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α-Lipoic Acid in Soluplus® Polymeric Nanomicelles for Ocular Treatment of Diabetes-Associated Corneal Diseases

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ABSTRACT

α-Lipoic acid (ALA) is a powerful antioxidant valuable for prevention and treatment of ophthalmic complications such as diabetic keratopathy and retinopathy. The aim of this work was to develop micelle-based formulations that can enhance the solubility, stability, and corneal permeability of ALA. Compared to a conventional surfactant (sodium dioctylsulfosuccinate), Soluplus® (polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol copolymer) led to smaller micelles (70-80 vs. 240-528 nm) with improved ability to solubilize ALA, maintaining ocular compatibility (Hens Egg Test on the Chorio-Allantoic Membrane). Soluplus nanomicelles enhanced more than 10-fold ALA solubility compared to common eye drops and withstood strong dilution in lachrymal fluid, filtration through sterilizing membranes, and freeze-drying. Interestingly, Soluplus nanomicelle formulation prepared with 1 or 2 mM block copolymer concentration exhibited *in situ* gelling capability and transformed into weak gels at 35°C, which is expected to increase corneal residence time. Bovine corneal permeability revealed that Soluplus nanomicelles notably enhanced ALA accumulation into the cornea as well as flux of drug toward the receptor chamber. Overall, these findings point out Soluplus nanomicelles as suitable carriers of ALA that may exhibit improved performance compared to currently available eye drop solutions.

Introduction

Diabetes is a highly prevalent disease that affects nearly 10% of the world population and is characterized by severe acute and chronic complications.[1] Affectation of ocular structures may lead to retinopathy, papilopathy, glaucoma, cataracts, and corneal damage, with an increased risk of loss of vision.[2] Damage of anterior eye segment structures is shown as corneal neuropathy and keratopathy, which are characterized by corneal epithelium fragility, abnormalities in corneal endothelium and conjunctival Goblet cells, dysfunctions in the repair mechanisms, instability of lachrymal film, and increased incidence of infections.[3,4] Diabetic keratopathy can be detected by non-invasive techniques and usually precedes a further development of diabetic retinopathy. Therefore, early treatment of cornea and conjunctiva damage may benefit overall ocular structures. Current pharmacological strategies involve prevention of sorbitol accumulation, vascular proliferation and inflammatory conditions, and delivery of growth factors for healing.[5] Most treatments for diabetic eye conditions rely on systemic (oral) or intravitreal administration, and there is still a demand for efficient ocular dosage forms that provide therapeutic levels in the anterior eye segment in a comfortable way for the patient.

α-Lipoic acid (ALA; Fig. 1) is an octanoic acid derivative that presents an intramolecular disulfide bound. Its high antioxidant potential along with its excellent security profile (it is an endogenous compound) determines its wide use as food supplement.[6] Moreover, ALA has hypoglycemic properties as well as favors corneal epithelium reparation. Currently, ALA is orally given for treatment of diabetic polyneuropathy and other complications solely or in combination with other drugs.[7,8] Antioxidant activity of ALA is due to a variety of mechanisms, which include formation of chelates with metallic ions, inhibition of lipid peroxidation, inactivation of reactive oxygen species, reduction of oxidized forms of other antioxidants such as vitamin C and E, or increment of glutathione intracellular levels because of the activation of

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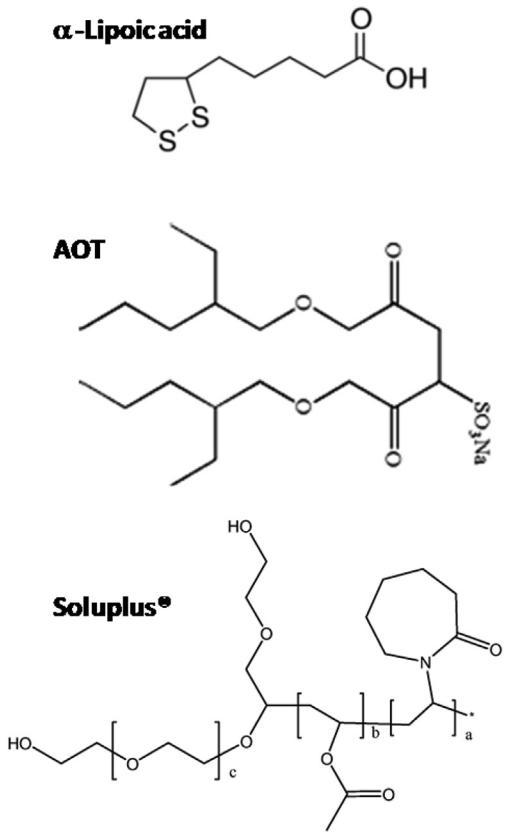

Figure 1. Structure of ALA, AOT, and Soluplus.

transcription factor Nrf2.[7] On the ocular surface, ALA reduces capillary injuries,[9] and subretinal space and rods alterations caused by oxidative stress.[10] ALA also attenuates vascular proliferation (lowering vascular endothelial growth factor level) and damage in the membrane of the corneal nerve fibers (keeping the efficiency of the nerve endings) by means of the reduction of malonaldehyde and other lipid peroxidation products.[11,12] Anti-inflammatory activity of ALA makes it an alternative to non-steroidal anti-inflammatory drugs.[13] However, prolonged oral intake of high ALA doses may have some detrimental (pro-oxidant) effects on cardiac function.[14] Formulation of ALA as eye drops has to face up to its low solubility in water, instability against light, and reactivity with other components. In some European countries, ALA (0.1%) ophthalmic solution containing hydroxypropyl methylcellulose (0.3%), tromethamine (0.15%), and ethylenediaminetetraacetic acid sodium salt (0.1%) is commercially available for topical treatment of corneal-conjunctival alterations associated to diabetic retinopathy (e.g., Tioretin® A).[9] When ALA is instilled on the ocular surface, it can permeate through the cornea and get access to the aqueous humor and even to the vitreous humor as demonstrated in some animal species.[9,15] However, the eye defense mechanisms and the limited drug concentration gradient on the human cornea determine that the ocular bioavailability is low.[16]

The aim of this work was to elucidate whether encapsulation of ALA in micelles could enhance drug solubility and corneal permeability compared to commercially available eye drops. ALA has been tested as a component of copolymers for polymeric micelles owing to its low solubility, self-assembling features, and the labile disulfide bond which can endow the systems with responsiveness to heat, light, or redox conditions.[17] However, ALA formulation as free molecule in micelles has not been explored yet. Nanocapsules with a shell of inorganic salts and polyethylenglycol chains have been designed for improved stability of ALA in cosmetic applications.[18] Recently, nanomicelles ($\leq$100 nm) have been pointed out as advantageous delivery systems for both anterior and posterior segment ocular drug delivery, being able to prolong the permanence of the formulation on the cornea and overcome a variety of barriers.[19,20] Thus, to carry out the work, polymeric nanomicelles were prepared with polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol copolymer (Soluplus®; Fig. 1) and their ability to solubilize ALA was compared with both that of the marketed eye drops and that obtained with conventional micelles of sodium dioctylsulfosuccinate surfactant (AOT; Fig. 1). Soluplus is one of the copolymers with higher drug solubilization capacity in solid dispersions,[21] but so far it has only been tested for eye drops formulations in one recent report for topical delivery of cyclosporine A.[22] Soluplus micelles (0.13 mM or 15 mg/mL copolymer concentration) delivered notably greater cyclosporine A amount inside the cornea than the conventional oil-based ophthalmic formulation.[22] An additional feature of Soluplus aqueous dispersions, still barely investigated, is the ability to undergo sol-to-gel transitions triggered by changes in temperature when the copolymer concentration is sufficiently high.[23] Thus, an additional aim of the work was to elucidate whether Soluplus nanomicelle dispersions suitable for drug solubilization undergo sol-to-gel transition under relevant conditions for ophthalmic purposes, which in turn may be useful for predicting *in situ* gelling ability on ocular surface. Ocular toxicity and corneal permeability were evaluated following the EU Reference Laboratory for Alternatives to Animal Testing[24] and the US Interagency Coordinating Committee on the Validation of Alternative Methods.[25]

Materials and Methods

Materials

ALA $\geq$ 99%, AOT 98% (444.56 g/mol), and phosphate buffer saline were from Sigma-Aldrich Company (Steinheim, Germany); Soluplus (115,000 g/mol) from BASF (Ludwigshafen, Germany); NaCl 99%, NaOH 98%, KCl 99.5%, and NaH_2PO_4 98% from Panreac Quimica SLU (Castellar del Valles, Spain); $NaHCO_3$ from Guinama (La Pobla de Vallbona, Spain), $CaCl{\cdot}2H_2O$ 99.5% from Merck (Darmstadt, Germany); and ethanol absolute from Panreac (Barcelona, Spain). Water was purified using reverse osmosis (resistivity >18 MΩ·cm, MilliQ, Millipore® Spain). Simulated lachrymal fluid (SLF) was prepared with the following composition: 6.78 g/L NaCl, 2.18 g/L $NaHCO_3$, 1.38 g/L KCl, and 0.084 g/L $CaCl{\cdot}2H_2O$ with pH 7.8.[26] Carbonate buffer pH 7.2 was prepared by mixing buffer solution A (100 mL; 1.24 g NaCl, 0.071 g KCl, 0.02 g NaH_2PO_4, 0.49 g $NaHCO_3$) and buffer solution B (100 mL; 0.023 g $CaCl_2$, 0.031 g $MgCl_2$).

Micelles Preparation and Characterization

AOT solution (20 mM) was prepared in water and diluted to 4, 8, 12, and 16 mM. Soluplus solution (2 mM) was prepared in water, carbonate buffer pH 7.2, or SLF by adding the adequate amount of copolymer to the medium and keeping the system under magnetic stirring for 48 h. Then, the solution was diluted to 0.4, 0.8, 1.2, and 1.6 mM in the corresponding medium. Size and Zeta potential were measured in triplicate in a Zetasizer® 3000HS (Malvern Instruments, UK).

HET-CAM Test

Hens Egg Test on the Chorio-Allantoic Membrane (HET-CAM)[24] was carried out using fertilized hen's eggs (50-60 g; Coren, Spain)