an Eppendorf cup and subsequently incubated at 37 °C. After 5, 10, and 20 min, the Eppendorf cups were inverted and solidification was assessed.

2.2.3. Rheological characterization

The reversibility of the sol–gel transition was investigated by placing the cold formulation (4 °C) into an Eppendorf cup, incubating for 15 min at 37 °C to become rigid, and then storing the gel in a refrigerator to liquefy again. This procedure was repeated 10 times. The state of aggregation was assessed after inverting the cup.

The hydrogel's rheological characteristics were examined with a Modular Compact Rheometer MCR 302 equipped with a cone/plate system (CP50-1-SN27364; 50 mm/1° cone plate; Anton Paar, Graz, Austria). All measurements were done in a Peltier controlled hood, and the data were processed using the Rheoplus/32 V3.61 software. The storage modulus G' and the loss modulus G'' were calculated from the complex shear modulus G^* as follows:

$$G' = G^* \cos(\delta) \tag{1}$$

$$G' = G^* \sin(\delta) \tag{2}$$

$$\tan(\delta) = \frac{G''}{G'} \tag{3}$$

To define the linear viscoelastic region (LVR) shear strain amplitude sweeps were carried out increasing the deformation from 0.001 to 100% at an angular frequency of 3 rad s^{-1} (Grüning and Müller-Goymann, 2008).

Frequency sweep experiments were performed at a constant deformation of 0.1% (20% POX 407; w/v) or 0.01% (20% POX 407 containing 30% TAAc; w/v) and decreasing the angular frequency from 100 to 0.01 rad s^{-1}.

Temperature-dependent characteristics of the preparations were elucidated in the range from 4 °C up to 42 °C at a heating rate of 1 °C min^{-1} in the oscillatory mode setting the angular frequency at 3 rad s^{-1} and the deformation at 0.1% without and 0.01% with TAAc, respectively.

Additionally, an oscillation–rotation–oscillation (ORO) test was accomplished at the same oscillation settings as above. In brief, during the first oscillatory mode the formulation was maintained for 200 s at 4 °C until the temperature increased up to 10 °C within 30 s. Subsequently, the rotation mode was performed at 10 °C for 30 s at the shear rate of 5000 s^{-1}, which simulates the injection process (injection needles LUER G27: 0.4 × 42 mm) and was calculated according to Eq. (4):

$$\gamma = \frac{4 \times V}{\pi \times R^3 \times t} \tag{4}$$

where γ is the calculated shear rate, V is the effluent volume, R is the radius, and t is the time.

Finally in the second oscillatory mode, the bottom plate was warmed up to 37 °C (0.2 °C s^{-1}) within 135 s and maintained at 37 °C for 120 s, subsequently the temperature was increased up to 42 °C.

All experiments, performed with a sample volume of 600 μl and at a cone gap of 0.101 mm, were repeated at least 3 times.

2.2.4. Release studies

In a preliminary attempt to establish a release model without a membrane barrier, 150 μl of a 20% POX 407 hydrogel colored by addition of 0.005% aqueous Evans Blue were transferred into an Eppendorf cup and incubated for 15 min at 37 °C. The solidified gel was overlain with 70 μl 1% aqueous 2-ethoxy-6,9-diaminoacridine lactate solution of 37 °C and further incubated at body temperature for 24 h. The images were acquired at defined times.

As a release model including a barrier, translucent filter inserts in 24-well plates were applied using artificial perilymph as an acceptor medium. The aqueous artificial perilymph was composed of 137 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM $NaHCO_3$, but without 11 mM glucose to avoid bacterial contamination.