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Incorporation of cyclosporin A in solid lipid nanoparticles (SLN)

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Abstract

The cyclic undecapeptide cyclosporin A (CyA) a potent immunosuppressive drug used in many therapies, is extremely hydrophobic. Commercial products employ solubilising agents to improve gastrointestinal absorption. In the present study CyA solid lipid nanoparticles (SLN) are prepared from warm o/w microemulsion, dispersed in cold water. The matrix chiefly consists of stearic acid, phosphatidylcholine and taurocholate; up to 13% of CyA can be incorporated. The average diameter of CyA-loaded SLNs is below 300 nm and transmission electron microscopy (TEM) analysis shows them to be spherical. In vitro release of CyA from SLNs is low. CyA-loaded SLNs can be proposed for most administration routes, in particular for the duodenal route.

Keywords: Cyclosporin A; Solid lipid nanoparticles; Colloidal carriers

1. Introduction

Cyclosporin A (CyA) is an extremely hydrophobic cyclic undecapeptide that is practically insoluble in water, used as first-line therapy in the prevention of xenograft rejection following organ transplantation. Many delivery systems have been proposed, such as liposomes (Venkataram et al., 1990), a commercial microemulsion such as Neoral®, lecithin vesicular carriers (Guo et al., 2000); the drug has been incorporated in solid lipid nanoparticles (SLN) (Zhang et al., 2000; Radtke and Müller, 2001a,b) obtained by high pressure homogenisation; Ford and co-workers (Ford et al., 1999) prepared spherical solid nanospheres, obtained by a precipitation method, constituted of the drug alone, to improve its bioavailability. Müller and co-workers obtained two patent applications (Müller et al., 1998; Penkler et al., 1999).

We prepare SLN from warm microemulsions: in laboratory we have monitored the administration of SLNs incorporating different drugs, by the parenteral, intravenous, ocular and duodenal routes, obtaining AUCs higher than those of the respective solutions, as well as sustained releases (Gasco, 1997, 2001).

The aim of the present study was to investigate whether it was possible to incorporate a lipophilic

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peptide as CyA in SLNs obtained from warm o/w microemulsion and to verify its in vitro release.

2. Materials and methods

2.1. Materials

CyA was obtained from Fluka (Buchs, Switzerland). Stearic acid and butyric acid were purchased from Merck (Darmstadt, Germany). Epikuron 200® (containing about 95% soya phosphatidylcholine) was a kind gift from Lucas Meyer Co. (Hamburg, Germany), taurocholic acid sodium salt (TC) was kindly provided by PCA (Basaluzzo, Italy). The other chemicals were of analytical reagent grade. Water from a Millipore Milli-Q® ultrapure water purification unit was used.

2.2. Preparation of solid lipid nanospheres

Two different amounts of CyA were added to a mixture of stearic acid and Epikuron 200®, melted together at 70 °C. A warm water solution of sodium taurocholate, butyric acid, ethanol and isopropanol was then added to obtain an optically transparent system. The two different microemulsions were immediately dispersed in cold water (2–3 °C), under mechanical stirring, obtaining SLN 1 and SLN 2, respectively. The microemulsion composition and the ratio of microemulsion dispersion in aqueous medium are reported in Table 1. The dispersions obtained were washed using an Amicon TCF2A ultrafiltration system (Amicon Grace, Beverley, MA, USA; membrane Amicon Diaflo YM 100). Both dispersions of SLNs and all the washing waters were freeze-dried using a Modulyo freeze-dryer (Edwards, Crawley, UK).

2.3. Photon correlation spectroscopy

The average diameters and polydispersity indices of SLNs were determined by photon correlation spectroscopy (PCS) using a 90 PLUS Particle Size Analyzer (Brookhaven Instrument Corporation, Holtsville, NY, USA) at a fixed angle of 90° and a temperature of 25 °C. The wavelength of the laser light (He/Ne) was 678 nm.

The SLNs were dispersed in either ultrapure water or 2.6% isotonic glycerol solution before analysis.

2.4. Transmission electron microscopy

Transmission electron microscopy (TEM) analysis was performed using a CM10 Philips instrument (Eindhoven, Netherlands). TEM samples were diluted 1:25 with ultrapure water and stained with a 2% solution of osmium tetraoxide before analysis.

2.5. Determination of cyclosporin A

The amount of CyA incorporated in the SLNs was determined by HPLC using a LC-6A pump unit control, a C-R5A Chromatopac integrator and an SPD-2A UV detector (Shimadzu Corporation, Kyoto, Japan) set at 210 nm. A reverse-phase Waters Spherisorb® 5 μm ODS2 column (4.6 × 250 mm) was used. The mobile phase consisted of acetonitrile–water (80/20, v/v), pH adjusted to 2.8 with phosphoric acid. The flow rate was 1.0 ml/min.

2.6. In vitro release kinetics of cyclosporin A

The release rates of CyA were determined in a phosphate buffer 0.025 M (pH 7.4). Sink condi-

Table 1
Composition % (w/w) of the microemulsions and ratio of microemulsion dispersion in aqueous medium

	Microemulsion 1	Microemulsion 2
CyA	2.2%	4.0%
Stearic acid	9.8%	9.1%
Epikuron 200®	4.9%	4.6%
Sodium taurocholate	17.1%	15.9%
Butyric acid	14.2%	14.0%
Water	49.1%	45.6%
Ethanol	2.7%	3.2%
Isopropanol	–	3.6%
Dispersion ratio	1:10	1:20

Table 2
Average diameter, polydispersity index of SLNs, and percentage (w/w) of CyA incorporated

	Average diameter (nm)	Polydispersity index	CyA in SLNs (% w/w)
SLN 1	250 nm	0.25	6.0
SLN 2	290 nm	0.32	13.0

tions were achieved by using a multicompartment rotating cell system with two identical units. The donor phases were buffered saturated solution (14.4 μg/ml) and SLN 2 dispersion (15.1 mg/ml at 13% containing CyA 1.96 mg/ml) in phosphate buffer. The acceptor phase was phosphate buffer. The compartments were separated by a Servapor® hydrophilic dialysis membrane (cut-off = 12000–14000 Da). At fixed times the acceptor phase was removed and analysed by HPLC; the compartment was simultaneously refilled with fresh acceptor phase.

3. Results

3.1. Size analysis

The average diameters and the polydispersity indices of SLNs prepared with two different amounts of CyA and dispersed in water are reported in Table 2. The results showed that increasing the amount of drug incorporated the sizes of SLNs increase; similar results were obtained in the isotonic glycerol solution (data not shown).

TEM analysis confirmed the spherical shape and colloidal sizes of the SLNs (Fig. 1).

3.2. Percentage of cyclosporin A incorporated

The amounts of CyA incorporated in the SLNs determined by HPLC analysis are reported in Table 2.

3.3. Release kinetics of cyclosporin A

After 120 min, the percentage of CyA released from SLN 2 was below 4% (Fig. 2), while from the saturated solution it was above 60%.

4. Discussion

The bioavailability of CyA by the oral route is always low, about 30% (Fahr, 1993; Noble and Markham, 1995). Furthermore, the rate and extent of absorption is dependent on many factors, including food intake, bile production and gastrointestinal motility. Many attempts have been made over time to enhance the drug's bioavailability using different dosage forms. The commercial microemulsion Neoral®, commonly administered in many therapies, is a microemulsion concentrate and consist of oil, propylene glycol and, as surfactant, polyoxyl-40 hydrogenated castor oil; the amount of cyclosporine in Neoral® is about 10% (Noble and Markham, 1995).

To develop an alternative carrier, we studied the incorporation of CyA into SLNs, which have been suggested for duodenal administration; in-

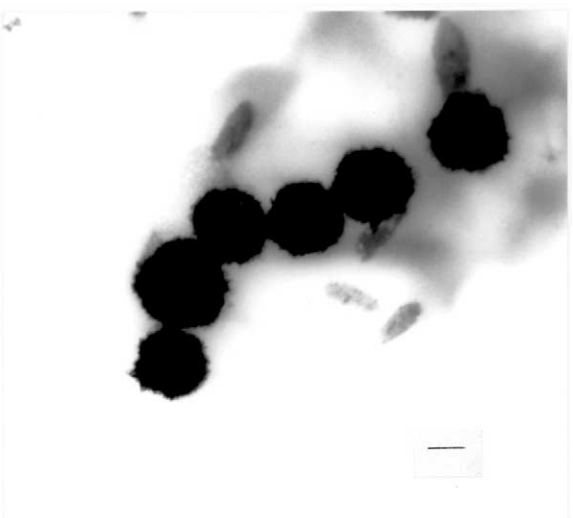

Fig. 1. Transmission electron micrograph of SLN 2 (Bar, 100 nm).

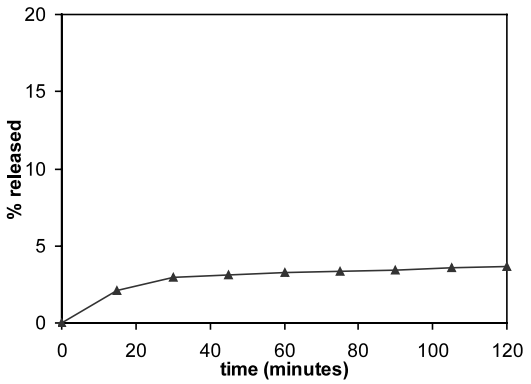

Fig. 2. Percentage of CyA released from SLN 2 vs. time.

deed tobramycin—a drug not absorbed by the oral route—when incorporated in SLNs and administered duodenally, was targeted to the lymph and absorbed with a high AUC and a sustained release (Bargoni et al., 1998; Cavalli et al., 2000).

CyA-loaded nanoparticles were prepared from warm oil-in-water microemulsions; the surfactant used to obtain the microemulsions was soya lecithin, containing about 95% phosphatidylcholine. The SLN dispersions were washed by diafiltration, obtaining a final product containing only biocompatible components.

The CyA-loaded SLNs were in the colloidal range; Fig. 1 shows their spherical shape. The amount of drug incorporated differs with the composition of the warm microemulsion; in SLN 2 13% of CyA was incorporated, above that of commercial preparations.

A sustained release of CyA from SLNs was performed: the amount of CyA released over time was very low, avoiding its precipitation.

In conclusion, CyA-loaded SLNs are a slow release carrier of CyA that can be proposed for most administration routes, particularly for the duodenal one.

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