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Research paper

Novel micelle carriers for cyclosporin A topical ocular delivery: *In vivo* cornea penetration, ocular distribution and efficacy studies

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ABSTRACT

Cornea transplantation is one of the most performed graft procedures worldwide with an impressive success rate of 90%. However, for "high-risk" patients with particular ocular diseases in addition to the required surgery, the success rate is drastically reduced to 50%. In these cases, cyclosporin A (CsA) is frequently used to prevent the cornea rejection by a systemic treatment with possible systemic side effects for the patients. To overcome these problems, it is a challenge to prepare well-tolerated topical CsA formulations. Normally high amounts of oils or surfactants are needed for the solubilization of the very hydrophobic CsA. Furthermore, it is in general difficult to obtain ocular therapeutic drug levels with topical instillations due to the corneal barriers that efficiently protect the intraocular structures from foreign substances thus also from drugs.

The aim of this study was to investigate *in vivo* the effects of a novel CsA topical aqueous formulation. This formulation was based on nanosized polymeric micelles as drug carriers. An established rat model for the prevention of cornea graft rejection after a keratoplasty procedure was used. After instillation of the novel formulation with fluorescent labeled micelles, confocal analysis of flat-mounted corneas clearly showed that the nanosized carriers were able to penetrate into all corneal layers. The efficacy of a 0.5% CsA micelle formulation was tested and compared to a physiological saline solution and to a systemic administration of CsA. In our studies, the topical CsA treatment was carried out for 14 days, and the three parameters (a) cornea transparency, (b) edema, and (c) neovascularization were evaluated by clinical observation and scoring. Compared to the control group, the treated group showed a significant higher cornea transparency and significant lower edema after 7 and 13 days of the surgery. At the end point of the study, the neovascularization was reduced by 50% in the CsA-micelle treated animals. The success rate of cornea graft transplantation was 73% in treated animals against 25% for the control group. This result was as good as observed for a systemic CsA treatment in the same animal model. This new formulation has the same efficacy like a systemic treatment but without the serious CsA systemic side effects. Ocular drug levels of transplanted and healthy rat eyes were dosed by UPLC/MS and showed a high CsA value in the cornea (11710 ± 7530 ng_{CsA}/g_{tissue} and 6470 ± 1730 ng_{CsA}/g_{tissue}, respectively).

In conclusion, the applied formulation has the capacity to overcome the ocular surface barriers, the micelles formed a drug reservoir in the cornea from, where a sustained release of CsA can take place. This novel formulation for topical application of CsA is clearly an effective and well-tolerated alternative to the systemic treatment for the prevention of corneal graft rejection.

1. Introduction

The cornea is the outside part of the eye, thus the first barrier for protection of the intraocular structures against foreign substances. In addition, the cornea has the important physiological

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role for the transmission of the incident light. It represents 60% of the total refractive power of the eye [1]. Many ocular diseases, such as keratoconus, corneal dystrophy, keratopaties, failed previous graft, need a total or partial replacement of the cornea [2]. In all these diseases, a cornea transplantation is performed to provide clear vision, structural support, or to alleviate pain [3]. In many cases, the surgery is a penetrating keratoplasty (PKP), in which the full thickness of the cornea is replaced. Every year 120,000 corneal grafts are performed worldwide [4]. Actually, it is one of the most successful transplantation procedures. The graft survival rate is of 90% for "low-risk" patients, those receiving the cornea transplantation for the first time and without additional ocular diseases besides the corneal problems requiring transplantation [5]. For patients considered "high risk," because they have other diseases in addition to the corneal ones, such as glaucoma, neovascularization, or because they undergo transplantation for the second or third time, the graft survival rate is drastically reduced to approximately 50% [6]. In the case of high-risk patients, a systemic immune-suppression with corticosteroids (the most commonly used is prednisolone), antimetabolite (i.e., mycophelonate mofetil), or cyclophillin binding T-cells inhibitors (i.e., cyclosporin A) is applied [2]. However, this systemic treatment is associated with systemic side effects, especially for the potent immunosuppressant cyclosporin A for which nephrotoxicity and hepatotoxicity are reported [7]. Thus, a topical ophthalmic CsA formulation is desirable, not only for the prevention of cornea graft rejection but also for the treatment of dry eye syndrome and uveitis [8].

CsA is a very hydrophobic drug with poor water solubility (0.012 mg/mL at 25 °C) [9], which leads to formulation problems. To obtain an aqueous CsA formulation, many approaches can be considered such as co-solvency, prodrugs, emulsions, and colloidal systems. A topical CsA pro-drug was evaluated by Bourges et al. in a rat model of penetrating keratoplasty, showing promising results with the same efficacy as the systemic treatment, avoiding the drawbacks of the before-mentioned side effects [10]. Emulsion and colloidal CsA formulations have been developed for ophthalmic applications, and despite the encouraging results for increased corneal penetration, no efficacy for the prevention of corneal graft rejection was proven [11–14]. For example, poly ε-caprolactone nanocapsules loaded with 1% CsA were ineffective for rejection prevention [14]. Restasis®, a commercial 0.05% CsA emulsion, was investigated in human transplantation in addition to the corticosteroid therapy, without an improvement of the graft survival [15,16].

Recently, our group investigated novel micelle formulations based on methoxy poly(ethylene) glycol-hexylsubstituted poly(lactides) (MPEG-hexPLA) copolymers [17–19]. These nanocarriers are interesting for topical ophthalmic administration, because of their excellent biocompatibility and ocular tolerance [20].

The aim of the present study was (i) to investigate the capacity of these nanosized carriers to overcome the corneal barriers after topical application for an effective CsA controlled release; (ii) to test the efficacy of a 0.5% CsA/MPEG-hexPLA micelle formulation for the prevention of corneal allograft rejection in a rat model of cornea transplantation; and (iii) finally to investigate the ocular CsA distribution in rat eyes in order to prove the suitability of aqueous micelle formulation in the delivery of CsA into ocular structures in comparison with an oil solution.

2. Materials and methods

2.1. Materials

Methoxy poly(ethylene) glycol (MPEG) with a molecular weight of 2000 g/mol was purchased from Union Carbide Corporation (USA). α-hydroxyoctanoic acid, 3,6-dihexyl-1,4-dioxane-2,5-dione (dihexylsubstituted lactide, hexLA), and methoxy poly(ethylene) glycol-hexylsubstituted poly(lactides) (MPEG-hexPLA) were synthesized as described previously [17,21]. The Nile Red labeled MPEG-hexPLA was synthesized in the laboratory [22].

The following products were purchased from:

Sigma–Aldrich (D): stannous 2-ethylhexanoate, and sucrose; Biotium (USA): 3,3-Dioctadecyloxacarbocyanine perchlorate ($DiOC_{18}(3)$); Théa (F): 0.5% tropicamide and oxybuprocaine chloridrate; Virbec (F): ketamine 1000; Sanofi-Aventis (F): chloropromazine and sodium pentobarbital; Dynapharm Distribution (CH): cyclosporin A; Gifrer (F): physiological saline solution; Stiefel (D): trephine; Healonid Pharmacia (S): hyaluronic acid; Ethicon (B): suture thread; Fluka (D): analytical grade acetone; VWR (F): methanol for HPLC; Biosolve (CH): methanol for UPLC/MS. MilliQ water was used in each experiment. 0.5% CsA oil formulation was provided by the Pharmacy of Hotel-Dieu Hospital in Paris, (F). Eight mL of this latter formulation were composed of 40 mg of CsA, 50 mg absolute ethanol, 7.6 mL castor oil, corn oil and inter-esterified corn oil to an overall of 8 mL solution.

2.2. Preparation and characterization of the formulations

CsA/MPEG-hexPLA micelle formulations were prepared as previously described [20,23].

Briefly, 120 mg of MPEG-hexPLA copolymer and 26.4 mg of CsA were dissolved in 2 mL acetone and added dropwise under sonication to 4 mL 10 mM phosphate buffer with 10% sucrose. The acetone was evaporated under vacuum. After sterile filtration with a 0.22-μm filter under laminar flow, the formulations were characterized in terms of size, morphology, and drug loading, using Dynamic Laser Scattering (DLS) (Zetasizer HS 3000, Malvern Instruments, UK), Transmission Electron Microscopy (TEM) (FEI Tecnai™ G2 Sphera, USA), and High-Pressure Liquid Chromatography (HPLC) (Waters, USA), respectively, with the methods and conditions reported earlier [20,23].

Two formulations were prepared: (a) MPEG-hexPLA formulation with 0.5% CsA for the transplantation study and the CsA ocular distribution study in healthy eyes and (b) a fluorescent micelle formulation prepared with Nile Red labeled MPEG-hexPLA copolymer:MPEG-hexPLA copolymer weight ratio of 97:3 and loaded with the dye 3,3-dioctadecyloxacarbocyanine perchlorate (DiO) at 0.1% w/w concentration, for the corneal penetration study. For both formulations, the copolymer concentration was 30 mg/mL.

2.3. Animals

Thirty-four Lewis and nine Brown-Norway 8-week-old female rats were purchased from Janvier (F). Four Lewis rats were used for the corneal penetration study, eighteen Lewis rats and nine Brown-Norway were used for the transplantation study, and twelve Lewis for the test of CsA ocular distribution. All rats were cared for in accordance with the European Committee Directives (Authorization numbers: A 75-06-12 and 75-580) and with the Association for Research in Vision and Ophthalmology resolution concerning the use of animals in ophthalmological research.

2.4. Evaluation of micelle penetration into the cornea

The corneal penetration was studied for two formulations, (a) fluorescent micelles on six eyes of three rats and (b) physiological saline solution on the two eyes of 1 rat. For the fluorescent micelle formulation, the two wavelengths of maximal fluorescence were determined by Confocal Laser Microscopy (CLSM) (Zeiss LSM T-PMT, equipped with a Laser Zeiss LSM 710, D).