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Formulations and toxicologic *in vivo* studies of aqueous cyclosporin A eye drops with cyclodextrin nanoparticles

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ABSTRACT

Cyclosporin A (CyA) is an immunosuppressive drug used topically to treat ocular inflammatory disorder such as dry eye disease (DES). It is a lipophilic cyclic peptide with molecular weight of 1202.6 Da. The aim of this study was to develop surfactant free aqueous 0.2% (w/v) CyA eye drops where the drug is present in an aqueous vehicle containing CyA/cyclodextrin (CyA/CD) nanoparticles and then do three-month toxicological testing in rabbits. Five formulations of different CD concentrations were studied, all of them contained 12.5% (w/v) of α-cyclodextrin (αCD) and various amounts of γ-cyclodextrin (γCD) (ranging from 0 to 12.5% w/v). αCD was used to solubilize the drug and γCD to promote formation of complex aggregates. CyA/CD complex aggregates were formed in all the formulations tested. However, the formulation containing 12.5% (w/v) αCD and 12.5% (w/v) γCD created more CyA/CD nanoparticles of suitable size and was therefore tested *in vivo*. The eye drops did not cause ocular irritation or toxic side effects upon topical administration to rabbits once or twice a day for three months.

1. Introduction

Cyclosporin A (CyA) is an immunosuppressive peptide that has proved useful for patients with dry eye syndrome (DES) (Di Tommaso et al., 2012; Nussenblatt and Palestine, 1986; Tatlipinar and Akpek, 2005). The lipophilic peptide is typically formulated in oil based eye drops (Restasis®, Ikervis®). The oily eye drops can be irritating to the eye. We propose that oil free water based eye drops would be better tolerated and therefore more beneficial to dry eye patients. In this study we formulated CyA eye drops as nanoparticle suspension in water and tested their tolerability in rabbits.

DES is a common ocular disorder caused by decreased tear production resulting in discomfort, visual disturbance and tear film instability, with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface (Anon., 2007). CyA decreases the number of activated T cells and expression of inflammatory markers in the conjunctiva of dry eye patients and, thus, decreasing the local inflammation (Rao, 2010). To reach therapeutic ocular drug level, high systemic concentration of CyA must be administered that can cause serious side effects, such as nephrotoxicity and hypertensions, and consequently topical CyA administration to the eye is preferred (Di Tommaso et al., 2012). This can be challenging both because of the ability of the eye to get rid of foreign substance *via* tear fluid drainage and blinking (Urtti, 2006), as well as the high molecular weight of CyA, its poor aqueous solubility and hydrophobic nature. CyA is a neutral, cyclic oligopeptide drug, with molecular weight of 1202.6 Da. It is formed by 11 amino acids, seven of which are *N*-methylated, which makes the peptide highly lipophilic (Survase et al., 2011). It has the aqueous solubility of 0.008 mg/ml at ambient temperature and $\log P_{octanol/water}$ (*i.e.* logarithm of the octanol-water partition coefficient) of 2.92 at 21 °C (Loftsson and Hreinsdôttir, 2006; Tayar et al., 1993).

Previously we described development of 0.05% cyclodextrin-based aqueous CyA eye drop formulation, where we used the natural α-cyclodextrin (αCD) to increase the solubility of the drug to 0.5 mg/ml and the natural γ-cyclodextrin (γCD) to promote formation of nano-sized CyA/CD particles (Johannsdottir et al., 2015). The nanoparticles can increase the contact time of CyA with the eye surface and thus increase the therapeutic drug level in ocular tissues.

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Cyclodextrins (CDs) are pharmaceutical excipients which are used to solubilize poorly soluble drugs. They are cyclic oligosaccharides derived from starch containing six (αCD), seven (βCD), eight (γCD) or more (α-1,4)- glucopyranose units (Fig. 1). They are cone shaped with primary hydroxyl groups extending from the narrow edge of the cone and secondary groups from the wider edge. In aqueous environment these hydroxyl groups can form hydrogen bonds with surrounding water molecules, which gives the molecule hydrophilic outer surface. The inside of the cone is lined with skeletal carbons and ethereal oxygens, which gives the CD cavity hydrophobic character. In aqueous solution hydrophobic drugs, or hydrophobic moieties of larger drug molecules, can enter the cavity to form inclusion complexes that can lead to increased aqueous solubility of poorly soluble drugs (Brewster and Loftsson, 2007; Loftsson and Brewster, 1996).

No covalent bonds are formed or broken upon formation of inclusion complexes, and in aqueous solutions drug molecules located within the CD cavities are in dynamic equilibrium with free drug molecules (Stella et al., 1999). Just like CDs can form inclusion complexes with lipophilic molecules in aqueous solutions, they are also able to form non-inclusion complexes with surrounding CDs and/or CD/drug complexes. This happens when hydroxyl groups on the surface of the CD molecules form hydrogen bonds with other CD molecules and/or CD/drug complexes. CDs and CD/drug complexes are able to self-assemble in aqueous solution to form nano-size aggregates and micellar-like structures (Brewster and Loftsson, 2007; Loftsson and Duchêne, 2007; Messner et al., 2010). The bonds holding these aggregates together are weak and the aggregates will disassemble upon dilution (Messner et al., 2011). All of the natural CDs are able to form these self-assembled aggregates, the natural γCD has however grater tendency to do so. γCD does also have the highest water solubility of the natural CDs and the most favorable toxicological profile of the pharmaceutical acceptable CDs (Saokham and Loftsson, 2017). This makes γCD an attractive pharmaceutical solubilizer in eye drop formulations. To summarize, CDs are able to increase the aqueous solubility of poorly soluble drugs through inclusion complex formation. These complexes can then self-assembly to form nanoparticles and small microparticles. In aqueous eye drops CDs can both increase the solubility of poorly soluble, lipophilic drugs and increase their contact time with the eye surface.

Here we describe formulation of eye drops containing 0.2% (w/v) CyA in an aqueous mixture of αCD and γCD, and the toxicological evaluation of the eye drops in rabbits.

2. Materials and methods

2.1. Material

Cyclosporin A (CyA) was purchased from GenWay BioTech Inc. (San Diego, CA, USA). α-Cyclodextrin (αCD) and γ-cyclodextrin (γCD) were purchased from Wacker Chemie (Munich, Germany). Poly(vinyl alcohol) (PVA) 87–91% hydrolyzed (average MW 30–70 kDa) and benzalkonium chloride from Sigma-Aldrich (St. Louis, MO, USA), disodium edetate dehydrate (EDTA) was purchased from Merck (Darmstadt, 120 Germany), and pentobarbital sodium 400 mg/ml (Exagon®) was purchase from Richter Pharma ag (Wells, Australia). Milli-Q water (Millipore, Billerica, MA) was used for the preparation of all solutions.

2.2. Formulation of eye drops

The first step in preparation of the aqueous 0.2% (w/v) CyA eye drop suspensions was preparation of a stock solution containing 12.5% (w/v) αCD, 0.02% (w/v) benzalkonium chloride and 0.1% (w/v) EDTA in aqueous 1.4% (w/v) PVA solution. The next day 20 ml of 5

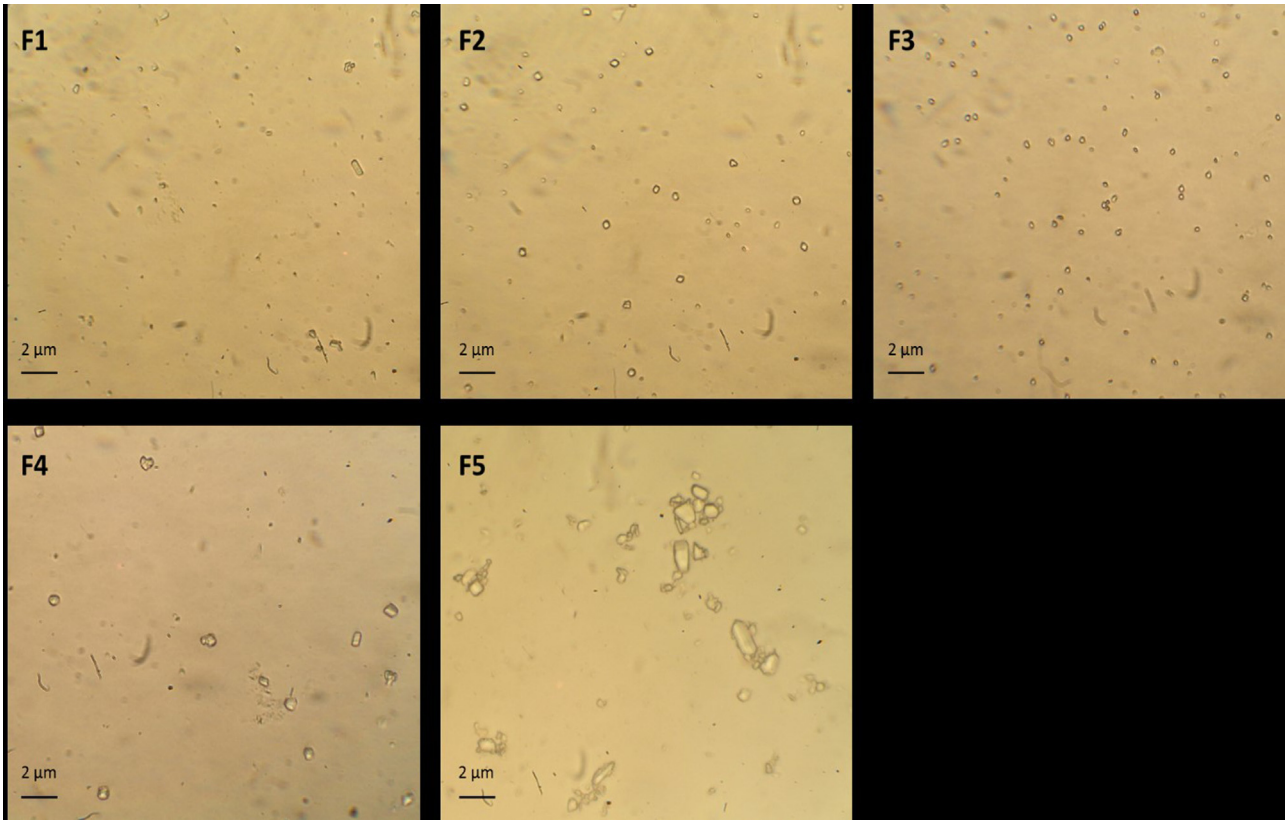

Fig. 1. Microscope pictures of formulations F1 to F5.