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Development of a novel injectable drug delivery system for subconjunctival glaucoma treatment

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Phosphoric acid (PubChem CID: 1004)
Hexamethylene diisocyanate (PubChem CID: 13192)
Dimethylsulfoxide (PubChem CID: 679)
Poly(L-lactide) (D-lactic acid?) (PubChem CID: 61503)
Latanoprost (PubChem CID: 5311221)
Egg white lysozyme (19–36) [*Gallus gallus*] (PubChem CID: 71581463)
Hyaluronic acid sodium salt (PubChem CID: 53398704)
Sodium chloride (PubChem CID: 5234)
DMEM: Dulbecco's Modified Eagle's Medium (PubChem SID: 56312938)
with D(+)-glucose (PubChem CID: 5793)
L-glutamine (PubChem CID: 5961)
Sodium chloride: isotonic (PubChem CID: 103770557)
with sodium chloride (PubChem CID: 5234)
Penicillin/streptomycin Pen-Strep (PubChem SID: 162248774)
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ABSTRACT

In this study we present the development of an injectable polymeric drug delivery system for subconjunctival treatment of primary open angle glaucoma. The system consists of hyaluronic acid sodium salt (HA), which is commonly used in ophthalmology in anterior segment surgery, and an isocyanate-functionalized 1,2-ethylene glycol bis(dilactic acid) (ELA-NCO). The polymer mixtures with different ratios of HA to ELA-NCO (1/1, 1/4, and 1/10 (v/v)) were investigated for biocompatibility, degradation behavior and applicability as a sustained release system. For the latter, the lipophilic latanoprost ester pro-drug (LA) was incorporated into the HA/ELA-NCO system.

In vitro, a sustained LA release over a period of about 60 days was achieved. In cell culture experiments, the HA/ELA-NCO (1/1, (v/v)) system was proven to be biocompatible for human and rabbit Tenon's fibroblasts. Examination of *in vitro* degradation behavior revealed a total mass loss of more than 60% during the observation period of 26 weeks.

In vivo, LA was continuously released for 152 days into rabbit aqueous humor and serum. Histological investigations revealed a marked leuko-lymphocytic infiltration soon after subconjunctival injection. Thereafter, the initial tissue reaction declined concomitantly with a continuous degradation of the polymer, which was completed after 10 months.

Our study demonstrates the suitability of the polymer resulting from the reaction of HA with ELA-NCO as an injectable local drug delivery system for glaucoma therapy, combining biocompatibility and biodegradability with prolonged drug release.

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1. Introduction

Glaucoma encompasses a heterogenic group of ophthalmic diseases that damage the optic nerve, resulting in gradual visual impairment and potentially irreversible vision loss.

Worldwide, over 60 million individuals are affected, and this number is expected to rise to 80 million by 2020 [1]. Elevated intraocular pressure (IOP) is often associated with primary open angle glaucoma. Results from long-term clinical studies show that lowering the IOP to a normal level can slow down or even stop the progression of the disease [2]. This can be achieved either surgically or pharmacologically with eye drops, which reduce the production of the aqueous humor (*e.g.* carbonic anhydrase inhibitors and β-adrenergic receptor antagonists) and/or increase its outflow (*e.g.* α2-adrenergic receptor agonists, parasympathomimetics, sympathomimetics, and prostaglandin analogs) [3]. Topically instilled drugs penetrate through the corneal or the scleral route, additionally some conjunctival contribution is observed [4].

A major disadvantage of eye drops is poor patient adherence to daily medication dosing instructions. Especially elderly people have difficulties with routine daily applications which are necessary to reach a permanent IOP decrease. Approximately 20% of glaucoma patients exhibit poor treatment compliance [5,6]. Previous studies have demonstrated that inadequate patient adherence is often associated with more severe visual field loss [7]. The common use of preservatives like benzalkonium chloride in the antiglaucoma medication has been shown to exert cytotoxic and inflammatory effects on corneal and conjunctival tissue [8]. Studies have shown that long-term application of glaucoma eye drops may result in ocular discomfort, dry eye, burning, foreign body sensation, and red eye [9]. Moreover, medications used to treat glaucoma can be absorbed systematically and induce clinically relevant systemic effects [10].

A promising approach addressing poor compliance and resulting fluctuations of IOP is the administration of drugs via local drug delivery (LDD) systems, thereby ensuring a sustained drug concentration over an extended period of time. Continuous drug release will reduce the need for daily drug administration which could improve patient adherence and treatment outcome. Using such systems could also be more economic than application of eye drops since smaller amounts of drugs might be needed to achieve the same effect.

In medical literature, different approaches have been described utilizing topical, implantable, and injectable LDD systems [11]. Topical ocular systems administer drugs from the outside of the eye, while implantable and injectable systems release drugs within the eye. Examples for topical applications are mucoadhesive formulations, hydrogels and particles [12,13]. Latanoprost-loaded contact lenses have been shown to release the drug over a period of up to 4 weeks [14]. Implantable and injectable devices can extend the release time up to several weeks or even months [15].

Subconjunctival administration is well tolerated in patients, considered to be safe, and is already used routinely in clinics for the delivery of different medications. Experimentally, a sustained *in vivo* drug release for up to 4 weeks was shown following subconjunctival implantation of dorzolamide-loaded polymer disks in rabbit eyes [16]. A therapeutic lowering of IOP beyond 50 days has been demonstrated in rabbit experiments after a single subconjunctival injection of latanoprost (LA)-loaded liposomes [17].

An ideal LDD is expected to combine consistent delivery of an appropriate drug dosage for up to 4 months with a complete degradation within this time and minimal side effects on the surrounding tissue.

Within this context, the objective of the present work was to develop a drug-loaded, biodegradable, and biocompatible LDD system which would provide localized, long-term release of IOP-lowering medication. The developed LDD system consists of 2 components: hyaluronic acid sodium salt (HA) and a more hydrophobic hexamethylene diisocyanate (HDI)-functionalized 1,2-ethylene glycol bis(dilactic acid) (ELA-NCO). HA is a naturally occurring polysaccharide with distinct physicochemical characteristics which is commonly used in ophthalmic microsurgery due to its viscoelastic and hydrophilic properties [18]. ELA-NCO has been previously introduced by our group as a biodegradable tissue adhesive with high adhesive strength and good *in vivo* biocompatibility [19,20].

Hydrophobic latanoprost ester pro-drug (LA), which is successfully used in glaucoma therapy, was selected as IOP-lowering model drug to be incorporated into the HA/ELA-NCO system.

Here, we describe the drug release of LA-loaded HA/ELA-NCO polymer samples as well as the degradation behavior *in vitro* and *in vivo*. The biocompatibility of the LDD system was investigated *in vitro* using ocular fibroblasts and *in vivo* after subconjunctival injection in rabbits.

2. Material and methods

2.1. Materials

Hyaluronic acid sodium salt (HA) from *Streptococcus equi* was purchased from Sigma-Aldrich (Taufkirchen, Germany, Cat. # 53747). 1,2-Ethylene glycol bis(dilactic acid) (ELA) was prepared as described by Heiss and colleagues [21]. In brief, to a mixture of 1 mol of ethylene glycol and 2 mol of lactide 1.5 g of 85% phosphoric acid was added as catalyst. After heating at 100 °C for 1 h the temperature was raised to 130 °C and held for at least 6 h. Subsequently, ELA was endowed with terminal reactive isocyanate groups by a stoichiometric reaction (ratio of 1/2 (n/n)) with aliphatic hexamethylene diisocyanate (HDI) at 50 °C to yield ELA-NCO according to Sternberg and colleagues [22]. To reduce its viscosity and enhance handling, ELA-NCO was diluted with absolute dimethyl sulfoxide (DMSO, 15% w). Poly(L-lactide) (PLLA, Resomer® L214, $M_w = 650{,}000$ g/mol) was purchased from Boehringer Ingelheim Pharma GmbH & Co. KG (Ingelheim, Germany).

Lysozyme from chicken egg white (45,100–48,200 U/mg), organic solvents, and all other reagents were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 4.5 g/L glucose was used from AppliChem (Darmstadt, Germany), whereas fetal calf serum, penicillin G, and streptomycin were obtained from PAA Laboratories GmbH (Cölbe, Germany).

2.2. Fourier-transform-infrared (FTIR) spectroscopy

To monitor the polymerization reaction between HA and ELA-NCO FTIR spectroscopy (spectral range between 4000 and 700 cm^{-1}, EQUINOX 55 FTIR equipped with ATR measuring cell, Bruker Optik GmbH, Ettlingen, Germany) was used. The samples were stored in sealable glass vials at 20 °C before measurement. The *in situ* reaction progress was traced by decrease in the isocyanate's (–NCO group) absorption band at 2270 cm^{-1}.