European Journal of Pharmaceutical Sciences 49 (2013) 233–240

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

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

Design and *in vitro* assessment of L-lactic acid-based copolymers as prodrug and carrier for intravitreal sustained L-lactate release to reverse retinal arteriolar occlusions

Marieke Veurink [a], Lutz Asmus [a], Maren Hennig [b], Béatrice Kaufmann [a], Lena Bagnewski [b], Arnd Heiligenhaus [b], Efstratios Mendrinos [c], Constantin J. Pournaras [c], Robert Gurny [a], Michael Möller [a,*]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland
[b] Department of Ophthalmology, St. Franziskus Hospital, Muenster, Germany
[c] Department of Ophthalmology, Geneva University Hospitals, 1211 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form 21 February 2013
Accepted 27 February 2013
Available online 14 March 2013

Keywords:
L-lactate
Retinal vasodilation
Biocompatibility
Biodegradable copolymers
Sustained release
Intravitreal injection

ABSTRACT

Ophthalmic conditions in which the retinal vasculature is obstructed generally lead to vision loss. Administration of the vasodilator L-lactate might offer a treatment strategy by restoring the blood flow, but unfortunately its effect after single intravitreal injection is short-lived. This study describes a concept in which the sustained release of L-lactic acid from a biodegradable copolymer system is investigated. The 50:50 (n/n) copolymer system, composed of L-lactic acid and L,D-2-hydroxyoctanoic acid, is a viscous injectable that will form an intravitreal drug depot. Hydrolysis of the copolymer will automatically lead to the release of L-lactic acid, which will convert to L-lactate at physiological pH, thereby providing a carrier and pro-drug in one. *In vitro* and *ex vivo* release studies demonstrate an L-lactic acid release over several weeks. Biocompatibility of the co-polymer and its degradation products is shown on a human retinal pigment epithelial cell line and on *ex vivo* retinal tissues. A low molecular weight copolymer (1200 g/mol) with low polydispersity has promising properties with a constant release profile, good biocompatibility and injectability.

1. Introduction

Many sight threatening ophthalmic conditions cause obstruction of the retinal vasculature. Such posterior segment diseases might be treated by administration of vasodilative drugs, which restore the blood flow in the obstructed vessels. The retinal vasculature lacks autonomic innervations (Ye et al., 1990) and therefore, retinal blood flow is most probably controlled locally through the release of vasoactive substances by the retinal tissues (Brown and Jampol, 1996). Although the exact mechanism of retinal vasodilation has not been fully elucidated, the endogenous compound L-lactate is involved in this mechanism because its intravenous administration results in dilation of retinal vessels (Garhöfer et al., 2003). Moreover, intravitreal injections of L-lactate in minipigs induce vasodilation of retinal arteries both in healthy eyes and in eyes in which acute branch retinal vein occlusion is evoked (Brazitikos et al., 1993; Mendrinos et al., 2008, 2011). Based on these observations and on the fact that L-lactate is an endogenous substance present in the eye, it is envisaged a potential drug candidate for the treatment of posterior segment diseases in which the retinal vasculature is obstructed. However, one major disadvantage of a single intravitreal injection of an L-lactate solution is its limited duration of action of approximately 15 min (Brazitikos et al., 1993). This is due to the fact that elimination from the eye depends on the size of the molecule, resulting in short half-lives for molecules like L-lactate (Mw 89 Da) with a molar mass <500 Da. Consequently, frequent injections are necessary to maintain therapeutic drug levels (Trimawithana et al., 2011). In order to prolong the therapeutic effect, a sustained intraocular release system is envisaged, ideally releasing L-lactate over a period of several weeks. The therapeutic benefit of such a system would be an immediate intervention to overcome the vascular obstruction, thereby preventing angiogenesis and giving time to plan further surgery.

For a suitable intraocular sustained release formulation, several challenges and limitations should be taken into account. First, the volume of injection is linearly related to the intraocular pressure (IOP) (Pallikaris et al., 2005), limiting the formulation volume that can be administered. Currently used volumes do not exceed 0.1 ml per injection (Bakri et al., 2009). Second, posterior segment diseases are difficult to treat because of the layer-like structure of the eye, which serves as protection barrier for the sensitive inner

* Corresponding author. Address: School of Pharmaceutical Sciences, University of Geneva, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland. Tel.: +41 22 379 31 32; fax: +41 22 379 65 67.
E-mail address: Michael.Moeller@unige.ch (M. Möller).

0928-0987/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejps.2013.02.021

environment, reducing its accessibility to drugs. The most successful routes of administration are intravitreal injections and implants that are placed in the vitreous humor or sutured onto the sclera (Duvvuri et al., 2003; Shah et al., 2010). The advantage of implants is that they offer a sustained release of drugs over a prolonged period of time, up to years. However, drawbacks of most implants that are currently on the market, like Vitrasert® and Retisert®, include high costs and invasiveness, due to the fact that they need to be surgically introduced in the vitreous (Lavik et al., 2011). Furthermore, they are not biodegradable and eventually need to be removed from the eye. Thus, to avoid invasiveness and high costs associated with surgery, we opt for a sustained release formulation that can be administered by conventional intravitreal injection, keeping the injection volume low to prevent an undesirable rise in IOP.

The sustained release of L-lactic acid from a polymer built up with L-lactic acid subunits, like poly(L-lactic acid) (PLA), is selected as a starting point for the sustained release formulation. The idea behind this choice is the fact that L-lactic acid, being a subunit of the polymer chain, will be released automatically through hydrolysis of the ester bonds, directly forming free L-lactate at physiological pH. Therefore, such a polymer system may serve as a carrier and a prodrug in one. However, PLA itself is a solid, and degrades too slowly for a sufficient release. Moreover, to change the polymer from a solid into an injectable formulation, solvents or plasticizers would be needed.

Herein, the concept of an L-lactic acid based copolymer system is investigated, in which 50% (n/n) of the methyl groups of PLA are substituted by hexylgroups, serving as internal plasticizers (Asmus et al., 2011). The result is a viscous biodegradable copolymer system, poly(L-lactic acid-*co*-L,D-2-hydroxyoctanoic acid) that in theory meets the requirements for the intravitreal sustained release of L-lactic acid, being:

(1) A viscous injectable suitable for conventional intravitreal injection.
(2) An L-lactate prodrug/carrier system that forms a drug depot in the vitreous humor, releasing L-lactic acid upon hydrolysis of the copolymer.

The degradation and degradation profile of poly(2-hydroxyoctanoic acid) by hydrolysis were demonstrated in earlier work by Trimaille et al. (2007). *In vitro* and *ex vivo* investigations are performed, aiming at the development of a system that is biocompatible with retinal tissues, releases therapeutic doses of L-lactic acid and is injectable by intravitreal injection.

2. Materials

2-Hydroxypropionic acid (L-lactic acid), 3,6-dimethyl-1,4-dioxane-2,5-dione (dilactide) and sodium L-lactate were obtained from Sigma Aldrich (St. Louis, USA). 2-hydroxyoctanoic acid was synthesized as described by Trimaille et al. (2007).

3. Methods

3.1. Polymerization and formulation preparation

Polymerization and purification of the random-order poly (L-lactic acid-*co*-L,D-2-hydroxyoctanoic acid) copolymers were performed as described for hexylsubstituted poly(lactic acid) by Asmus et al. (2011), whereby L-lactic acid was reacted with 2-hydroxyoctanoic acid in a 50:50 M ratio. In short, a melt polycondensation was performed at 150 °C under vacuum, leading to a quantitative yield. At this reaction temperature, preservation of the L-form of lactic acid is expected (Hiltunen et al., 1997). In the case of low polydisperse copolymers, monomers and short oligomers were removed by precipitation into cold 1 M sodium bicarbonate solutions followed by decantation and drying under vacuum. The molecular weight (M_w) and polydispersity index (PDI) (M_w/M_n) of the copolymers were determined by gel permeation chromatography. A calibration was done with eight polystyrene standards (PSS, Mainz, Germany) with known M_w and M_n. A Waters 515 HPLC pump, Waters 410 injector, Styragel HR 1–4 columns and Waters 2414 refractive index detector (Waters Corporation, Milford, USA) were used with tetrahydrofuran as continuous phase.

Three copolymer systems were compared:

(1) 50:50 n/n poly(L-lactic acid-*co*-L,D-2-hydroxyoctanoic acid), M_w of 1200 g/mol, PDI of 1.5.
(2) 50:50 n/n poly(L-lactic acid-*co*-L,D-2-hydroxyoctanoic acid), M_w of 1400 g/mol, PDI of 2.6.
(3) 50:50 n/n poly(L-lactic acid-*co*-L,D-2-hydroxyoctanoic acid), M_w of 2500 g/mol, PDI of 1.5.
    a. With 5% of additional sodium L-lactate.
    b. With 10% of additional sodium L-lactate.

Sodium L-lactate incorporation into the copolymer was achieved by cryomilling (SPEX 6700 freezer/mill, SPEX SamplePrep, Metuchen, USA) during 5 min.

3.2. Injectability

The injectability of the copolymers was tested *in vitro*, using a 1 ml Tuberculin syringe (Norm-Ject, Henke-Sass, Wolf GmbH, Tuttlingen, Germany) with a 21-gauge needle (0.8 mm × 25 mm) (BD Medical, Franklin Lakes, USA). All formulations were warmed to 37 °C and 100 µl of copolymer were injected into sodium phosphate buffer to mimic an application in clinic with minimal discomfort for the patient and to exclude an influence of the temperature on viscosity (Asmus et al., 2011). Injectability was considered sufficient if the formulation could be manually pushed through the 21-gauge needle.

3.3. In vitro and ex vivo L-lactic acid release studies

3.3.1. Study design

L-lactic acid release from the different copolymers as described in Section 3.1 was studied over time *in vitro*. A quantity of 100 µl of copolymer, equivalent to the quantity that would be used *in vivo*, was placed in 25 ml 150 mM sodium phosphate buffer at pH 7. This buffer strength and volume were chosen to maintain a constant pH, which simulates the ocular buffering capacity. Because of the good solubility of L-lactic acid at pH 7, sink conditions were met during all studies. The release tests were performed at 37 °C under light shaking conditions during the entire study period. Samples of 50 µl of release medium were taken at different time points to measure the amount of released L-lactic acid; this volume was replaced by fresh medium after each sample collection. A similar study was carried out in porcine vitreous humor with 0.2% sodium azide to avoid bacterial growth (*ex vivo* tests), in order to study the influence of enzymes on the release rate.

3.3.2. Sample analysis

The amount of L-lactic acid released from the copolymers was quantified by ultra performance liquid chromatography (UPLC Acquity system, Waters Corporation, Milford, USA) coupled with UV detection at 210 nm. Chromatographic separation was accomplished at 25 °C using an Acquity HSS T3 column (1.8 µm, 2.1 × 50 mm). A phosphate buffer at pH 2.5 was used as mobile phase with a flow rate of 0.2 ml/min. In a first step of method