Design of an Anti-Inflammatory Composite Nanosystem and Evaluation of Its Potential for Ocular Drug Delivery

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ABSTRACT: This study compared two specific embodiments of an ocular nanosystem (NS): one portraying a purely polymeric system, referred to as the chitosan–poly(ε-caprolactone) nanosystem, and the other based on a composite lipoidal–polymeric NS architecture utilizing phospholipids—the lipoidal–chitosan–poly(ε-caprolactone) nanosystem. Investigations undertaken were implicit to warrant inclusion in an implantable system for the intelligent treatment of inflammatory disorders (specifically ocular afflictions). Results obtained highlighted the enhanced efficacy of both NS to an indomethacin suspension in terms of tissue permeation, cell uptake, and anti-inflammatory activity. Furthermore, the size (134.3 vs. 140.7 nm); surface charge (+49.4 vs. +55.7 mV); drug incorporation efficiency (75.00% vs. 67.20%); flux across the retinal pigment epithelium–choroid–sclera (0.002951 vs. 0.001255 mg cm $^{-2}$ h^{-1}); anti-inflammatory efficacy, demonstrated by a decrease in 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole complex formation (0.0031 vs. 0.0023 mmol L^{-1}) and decrease in NFκB formation (decrease in relative optical density of 0.2027 vs. 0.2420); and enhanced inflammatory cell uptake, visualized via high-speed fluorescence and confocal microscopy, all highlighted the enhanced potential of the lipoidal system compared with the purely polymeric NS for potentially targeting inflammatory disorders of the posterior segment of the eye. Mechanics energy relationships revealed the favorable hydrophilic–lipophilic balance of the composite NS compared with the purely polymeric NS. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2780–2805, 2013

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INTRODUCTION

In terms of research spurring the pharmaceutical scientist, ophthalmic drug delivery is an increasingly demanding sector.[1] In their investigations, authors have pointed to inflammatory posterior segment ocular (vitreoretinal) disorders, such as uveitis, as the foremost contributors to visual impairment, and ultimately blindness.[2,3] Ensuring delivery of the indicated bioactive to the posterior segment of the eye is fundamental for the effectual treatment of internal eye structure disorders.[3] Nonetheless, anterior segment drug delivery systems, most notably, eye drops, still lead trends in the ocular market. The goal in ocular therapeutics is to maintain an effective drug concentration at the site of action for an appropriate period of time, to achieve the expected pharmacological response.[4] Drug delivery systems undoubtedly form a crucial component of the "therapeutic armamentarium" in ophthalmology. Accordingly, investigators have stated that, "ophthalmic drug delivery, probably more than any other route of administration, may benefit from the characteristics of nanotechnology-based drug delivery systems."[5,6] Knowledge derived

Additional Supporting Information may be found in the online version of this article. Supporting Information

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from drug delivery systems using nonocular routes of administration has stimulated researchers to find applications for nanotechnology in ophthalmology.

The inherent advantages of nanopharmaceutical systems (nanosystems; NS) are intrinsic to their colloidal nature. NS are poised, and have thus been conceptualized and fabricated, to circumvent the problems associated with conventional ocular systems to essentially increase the ocular bioavailability of drugs (especially of poorly water soluble or poorly permeable drugs) and maintain activity at the site of action, thus enhancing the therapeutic effect (because of reduced cellular and tissue clearance of drugs, sustained drug delivery, enhanced precorneal residence, and uptake of drugs by ocular epithelia). NS have the ability to protect the encapsulated molecule while facilitating its transport to the different compartments of the eye, as well as providing targeted delivery of bioactives.[7–13] This is a result of the capability to overcome blood-ocular barriers, and efflux-related issues associated with the parent drug. Further, these carriers can also bypass or overcome various stability-related problems of drug molecules, as evidenced for proteins and peptides.[14] Additionally, NS can provide controlled drug delivery for extended periods of time, an attractive benefit for the treatment of some chronic ocular diseases.[13,15,16] Furthermore, NS purportedly greatly reduce toxicity compared with the free drug, and they avoid the discomfort associated with the application of viscous or sticky preparations such as ointments, which cause total blurring of vision on correct administration.[17]

Few reports emphasize the intravitreal delivery of drugs through ocular barriers via NS; however, some recent studies have shown commendable results on the use of intravitreally injected nanoparticles (NPs). For example, ganciclovir-loaded albumin NPs have been developed, as ganciclovir is one of the standard treatments for cytomegalovirus (CMV) retinitis, a prevalent infectious retinal disease in immunosuppressed patients (e.g., HIV/AIDS patients). Sustained drug release was evident following *in vitro* experiments,[18] as well as a significant improvement of drug uptake by CMV-infected human cells.[19,20] Following single intravitreal injections in rats, safety and tolerance was demonstrated and drug levels were detected in the vitreous and ciliary body for at least 2 weeks.[21] Bourges et al.[8] developed intravitreal poly(lactic acid) (PLA) NPs loaded with fluorochromes and tested them in rats; they demonstrated a preferential localization at the retinal pigment epithelium (RPE) cells after 24 h. Notably, the RPE cells retained the NPs, allowing continuous delivery of the fluorochrome for months following a single injection. Bejjani et al.[22] undertook *in vitro* and *in vivo* investigations on PLA and copolymers of lactic and glycolic acids (PLGA) NPs loaded with fluorochromes and model plasmids. Twenty-four hours following intravitreal injection in rats, NPs encapsulating a plasmid encoding red nuclear fluorescent protein were localized in the RPE, and plasmid expression was achieved after 4 days of injection. Other intravitreal systems include injectable NPs for gene therapy as extensively reviewed by Conley and Naash.[23] Despite the undeniable discomfort, the risks associated with injectable systems, however, must be borne in mind (i.e., cataract formation, retinal detachment, endophthalmitis, and vitreous hemorrhage).[2] The design of an ocular NS, possessing the capabilities to penetrate diverse ocular barriers and target inflammatory tissues of the posterior segment following presentation at various ocular sites, could be perceived as a significant advance in ocular drug delivery.

Through our published investigations,[24] we have identified the combinatory advantages of polymerically enhanced lipoidal NS, which include: (a) incorporation of poorly water-soluble drugs, which is largely independent of the liposome bilayer physicochemical properties, (b) prolonged lifetime attributed to the polymeric component, (c) tissue distribution, which will be largely lipid dose independent, and (d) inflammatory tissue targeting based on the careful selection of lipoidal and polymeric components.

On the basis of the aforedescribed findings, and elaborated via the principles of liposomology and ionotropic gelation, a composite lipoidal–polymeric NS was thus developed, which incorporated suitable phospholipids, the hydrophobic poly(ε-caprolactone) (PCL), and the mucoadhesive, permeation-enhancing chitosan. Indomethacin was selected as the model nonsteroidal, anti-inflammatory drug, being a potent inhibitor of all isoforms of cyclooxygenase (COX), a significant enzyme in the inflammatory process.[25]

To establish whether inclusion of the phospholipid component in the NS conferred a notable advantage to the NS for targeting ocular inflammatory diseases, it was compared with a purely polymeric NS. This investigation, therefore, compared the potential of two specific embodiments of an ocular NS: one portraying a purely polymeric system (including chitosan and PCL), referred to as the Chit–PCL NS, and the other based on a composite lipoidal–polymeric NS architecture utilizing phospholipids (DL-α-disteroylphosphatidylcholine, DSPC, and L-α-distearoylphosphatidylethanolimine, DSPE), referred to as the Lipo–Chit–PCL NS. The preferred NS would ultimately be incorporated within an intelligent intraocular implant to achieve long-term delivery to the posterior segment of the eye. The NS will be included in the cross-linked core of the solid implant. Implantation would be at the following proposed sites: sub-Tenon, intrascleral, or at the pars plana. The design of the final implant for NS delivery is discussed in subsequent investigations. The following attributes of