Colloids and Surfaces B: Biointerfaces 146 (2016) 624–631

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Sustained-release hydrogels of topotecan for retinoblastoma

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ARTICLE INFO

Article history:
Received 4 April 2016
Received in revised form 28 June 2016
Accepted 1 July 2016
Available online 2 July 2016

Keywords:
Topotecan
Hydrogels
Cytotoxicity
Retinoblastoma
Rabbits

ABSTRACT

Treatment of retinoblastoma, the most common primary ocular malignancy in children, has greatly improved over the last decade. Still, new devices for chemotherapy are needed to achieve better tumor control. The aim of this project was to develop an ocular drug delivery system for topotecan (TPT) loaded in biocompatible hydrogels of poly(ε-caprolactone)-poly(ethyleneglycol)-poly(ε-caprolactone) block copolymers (PCL-PEG-PCL) for sustained TPT release in the vitreous humor.

Hydrogels were prepared from TPT and synthesized PCL-PEG-PCL copolymers. Rheological properties and *in vitro* and *in vivo* TPT release were studied. Hydrogel cytotoxicity was evaluated in retinoblastoma cells as a surrogate for efficacy and TPT vitreous pharmacokinetics and systemic as well as ocular toxicity were evaluated in rabbits. The pseudoplastic behavior of the hydrogels makes them suitable for intraocular administration. *In vitro* release profiles showed a sustained release of TPT from PCL-PEG-PCL up to 7 days and drug loading did not affect the release pattern. Blank hydrogels did not affect retinoblastoma cell viability but 0.4% (w/w) TPT-loaded hydrogel was highly cytotoxic for at least 7 days. After intravitreal injection, TPT vitreous concentrations were sustained above the pharmacologically active concentration. One month after injection, animals with blank or TPT-loaded hydrogels showed no systemic toxicity or retinal impairment on fundus examination, electroretinographic, and histopathological assessments. These novel TPT-hydrogels can deliver sustained concentrations of active drug into the vitreous with excellent biocompatibility *in vivo* and pronounced cytotoxic activity in retinoblastoma cells and may become an additional strategy for intraocular retinoblastoma treatment.

1. Introduction

Retinoblastoma is the most common intraocular tumor in children [1]. Although significant improvements have been achieved over the past few years in terms of ocular survival, tumor recurrence in the vitreous is associated with worse ocular survival and remains a challenge [1]. The need to improve outcome is essential, especially for children with bilateral disease in whom both eyes are compromised with tumor and enucleation would lead to complete blindness [2].

An ideal scenario would be the use of an active chemotherapeutic agent delivered through a local route devoid of retinal and systemic toxicity that controls tumor growth while avoiding relapses. Topotecan (TPT) is a topoisomerase-I inhibitor that causes single-strand breaks in DNA and thereby interferes in cell replication, with extensive and well-documented *in vitro* and *in vivo* activity against retinoblastoma [3,4]. The antitumor efficacy is highly dependent on the drug and the schedule of drug administration [5]. The protracted administration of TPT in a five days on followed by two days off schedule has shown better tumor control and fewer severe systemic adverse effects than the single-dose administration of high-dose TPT in xenograft animals bearing pedi-

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http://dx.doi.org/10.1016/j.colsurfb.2016.07.001
0927-7765/

atric tumors and in children with solid tumors [6–8]. Specifically for ocular purposes, a favorable and prolonged vitreous TPT disposition of has been demonstrated after intravitreal injection of a single-dose aqueous solution of TPT in rabbits [7]. Although high vitreous exposure was attained using this local route, no retinal toxicity was found on electroretinographic and histopathological assessment in the animal model [7]. In this setting, TPT emerges as an ideal candidate for retinoblastoma treatment if incorporated in a sustained-drug-release device that allows for protracted delivery.

Different approaches have been studied for chemotherapy delivery into the vitreous humor of eyes with tumors. Periocular administration of an aqueous solution [8,9] or an episcleral implant [10] resulted in low TPT bioavailability in the vitreous of the treated eyes of rabbits and little benefit in terms of ocular survival in children [8]. Further research in the periocular administration of TPT using controlled-release devices, such as fibrin sealant, showed limited use in less advanced intraocular tumors probably due to low bioavailability in the vitreous due to rapid orbital clearance and limited *trans*-scleral penetration of TPT [11]. The development of a safe technique for intravitreal injection of chemotherapy preventing extraocular seeding of tumor cells marked a new era of retinoblastoma treatment and permitted to save eyes that were enucleated upfront in the past [12]. Nonetheless, its main shortcoming has been the need for weekly intravitreal injections of chemotherapy for tumor control. To overcome this limitation, a sustained-release formulation of TPT for intravitreal injection had to be developed.

Different materials, such as liposomes [13], lipid nanoparticles [14], polymeric implants [10], and hydrogels [15], have been studied for sustained TPT release, but none of them was indicated for ophthalmic applications. Disadvantages of previously developed liposome formulations of TPT included a low loading efficiency and a rapid elimination in animal models [13]. Others developed lipid nanoparticles of TPT with a promising cytotoxic effect but no available data on the toxic effect of the nanoparticle itself was published [13]. We hypothesized that PCL polymer, a commercial and FDA-approved inactive ingredient already employed in drug delivery systems, could be suitable for a sustained-release formulation of TPT based on its well-documented biocompatibility [16] and versatility to entrap hydrophilic drugs [15]. Moreover, the low cost of PCL favors its use allowing more affordable novel developments to be translated into the clinics of retinoblastoma.

Therefore, we aimed to design a TPT-loaded hydrogel to deliver sustained and pharmacologically active levels to the vitreous humor using a single intravitreal injection. We developed different biocompatible TPT-loaded hydrogels composed of PCL-PEG-PCL copolymer and characterized the physicochemical properties and the *in vitro* release of TPT from the hydrogels. We also studied the vitreous TPT disposition after intravitreal injection in rabbits and the potential toxicity of the drug and the blank hydrogel in the retinal tissue of the animals. In addition, we assessed the antitumor activity in retinoblastoma cell lines as a parameter of efficacy. Thus, the present is a multimodality approach to the development and *in vivo* and *in vitro* characterization of a new sustained-delivery TPT formulation with potential translation into the clinical treatment retinoblastoma.

2. Materials & methods

2.1. Materials

Poly(ethylene glycols) of different molecular weights (1 kg/mol, PEG1000; 4 kg/mol, PEG4000; 6 kg/mol, PEG6000) were provided by Merck Chemicals (Buenos Aires, Argentina), ε-caprolactone 99% (monomer, CL, Sigma, Argentina), tin (II) 2-ethyl-hexanoate 95% (catalyst, SnOct, Sigma, Argentina). TPT hydrochloride was kindly donated by Asofarma S.A. (Buenos Aires, Argentina). Stock solutions of TPT were prepared in methanol and stored at −20 °C to minimize degradation. High-performance liquid chromatography (HPLC) solvents (Sintorgan, Buenos Aires, Argentina) were used. HPLC-grade water was obtained using a Milli-Q system (Millipore Corporation (Billerica, MA). Multi-use floating dialysis bags (DispoDialyzer®) were purchased from Spectrum Labs (USA).

2.2. Synthesis of PCL-PEG-PCL copolymer

The three PCL-PEG-PCL derivatives were synthesized by ring opening polymerization (ROP) of CL in the presence of PEG (PEG1000, PEG4000 or PEG6000) catalyzed by SnOct and assisted by microwave radiation as described elsewhere [17]. Briefly, PEG was poured in a round-bottom flask (250 mL) and dried under vacuum conditions at 80–90 °C over 2 h in a glycerin bath. Then, the adequate amount of CL (10% molar excess) and the catalyst were added and mixed. Subsequently, the reaction mixture was poured inside a household microwave apparatus (Whirlpool®, WMD20SB, microwave frequency 2450 MHz, potency 800 W, Argentina) connected to a condenser where it was exposed to microwave radiation for 10 min under reflux. Finally, the crude was dissolved in dichloromethane (50 mL) and precipitated in 200 mL of *n*-hexane. The derivatives were isolated by filtration, washed with petroleum ether (40–60 °C) and dried at room temperature until constant weight. Copolymers with different average molecular weight were obtained varying the PEG molecular weight and the total amount of CL employed.

2.3. Copolymer characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained from deuterated chloroform (Sigma) solutions at room temperature on a Bruker MSL300 spectrometer (Karlsruhe, Germany) at 300 MHz. The hydrophobic/hydrophilic balance, as represented by the CL/ethylene oxide (EO) molar ratio, and the number-average molecular weight (Mn) of the different copolymers were calculated by rationing the integration area of the peaks of PCL protons (2H, triplet, 2.30 ppm) and PEG (4H, multiplet, 3.65 ppm). Number- and weight-average molecular weights (Mn and Mw) and molecular weight distributions (Mw/Mn polydispersity, PDI) were determined by gel permeation chromatography (GPC) using a Waters GPC instrument (Berlin, Germany) provided with a refractive index detector (Waters 2414), a Waters Styragel HR4 THF 7.8 × 300 mm column, and tetrahydrofuran as eluent. Polystyrene standards (Polymer Laboratories, Shropshire, UK) were used for calibration.

2.4. Preparation of TPT-blank and TPT-loaded hydrogels

In a first step, each PCL-PEG-PCL copolymer synthesized (35% w/w) was suspended in a deionized sterile water solution in a glass beaker with constant magnetic stirring (50 RPM) for 4 h at room temperature. Then, the suspension was heated at 65 °C for 1 min with gentle magnetic stirring. Once the copolymer gelation was completed, the stirring was continued until the gel cooled to room temperature (15 min) as previously described. In a second step, to evaluate the *in vitro* behavior of TPT-loaded hydrogels, they were prepared with three different loads (0.05, 0.1, and 0.2 mg TPT/g of hydrogel). Aqueous solutions of TPT were prepared by direct dissolution of commercial TPT (Topokebir®, Aspen, Argentina) [16,18].

2.5. Rheological analyses

Rheological behavior of the hydrogels prepared with the synthesized copolymers was analyzed using a rotational type viscometer