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Intravitreal injection of rapamycin-loaded polymeric micelles for inhibition of ocular inflammation in rat model

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ABSTRACT

The therapeutic efficacy of rapamycin conjugated monomethoxy poly(ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL) micelles (rapamycin micelles) was evaluated in a rat experimental autoimmune uveitis (EAU) model. Rapamycin micelles exhibited spherical morphology and had a mean particle size of 40 nm and a zeta-potential of −0.89 mv. The water solubility of rapamycin improved by more than 1000-fold in a micellar formulation. Intravitreal injection of MPEG-PCL micelles did not result in vitreous hemorrhage or retinal detachment. Fluorescence microscopy demonstrated that labeled micelles localized to the retinal pigment epithelium for at least 14 days following injection and the drug concentration of rapamycin micelles in the retinal tissue was significantly higher than unconjugated rapamycin over this period. At the optimal concentration of rapamycin micelles (9 μg/eye), clinical signs of EAU were abolished via the downregulation of the Th1 and Th17 response. There were no significant difference in T cell proliferation and delayed-type hypersensitivity between the treatment and control groups, suggesting that the therapeutic effect of rapamycin manifested locally in the eye and not systemically. These results indicate that intravitreal injection of rapamycin micelles is a promising therapy for controlling sterile intraocular inflammation.

1. Introduction

Posterior uveitis is an inflammatory disorder of the posterior segment of eye, often involving both the retina and choroid. Posterior uveitis has a complex etiology, is challenging to treat and accounts for up to 10% of all cases of blindness (de Smet et al., 2011; Selmi, 2014; Ozzello and Palestine, 2015). Currently, systemic and topical administration of steroids are the first choice treatments for acute posterior uveitis (Patel et al., 2012; Kempen et al., 2014; Prete et al., 2015). However, steroidal therapy normally has significant side effects, including glucose intolerance, bone demineralization, cataract and glaucoma, which greatly limits its long-term usage. Non-steroidal anti-inflammatory agents such as cyclosporine, methotrexate and azathioprine offer reduced side effects and can provide long-term therapeutic management for patients (Agrawal et al., 2014; Letko et al., 2015). However, such agents are limited by a narrow therapeutic window, which is a challenging scenario for the clinician.

Extensive study over the past two decades has demonstrated that T cell inhibitors, such as cyclosporine-A, tacrolimus and sirolimus (rapamycin), can effectively treat intraocular inflammation (Salzmann and Lightman, 2000; Zhang et al., 2010; Bauer et al., 2014). Rapamycin was initially discovered in the bacterium *Streptomyces hygroscopicus* and exhibits a unique immunosuppressive mechanism, as it inhibits T and B cell activation and proliferation via binding to the immunophilin FK protein 12 (Phillips and Wroblewski, 2011). It also suppresses T cell proliferation through the inhibition of specific lymphokines (IL-2, IL-4, IL-6 and IL-12), induced by both Ca^{2+}-dependent and Ca^{2+}-independent pathways. Owing to its favorable pharmacological properties, rapamycin has been widely used in the treatment of immune rejection after organ transplantation. Currently, two FDA-approved rapamycin formulations, Rapmune® and Cypher®, have been used clinically for renal transplant and symptomatic ischemic disease patients (Virmani et al., 2004; Min et al., 2006; Adelman, 2010). Compared with systemic administration (e.g. oral, intravenous), local administration, such as intravitreal injection, is preferred for the treatment of posterior uveitis (Dugel et al., 2012).

To increase the intraocular concentration of rapamycin and reduce systemic side effects, a new drug delivery system is required. However, rapamycin is extremely water insoluble

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(approximately 2.6 μg/ml in water) and lacks ionizable functional groups in the pH range of 2–10, rendering it unsuitable for noninvasive topical administration (Adelman, 2010; Lu et al., 2011; Sun et al., 2011). In recent years, the use of biodegradable polymeric micelles as a novel drug delivery system has gained considerable attention due to their high drug loading capacity, small particle size (<100 nm) and controllable drug release (Kataoka et al., 2001; Bae and Kataoka, 2009; Gong et al., 2013). Biodegradable polymeric micelles can deliver drugs directly to the target tissue, thus increasing drug bioavailability and reducing side-effects. A number of amphiphilic polymers composed of hydrophilic polyethylene glycol and biodegradable hydrophobic segments such as polylactic acid (PLA), poly(lactide-*co*-glycolide) and poly(ε-caprolactone) have been demonstrated to be capable of forming micelles/nanoparticles for delivery of various hydrophobic drugs (Dong and Feng, 2004; Li et al., 2012; Xue et al., 2012). Luderer et al. (2011) reported that rapamycin loaded PLA nanoparticles administered intraluminally were able to reduce in-stent restenosis after stent implantation. Our recent study also demonstrated that the monomethoxy poly(ethylene glycol)-poly (ε-caprolactone) (MPEG-PCL) block polymer could self-assemble into micelles for hydrophobic drug delivery (Li et al., 2012).

In the present study, we hypothesized that MPEG-PCL micelles are a promising nanocarrier for rapamycin. The MPEG-PCL micelles could greatly improve the water solubility and stability of rapamycin, along with extending the duration of drug release and enhancing bioavailability. The intraocular distribution of rapamycin loaded MPEG-PCL micelles (herein referred to as rapamycin micelles) was investigated in rats following intravitreal injection and their inhibition of intraocular inflammation in an experimental autoimmune uveitis (EAU) model was evaluated in comparision with rapamycin suspensions.

2. Materials and methods

2.1. Materials

MPEG-PCL (M_n = 5000; MPEG/PCL = 2000/3000) block polymer was synthesized according to our previous study (Li et al., 2012). Rapamycin (Rap) was purchased from Dalian Meilun Biology Technology Co., Ltd (Dalian, China). All other reagents were of analytical grade.

2.2. Preparation and characterization of rapamycin micelles

Rapamycin micelles were fabricated by a previously reported method (Li, Zhang et al., 2012). Briefly, 100 mg of rapamycin and 900 mg of MPEG-PCL block polymer were co-dissolved into 10 ml of acetone followed by evaporation of the organic solvent with rotary evaporators at 40 °C. Thereafter, 10 ml of distilled water was added and incubated at 50 °C to obtain rapamycin micelles. The rapamycin micelles were then filtered with a 0.22 μm filter and lyophilized for further usage. Finally, the drug loading capacity and drug loading efficiency were determined by HPLC analysis (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). Separation was performed on an Agilent Eclipse-C18 column (4.6 × 150 mm, 5 μm) (Agilent Technologies) at a flow rate of 1 ml/min. The mobile phase was composed of (A) acetonitrile and (B) water (75:25; v/v). The eluent was detected at 277 nm. The column temperature was set to 40 °C.

2.3. Size distribution, zeta potential and transmission electron microscope observation

The size distribution and zeta potential of the rapamycin micelles were analyzed using a Zetasizer Nano ZS-90 (Malvern Instruments, Malvern, UK). The morphology of the rapamycin micelles was determined by transmission electron microscopy (Tecnai F20, FEI, Hillsboro, OR, USA). Prior to the microscopy, the samples were negatively stained with 0.5 wt% phosphotungstic acid and placed on a copper grid.

2.4. Intraocular biocompatibility test via intravitreal injection

All animal experiments complied with the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources and were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University. Six Sprague

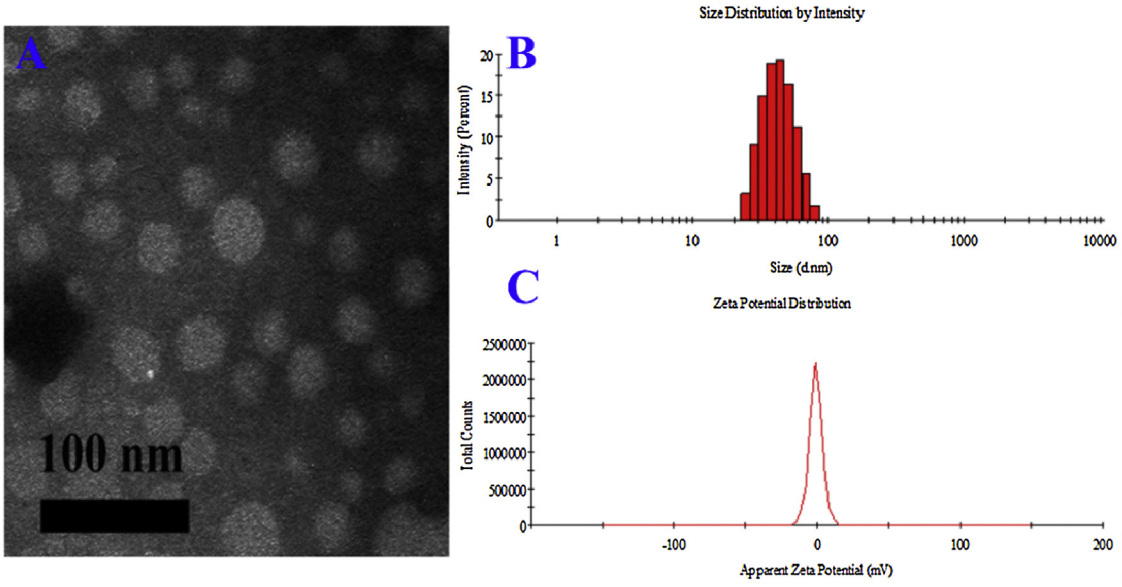

Fig. 1. (A) TEM image, (B) Size distribution and (C) Zeta potential of rapamycin loaded MPEG-PCL micelles.