Biomaterials 125 (2017) 38–53

Contents lists available at ScienceDirect

Biomaterials

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

Subconjunctival injectable dendrimer-dexamethasone gel for the treatment of corneal inflammation

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ARTICLE INFO

Article history:
Received 27 December 2016
Received in revised form
11 February 2017
Accepted 12 February 2017
Available online 16 February 2017

Keywords:
Corneal inflammation
Macrophages
Dendrimers
Dexamethasone
Hyaluronic acid
Injectable gel

ABSTRACT

Corneal inflammation is often encountered as a key pathological event in many corneal diseases. Current treatments involve topical corticosteroids which require frequent instillations due to rapid tear turnover, causing side-effects such as corneal toxicity and elevated intraocular pressure (IOP). Hence, new interventions that can reduce side effects, dosing frequency, and increase patient compliance can be highly beneficial. In this study, we explore a subconjunctival injectable gel based on G4-PAMAM dendrimer and hyaluronic acid, cross-linked using thiol-ene click chemistry, incorporated with dendrimer dexamethasone (D-Dex) conjugates as a potential strategy for sustained delivery and enhanced bioavailability of corticosteroids. The efficacy of the injectable gel formulation was evaluated in a rat mild alkali burn model. Fluorescently-labelled dendrimers (D-Cy5) incorporated in the gel release D-Cy5 *in vivo*. The released D-Cy5 selectively targets and localizes within corneal macrophages in inflamed rat cornea but not in healthy controls. This pathology dependent biodistribution was exploited for drug delivery, by incorporating D-Dex in the injectable gel. The attenuation of corneal inflammation by D-Dex gels was assessed using various clinical and biochemical parameters over a 2-week period. Subconjunctival D-Dex gel treatment resulted in favorable clinically-relevant outcomes with reduced central corneal thickness and improved corneal clarity compared to free-Dex and placebo gel controls. The extent of corneal neovascularization was significantly reduced in the D-Dex group. These findings suggest that D-Dex attenuates corneal inflammation more effectively than free-Dex by attenuating macrophage infiltration and pro-inflammatory cytokines expression. A significant elevation in IOP was not observed in the D-Dex group but was observed in the free-Dex group. This novel injectable D-Dex gel may be a potential drug delivery platform for the treatment of many inflammatory ocular surface disorders such as dry eye, autoimmune keratitis and post-surgical complications where frequent steroid administration is required.

1. Introduction

Corneal inflammation remains a major and common clinical problem underlying many disease processes, such as: severe dry eye, infectious keratitis, chemical burn related injuries, corneal graft rejection and others [1–6]. If left untreated at its initial stages, corneal inflammation often progresses to a chronic stage and this persistent infiltration of the cornea by white blood cells and macrophages may lead to neovascularization, corneal opacity, edema, and vision loss [7,8]. So, a timely treatment of inflammation in the

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http://dx.doi.org/10.1016/j.biomaterials.2017.02.016
0142-9612/

cornea is highly beneficial.

The standard of care for corneal inflammation is the topical administration of corticosteroids and this treatment paradigm has not changed significantly over the past few decades [9]. Many commercially available steroid eye drops, such as dexamethasone and prednisolone show potent anti-inflammatory effects [10,11]. However, due to the rapid tear turnover and clearance of the instilled drugs, repeated instillations are often required which results in patient incompliance particularly in elderly [11–14]. In addition, steroids are often associated with an increase in intraocular pressure (IOP) and the development of cataracts [15]. A clinically significant increase in IOP may require the use of antihypertensive eye drops which by themselves cause ocular surface toxicity [16]. Periocular administration of corticosteroids using the subconjunctival route as a depot can be an attractive alternative as it leads to high local drug levels that are necessary to alleviate postoperative inflammation, especially in the setting of a corneal transplant [17–19]. Several studies have reported better clinical outcomes, such as reduction in neovascularization, preservation of corneal clarity and reduction of inflammation, with subconjunctival depot injections of steroids [17,18,20–23]. A single subconjunctival corticosteroid administration results in higher drug concentration than several topical administrations [17,24,25]. However, an increase in IOP and rapid clearance of steroids is also a concern, irrespective of the route of administration [17]. Hence, developing a sustained steroid delivery system to the anterior segment is highly desirable.

Polyamidoamine (PAMAM) dendrimers are hyperbranched polymeric nanocarriers. They possess many favorable properties that make them excellent ocular drug delivery systems: they are nano-sized, multivalent, monodisperse and highly water-soluble particles [11,26]. Hydroxyl-terminated PAMAM dendrimers (G4-OH) due to their improved safety profile and near neutral surface charge significantly reduce non-specific retention and interactions in the tissues [27]. The multiple hydroxyl groups present on the surface can be easily manipulated for introducing drugs, imaging agents or for complexing biologics [11,28–31]. Dendrimer-triamcinolone acetonide (D-TA) conjugates enhances drug solubility and intracellular delivery of TA resulting in improved anti-angiogenic and anti-inflammatory activity [32]. Single intravitreal injection of dendrimer-fluocinolone acetonide conjugates attenuates neuroinflammation and provides sufficient neuroprotection for more than 30 days in rat model of retinitis pigmentosa at a 30-fold lower dose than free drug [33]. Upon systemic administration dendrimers selectively target, localize and remain in activated microglia/macrophages in the retina and brain in ischemia/reperfusion (I/R) injury mice, cerebral palsy (CP) rabbit model and canine model of hypothermic cardiac arrest [34–36]. When delivered topically, dendrimer-encapsulated with anti-glaucoma drugs resulted in higher concentrations of drugs in corneal layers with better efficacy compared to regular eye drops, suggesting they improve tissue permeability of drugs [37].

Subconjunctival injectable hydrogels can be a suitable option for sustained delivery and improving the bioavailability of steroids thereby avoiding frequent injections [38,39]. Additionally, targeting steroids to the inflammatory cell will be highly beneficial and may improve drug efficacy and reduce side effects. In this study, we designed an injectable and biocompatible hydrogel based on hydroxyl-terminated PAMAM dendrimers and hyaluronic acid cross-linked via thiol-ene click chemistry for subconjunctival injections. This injectable gel system can easily be loaded in to applicable syringes as solutions and can be crosslinked upon UV treatment. We further synthesized dendrimer dexamethasone (D-Dex) conjugates and incorporated them in injectable gel formulation. We hypothesize that sustained release of D-Dex from the injectable gel and the targeting ability of dendrimers to inflammation associated cells will lead to a synergistic effect and that D-Dex will be have a better anti-inflammatory effect than a free drug (dexamethasone). In this study we used a rat corneal mild alkali burn model in order to demonstrate the macrophage targeting ability of dendrimer conjugates released from an injectable gel; and to attenuate corneal inflammation. The evaluation included a combination of clinically-relevant and biochemical parameters.

2. Materials and methods

2.1. Chemicals and reagents

Hydroxyl-functionalized ethylenediamine core generation four PAMAM dendrimers (G4-OH; diagnostic grade; 64 end-groups) were purchased from Dendritech Inc. (Midland, MI, USA). Dexamethasone (Dex), Succinic anhydride (SA), *N,N'*-diisopropylethylamine (DIEA), trifluoroacetic acid (TFA), anhydrous dimethylformamide (DMF), dimethylacetamide (DMA), 3-(Tritylthio)propionic acid and 4-Pentenoic acid (Kosher) were purchased from Sigma-Aldrich (St. Louis, MO, USA). (Benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate (PyBOP) was purchased from Bachem Americas Inc. (Torrance, CA, USA). Cy5-mono-NHS ester was purchased from Amersham Biosciences-GE Healthcare (Pittsburgh, PA, USA). Dexamethasone 21-phosphate disodium salt was purchased from MP biomedicals (Santa Ana, CA, USA). ACS grade DMF, dichloromethane (DCM), diethylether, hexane, ethyl acetate, HPLC grade water, acetonitrile, and methanol were obtained from Fisher Scientific and used as received for dialysis, purification and column chromatography. Dialysis membrane (MW cut-off 1000 & 2000 Da) was obtained from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA).

The reactions were carried out under nitrogen. Thin-layer chromatography (TLC) was performed on silica gel GF_{254} plates (Whatman, Piscataway, NJ), and the spots were visualized with UV light. Proton NMR spectra of the final conjugates as well as intermediates were recorded on a Bruker (500 MHz) spectrometer using commercially available DMSO-d_6 solvent. Proton chemical shifts were reported in ppm (δ) and tetramethylsilane (TMS) used as internal standard. All data were processed using ACD/NMR processor software (Academic Edition).

2.2. Synthesis of dendrimer conjugates

2.2.1. Synthesis of dexamethasone-21-succinate (Dex-linker, 1)

Dexamethasone-21-succinate (Dex-linker, *1*) was synthesized using a modified synthesis procedure established previously [32]. A detailed synthesis description is provided as a part of supplementary information (S.1).

2.2.2. Synthesis of dendrimer-dexamethasone conjugates (D-Dex, 2)

Dexamethasone-21-succinate (Dex-linker, 255 mg, 0.541 mmol) was dissolved in anhydrous DMF (5 mL) in a 50 mL round bottomed flask under nitrogen at 0 °C to which PyBOP (703.9 mg, 1.35 mmol) dissolved in DMF (5 mL) and DIEA (300 μL) were added. The reaction mixture was allowed to stir for 1 h in an ice bath. PAMAM G4-OH (505 mg, 0.036 mmol) dissolved in anhydrous DMF (10 mL) was added dropwise to the reaction mixture above and stirred for 48 h under nitrogen. The solvent mixture was evaporated at 25 °C under vacuum. The crude product was re-dissolved in DMF (20 mL) and subjected to dialysis in DMF (membrane MW cutoff = 2 kDa) for 48 h, the DMF was evaporated under reduced pressure and D-Dex is subjected to water dialysis to remove solvent traces. The resultant water layer was lyophilized to get a fluffy white powder of