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Research paper

Solid lipid nanoparticles as promising tool for intraocular tobramycin delivery: Pharmacokinetic studies on rabbits

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ABSTRACT

Eye drops are widely accepted as formulations for targeting the anterior segment notwithstanding their limitations in terms of bioavailability. The unique structure of the eye requires specially-designed formulations able to favor the pharmacokinetic profile of administered drugs, mainly minimizing the influence of ocular barriers. Nanotechnology-based delivery systems lead to significant technological and therapeutical advantages in ophthalmic therapy.

The aim of the present study was to determine whether tobramycin as ion-pair incorporated in mucoadhesive Solid Lipid Nanoparticles (SLN) reaches the inner parts of the eye favoring drug activity.

After technological characterization of the tobramycin entrapped SLN formulation (Tobra-SLN), a pharmacokinetic study in rabbits after topical instillation and intravenous administration of the formulation has been carried out. In addition, the intracellular activity of Tobra-SLN formulation against phagocytosed *Pseudomonas aeruginosa* was investigated.

The SLN were spherical in shape, and showed a hydrodynamic diameter of about 80 nm, a negative zeta potential (−25.7 mV) with a polydispersity index of 0.15, representative of a colloidal dispersion with high quality, characterized by an unimodal relatively narrow size distribution. As demonstrated by FTIR and DSC, tobramycin ion-pair could be concentrated into lipid inner core of SLN, without interaction with the stearic acid, thus promoting a slow and constant drug release profile in the dissolution medium.

Surprisingly, the drug concentration was significantly higher in all ocular tissues after ocular and intravenous administration of Tobra-SLN formulation with respect to reference formulations and only Tobra-SLN allowed the penetration of drug into retina. Furthermore, the use of Tobra-SLN resulted in both higher intraphagocytic antibiotic concentrations in polymorphonuclear granulocytes and greater bactericidal activity against intracellular *Pseudomonas aeruginosa,* probably due to the ability of Tobra-SLN to penetrate either into phagocytic cells, or alternatively to cross bacterial barrier.

The present study broadens the knowledge on the use of SLN as carriers for ocular drug delivery to the posterior chamber and might open new avenues for treatment of ocular infections, representing a strategy to overcome the microbial resistance.

1. Introduction

The eye is considered a complex and delicate organ of the human body that represents a very challenging issue in drug delivery. Eye drops are widely accepted as formulations for targeting the anterior segment notwithstanding their limitations in terms of bioavailability for extensive pre-corneal loss due to blinking, rapid washout by tearing, drainage through the naso-lacrimal duct, non-productive absorption [1–4]. Furthermore, eye drops capability to treat vitreoretinal diseases, such as bacterial infections, endophthalmitis, cytomegalovirus retinitis (CMV), uveitis, proliferative vitreoretinopathy (PVR), diabetic retinopathy, and age-related macular degeneration (AMD) is worthless because of poor drug penetration in the posterior segment [1,4–6]. The main challenges in ocular therapy are represented by the reduction in pre-corneal drainage of the instilled formulations and the overcoming of the protective barriers of eye to reach a sufficient drug concentrations at the site of action. The unique structure of

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the eye requires specially-designed carriers able to optimize the pharmacokinetic behavior of topically administered drugs, mainly minimizing the influence of ocular barriers [1,3,5–9]. The corneal layers, in particular the epithelium and stroma, are considered the major barriers for absorption of topically applied drugs, which should have amphipathic nature, in order to permeate through the lipophilic/hydrophilic layers. In addition, the sclera, mainly consisting of collagen fibers and proteoglycans embedded in an extracellular matrix, is considered to be comparable in permeability to the corneal stroma. The permeability of drug molecules across the sclera is inversely proportional to the molecular radius and to the charge of the drug molecule: positively charged molecules exhibit scarce permeability, presumably for their binding to the negatively charged proteoglycan matrix [9–11].

The systemic route represents a successful treatment for ocular diseases involving the anterior segment or the ocular surface, but it is not suitable to treat vitreoretinal diseases. In fact, the outer and inner blood-retinal barriers, formed respectively by retinal pigment epithelium and retinal vascular endothelium, inhibit the intraocular penetration of drugs when applied systemically [3,12,13].

Nanotechnology-based delivery systems (i.e. nanocapsules, microemulsions, liposomes, nanomicelles and lipid nanoparticles) lead to significant technological and therapeutical advantages in ophthalmic therapy, as increase in precorneal drug retention time, sustained drug release, reduction in administration frequency, reduced drug toxicity, targeted delivery to specific eye tissues. Furthermore, micro/nanocarriers can be generally administered as dispersion in the form of eye drops without causing blurred vision and irritation [7,8,13–16]. All these aspects cause an improvement in bioavailability of drugs and in the patient compliance. Among all nanocarriers, lipid carriers, in particular SLN, were extensively investigated as promising delivery systems for hydrophilic or lipophilic ophthalmic drugs [8,17–23]. Besides being a droppable preparation and improving ocular bioavailability of drugs, lipid nanocarriers have the advantage to reduce both the leakage of drugs encapsulated, as for liposomes, and the instability during storage typical of emulsions, polymeric nanoparticles and liposomes.

In previous works, we showed that solid SLN modified the pharmacokinetic parameters and the distribution of several incorporated drugs in the biological tissues, increasing their passage through barriers such as the blood brain barrier [24–26]. In detail, we observed that a SLN dispersion containing the antibacterial tobramycin significantly enhanced the drug bioavailability in the aqueous humor [27] and that pilocarpine incorporated in SLN tripled the miotic effect of the drug in comparison with the commercial formulation [28]. Recent reports referred on the positive behavior of the association between the drug-entrapped SLN carriers prepared with different lipidic components and innovative "in situ" gel forming vehicles [29] and on the improved efficacy of SLN by incorporating hyaluronic acid and protamine to obtain a versatile carrier for gene therapy [30].

Even if in the last ten years SLN have been studied extensively and several manuscripts reported the advantage obtained by their use as delivery systems for many ophthalmic drugs (anti-inflammatory, antiglaucoma, antibacterial, antifungal), natural extracts, antioxidant molecules and gene therapy [9,20,21,30–35], in vivo studies are almost rare, while they should be helpful to establish the real therapeutical potential of SLN as ocular drug delivery.

The aim of the present study was to broaden the knowledge on the ocular delivery of tobramycin on rabbits by determining whether tobramycin incorporated in a mucoadhesive SLN dispersion is able to reach the inner parts of the eye. Hence, a pharmacokinetic study in rabbits after ocular instillation and intravenous administration of the SLN has been carried out. Since the entrance of antimicrobial agents into phagocytic cells in association with cellular bactericidal mechanisms is a prerequisite for their activity, we also investigated the intracellular activity of tobramycin in comparison with SLN formulations against phagocytosed *Pseudomonas aeruginosa*, which is responsible for numerous eye infections [36,37], in order to demonstrate all their possible benefits. Furthermore, the physical and chemical stability over the time of drug-loaded SLN has been investigated.

2. Materials and methods

2.1. Materials

Stearic acid and 1-fluoro-2,6 dinitrobenzene were from Fluka (Buchs, Switzerland); Epikuron 200 (soya phosphatidylcholine 95%) was kindly provided by Cargill (Hamburg, Germany); tobramycin base, mucin from porcine stomach type III, type VII alkaline phosphatase and fluorescamine were from Sigma Chemical Co. (Missouri, USA), and taurocholate sodium salt was kindly provided by PCA (Basaluzzo, Italy). Sodium hexadecylphosphate was prepared as indicated by Brown [38]. The commercial eye drops Tobral® (0.3% of tobramycin, Alcon Italia S.p.A., Italy) was used as control. The other chemicals were of analytical grade.

2.2. Animals

All experiments were carried out on male New Zealand albino rabbits of 2.8–3.5 kg (Pampaloni Rabbitry, Fauglia, Italy). The animals were housed in singly standard cages in a light-controlled room (10 h dark/14 h light cycle) at 19 ± 1 °C and 50 ± 5% RH and were given a standard pellet diet and water *ad libitum*. During the experiments the rabbits were placed in restraining boxes but their eyes movements were not restricted. The rabbits were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, following a protocol approved by the Ethical-Scientific Committee of the University of Pisa and under veterinary supervision.

Eight groups of animals, each of three rabbits, were used for the different treatments.

2.3. Preparation of Solid Lipid Nanoparticles incorporating tobramycin (Tobra-SLN)

Solid lipid nanoparticles containing the tobramycin as complex (Tobra-SLN) were prepared, as tuned previously by us, from o/w microemulsions consisting of stearic acid as internal phase (0.70 mmol), Epikuron 200 as surfactant (0.14 mmol), sodium taurocholate as cosurfactant (0.72 mmol), and deionized water as continuous phase (110.10 mmol). The drug was added to the microemulsion as ion-pair complex with hexadecylphosphate in a 1:2 tobramycin:hexadecylphosphate molar ratio to increase the lipophilicity of tobramycin. The final amount of tobramycin complex was 0.05 mmol [27]. The ion-pair complex favored partition of tobramycin in the oil phase of the microemulsion and was prepared using a co-precipitation method with hexadecylphosphoric acid as tobramycin counter ion [39,40]. Tobra-SLN aqueous dispersions were obtained by dispersing warm o/w microemulsion in cold water at a 1:10 microemulsion:water (v/v) ratio under mechanical stirring. Dispersions were washed three times by diaultrafiltration with TCF2 system (Amicon, Danvers, USA) using a Diaflo YM 100 membrane (cutoff 100,000 Da) to remove the majority of the cosurfactant used to obtain the microemulsion. After the purification, Tobra-SLN dispersion was freeze-dried and subsequently dispersed to obtain the required final concentration