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Technology of stable, prolonged-release eye-drops containing Cyclosporine A, distributed between lipid matrix and surface of the solid lipid microspheres (SLM)

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ABSTRACT

The aim of this study was to prepare solid lipid microspheres (SLM) with incorporated Cyclosporine A (Cs), suitable for ocular application. For this purpose, SLM were formulated by using different lipids and three different nonionic surfactants. The SLM were produced using a hot emulsification method. The SLM dispersions contained 10, 20 or 30% of lipid (w/w) and up to 2% (w/w) of Cs. The size of the microspheres with Cs ranged from 1 to 15 μm. Physically stable SLM with Cs were prepared using Compritol, as a lipid matrix, and Tween 80, as a surfactant. In contrast, dispersion with Precirol alone, formed semi-solid gels during storage, while in formulations with Precirol and Miglyol, crystals of Cs were observed. *In vitro* release profile of Compritol formulations showed that 40% of Cs is released within 1 h, while the release of the following 40% takes more time, depending on lipid content in the formulations. The large part of Cs, added to SLM formulations (from 45 to 80%), was found on the surface of microparticles, but no drug crystallization occurred during a long-term storage.

1. Introduction

Cyclosporine A (Cs) is a lipophilic, cyclic undecapeptide with a high molecular weight (1202.6 Da) used as an immunosuppressive agent (Gökce et al., 2009; Başaran et al., 2010). Over the past years, numerous studies have shown the potential applications of Cs in ophthalmology (Hingorani et al., 1999; Tang-Liu and Acheampong, 2005). It was reported that topical ocular administration of Cs could be used in treatment of the variety of immune-mediated ocular surface disorders and in the prevention of corneal allograft rejection (Perry et al., 2002; Gökce et al., 2008; Shen et al., 2010). Cs is also the first US Food and Drug Administration (FDA)-approved drug therapy for dry eye (Tang-Liu and Acheampong, 2005; Guzey et al., 2009).

Cs is practically insoluble in aqueous media (Lallemand et al., 2003). Various ophthalmic formulations of Cs, such as oily solutions (del Castillo et al., 1994), colloidal carriers (liposomes, nanoparticles, emulsions, micelles) (Milani et al., 1993; Van der Bijl et al., 2001; Lallemand et al., 2003), collagen particles and collagen shields (Reidy et al., 1990; Gebhardt et al., 1995) have been developed to enhance solubility and bioavailability of Cs (Gökce et al., 2009).

In hospitals, oily solutions are prepared using injectable or oral solution, which is solubilized by surfactants, and contain ethanol, which has to be evaporated before the oily eye drops are prepared. Oily solutions of Cs are poorly tolerated and provide a low ocular bioavailability (del Castillo et al., 1994; Shen et al., 2010). The only commercially available eye drops preparation with Cs in the United States is Restasis® (0.05% Cs), which is an oil-in-water emulsion. Unfortunately, Cs levels delivered by Restasis® are not sufficient to prevent rejection after corneal allograft. Optimmune®, 0.2% USP ophthalmic ointment, has been approved for veterinary use, but is not being used in humans, because of its poor tolerance by patients.

There is a growing interest in the use of lipid-based systems in drug discovery and product development to effectively overcome physical and biological barriers related to poor aqueous solubility and stability, membrane permeability and availability (Muller and Keck, 2004; Attama et al., 2009; Bunjes, 2010). The results of different studies show that solid lipid nanoparticles (SLN, mean size 200–500 nm) are promising systems (Gökce et al., 2008; Başaran et al., 2010). The particles are composed of triglycerides and/or fatty acids, as matrix lipids. It was reported that they can be administered to the eye and their use avoids blurred vision and is comfortable for the patient (Gökce et al., 2009). Although not studied so far as ocular drug carriers, solid lipid microspheres (SLM), which are in order of magnitude larger (generally from 200 nm to 50 μm in size) than SLN, are also developed (Reithmeier et al., 2001; Sanna et al., 2004).

SLM and SLN can be considered as physiologically compatible, physicochemically stable and allow for a large scale production

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at a relatively lower production cost than, for example, liposomes (Reithmeier et al., 2001; Muller and Keck, 2004; Sanna et al., 2004; Bunjes, 2010; Gökce et al., 2009). The release rate for the entrapped substance is controlled by the surfactant coating and the lipid carrier (Attama et al., 2009).

Cs was successfully incorporated into SLN, and such product was demonstrated to be a promising formulation to target the cornea (Ugazio et al., 2002; Gökce et al., 2008, 2009; Başaran et al., 2010; Shen et al., 2010). When Cs was administered in SLN, the corneal levels of Cs were shown to increase three to five times, compared to oily formulations.

Incorporation of Cs in SLM has not been reported up to date, although SLM, like SLN, can provide an alternative option for encapsulating lipophilic compounds. In recent years, biocompatible solid lipid microparticles (SLM) have been reported as potential drug carrier alternative to polymer microparticles, although they offer shorter release times. In contrast to numerous publications on SLN, limited data is currently available on SLM properties (Reithmeier et al., 2001; Pietkiewicz and Sznitowska, 2004; Sanna et al., 2004; Jaspart et al., 2005; He et al., 2006; Long et al., 2006; Pietkiewicz et al., 2006; Gökce et al., 2009; Shen et al., 2010; Nanjwade et al., 2011).

The aim of this work was to formulate Cs-loaded lipid microspheres, suitable for ocular application, for the purpose of sustained drug release, and will offer less frequent administration than emulsion-type eye drops and better comfort for the patient, in contrast to oily solutions.

2. Materials and methods

2.1. Materials

Cyclosporine A (Cs) was obtained from IVAX Pharmaceutical (Czech Republic); Precirol ATO 5 (glyceryl palmitostearate) and Compritol 888 ATO (glyceryl behenate) were a gift sample from Gattefossé (France); Witepsol H15 was purchased from Sasol (Germany); Miglyol 812 (medium chain triglycerides) from Caelo Caesar and Loretz (Germany); Tween 80 (Polysorbate 80) from Sigma–Aldrich (USA). Methanol and acetonitrile were purchased from Merck (Germany); sodium lauryl sulfate (SLS), sodium hydroxide and hydrochloric acid were purchased from POCH (Poland). All other chemicals used were of analytical reagent grade.

2.2. Solubility of Cs in lipids

The solubility of Cs in solid lipids – Precirol (P), Compritol (C) and their blend with liquid lipid, Miglyol (M) in ratio 4:1, was studied semi-quantitatively. Small amounts of pure Cs were added to 1 g of melted lipids at 80 ± 1 °C and stirred with magnetic stirrer. Solubility of Cs was estimated visually in the melted lipids (in a test tube) and after cooling (microscopic observations in thin layers).

2.3. Preparation of SLM suspension

The composition of placebo and Cs-loaded SLM formulations is listed in Table 1. SLM contained 10, 20 or 30% (w/w) of a lipid and 0.0 (placebo formulations) 0.7, 1.0 or 2.0% (w/w) of Cs. Tween 80 was used as an emulsifying agent alone (2–3%, w/w) or in a mixture with Cremophor EL, or Span 80. Isotonicity was adjusted with glycerol.

SLM formulations were prepared using a hot emulsification method, followed by cold re-solidification (Pietkiewicz et al., 2006). The aqueous phase (water, surfactant and glycerol), heated to 80 °C, was poured into the melted lipid phase and the homogenization was performed using a high-shear mixer, Ultra-Turrax (T25 Janke-Kunkel IKA Labortechnik, Staufen, Germany) under the stirring speed of 8000 rpm for 5 min. In order to prepare Cs-loaded SLM, the drug was dissolved in ethanol, in ratio 1:1.5 (w/v), and the solution was added to the melted lipid phase. The mixture was stirred for 30 min to evaporate ethanol and the aqueous phase was introduced to the lipid. In this case, homogenization was carried out at the constant temperature of 80 °C. Optionally, the product was discontinued from being heated during homogenization (temperature decreased to about 60 °C). SLM suspension was obtained when the flask with emulsion was cooled in water with ice after homogenization.

Immediately after cooling, the pH was adjusted to the required value. The effect of pH on the size of SLM particles was examined. The placebo SLM with Compritol (F3) was prepared with pH adjusted (with HCl or NaOH) within the range from 4 to 9. In all other formulations the pH was adjusted to 8.0 with NaOH. Glass vials (20 ml) with teflon stoppers were used as containers.

SLM were sterilized at 121 °C for 15 min in an autoclave. After thermal sterilization, hot vials with SLM were mixed for 1, 3 or 10 min using vortex. Some of SLM formulations were also exposed to ultrasounds for 5 or 15 min, to restore the homogeneity of the dispersions and to prevent particle aggregation.

Prepared SLM were stored in a refrigerator (4 °C).

2.4. Particle shape and size analysis

All formulations were examined using an optical microscope (Nikon Eclipse 50i, Nikon Corporation, Japan). The detailed surface characteristics of the selected placebo and Cs-loaded SLM formulations were observed using a scanning electron microscope (SEM) (Hitachi S-5500, Tokyo, Japan). The SLM sample was placed on an adhesive tape, dried and osmium-coated. Particle size distribution was measured by laser diffractometry (Mastersizer E, Malvern Instruments, UK): $d_{0.5}$, $d_{0.9}$ and d_{max} values were determined as measures of maximum diameter of 50%, 90% and 100% of the detected particles, respectively.

2.5. Total content of Cs in SLM and its distribution in the phases of SLM dispersion

To determine Cs content in the formulations, about 5 ml of methanol was added to 50 mg of Cs-loaded SLM, lipids were melted at 80 °C and the mixture was shaken. After cooling to room temperature, methanol was added to 10 ml and the mixture was filtered. The concentration of Cs in the obtained solution was analyzed by HPLC. The apparatus (Merck Hitachi, Tokyo, Japan) was equipped with a Lichrosphere 100 RP C_{18} column (250-4; Merck Hitachi, Darmstadt, Germany), UV detector (Hitachi L-4250) set at 210 nm and HPLC column temperature controller (Thermostat Lachrom L-7350). The column was maintained at 80 °C. The mobile phase consisted of acetonitrile/water/t-butyl methyl ether/ortophosphoric acid (520:430:50:1, v/v) with a flow rate of 2 ml/min (USP method slightly modified). The injected volume was 20 μl.

Distribution of Cs into the aqueous phase of SLM suspension was analyzed. The aqueous phase was separated by ultrafiltration using centrifuge filtration units – Microcon YM-100 (cut-off 100 kDa, Millipore, Bedford, USA) (Pietkiewicz et al., 2006), and the filtrate was analyzed by HPLC for Cs concentration. The fraction of free drug dissolved in the aqueous phase (A_p) of SLM was determined as a percentage of the total dose.

To check if the remaining Cs was incorporated in lipid cores of SLM, an additional experiment was performed. An accurately measured amount of Cs-loaded SLM dispersion was suspended in methanol (1:4, w/v), shaken by vortex and centrifuged for 5 min at 3500 rpm. The sample of the supernatant was analyzed by HPLC in order to determine the fraction of non-entrapped (localized on the SLM surface and dissolved in the aqueous phase) drug (M_p). The