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Development of cyclosporin A-loaded hyaluronic microsphere with enhanced oral bioavailability

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Abstract

To develop a hyaluronic microsphere with the improved oral bioavailability of poorly water-soluble cyclosporin A (CsA), the microspheres were prepared with varying ratios of sodium hyaluronate (HA)/sodium lauryl sulfate (SLS)/CsA using a spray-drying technique. The effects of HA and SLS on the dissolution and solubility of CsA in microspheres were investigated. The CsA-microsphere prepared with HA/SLS/CsA at the ratio of 4/2/1 gave the highest solubility and dissolution rate of CsA among those formulae tested. As solubility and dissolution rate of CsA were increased about 17- and 2-fold compared to CsA powder, respectively, this CsA-microsphere was selected as an optimal formula for oral delivery in rats. The CsA-microsphere and Sandimmun neoral sol® gave significantly higher blood levels compared with CsA powder alone. Moreover, the AUC, T_{max} and C_{max} values of CsA in CsA-microsphere were not significantly different from those in Sandimmun neoral sol® in rats, indicating that CsA–microsphere was bioequivalent to the commercial product in rats. Our results demonstrated that the CsA–microsphere prepared with HA and SLS, with improved bioavailability of CsA, might have been useful to deliver a poorly water-soluble CsA.

Keywords: Bioavailability; Cyclosporin A; Sodium hyaluronate; Sodium lauryl sulfate; Microsphere; Spray drying

1. Introduction

Cyclosporin A (CsA) is a lipophilic cyclic undecapeptide of fungal origin, which has the selective property of suppressing various T-lymphocyte functions, particularly the production of interleukin-2 (IL-2) (Christopher et al., 2001). CsA has also been applied in the treatment of patients with selected autoimmune diseases such as rheumatoid arthritis (Sajjadi et al., 1994; Thomson and Neild, 1991). However, it is known that the oral bioavailability of CsA is usually very low due to the poor absorption, which is related to the relatively high molecular weight, very high lipophilicity ($\log P = 2.92$) (Taylor et al., 1993) and poor solubility in aqueous medium (Ismailos et al., 1991). The conventional formulation of Sandimmun neural sol® is a microemulsion of pre-concentrated CsA designed to provide better consistent absorption of the drug than Sandimmun®. Although this orally administered CsA has more stable drug metabolism, but its gastrointestinal absorption is still incomplete and variable due to its hydrophobic character (Gennery et al., 1999; Tom et al., 2000). Various oral formulations of CsA such as a complexation with cyclodextrin (Miyake et al., 1999) and microsphere (Aberturas et al., 2002; Chacon et al., 1999; Kim et al., 2002a; Urata et al., 1999), nanoparticles (Chacon et al., 1996; Chen et al., 2002; Gref et al., 2001; Guzman et al., 1993; Molpeceres et al., 2000; Ugazio et al., 2002) and microemulsion (Drewe et al., 1992; Gao et al., 1998; Kim et al., 1997; Tejani, 1998) and emulsion (Kim et al., 2002b) have been developed to enhance the solubility, dissolution and bioavailability of CsA. In order to gain more satisfactory release rate, a wider range of

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loading materials is needed to be screened. However, there were still no reports on using sodium hyaluronate (HA) for controlling CsA release. Thus, the development of new oral formulation for the enhancing absorption and bioavailability of systemically effective but poorly absorbed CsA is urgently needed.

Microspheres, in general, have been used as a formulation for improving the solubility, release and bioavailability of poorly water-soluble drugs. Furthermore, spray drying has been commonly used in the pharmaceutical industries for increasing the solubility of poorly water-soluble drugs (Kawashima et al., 1975; Tsuda et al., 1988).

Hyaluronic acid (HA) is a chemically well-characterized linear polysaccharide consisting of alternating b(1 → 4) linked *N*-acetyl-D-glucosamine and b(1 → 3) linked D-glucuronic acid (Jouon et al., 1995; Lapcik et al., 1998). Hyaluronic acid is the only non-sulfated glycosaminoglycan in the extracellular matrix of all higher animals. This polyanionic polymer has a range of naturally-occurring molecular sizes from 1000 to 10,000,000 Da and has unique physicochemical properties and distinctive biological functions (Laurent et al., 1995). Ongoing pharmaceutical and medical research is concentrating on its use in drug delivery systems in addition to its present therapeutic indications in ophthalmology, dermatology and osteoarthritis (Goa and Benfield, 1994; Lapcik et al., 1998). In particular, it was used as carriers in various oral formulations such as microsphere and complex for the improved solubility and bioavailability of poorly water-soluble drugs (Jederstrom et al., 2004; Piao et al., 2007).

Sodium lauryl sulfate (SLS) is an anionic surfactant employed in a wide range of nonparenteral pharmaceutical formulations. SLS was also used as a solubilizer or co-carrier of solid dispersion systems to improve the solubility and dissolution rate of drugs (Ghosh et al., 1998; Khanfar et al., 1997). The CsA-loaded microsphere prepared with SLS and dextrin gave 1.7-fold higher AUC of CsA compared with CsA powder alone (Lee et al., 2001). Thus, in the formulation of CsA-loaded microsphere suitable for solid dosage form, water-soluble HA and SLS were used as a carrier and solubilizer, respectively.

To develop a CsA-loaded microsphere for improving the oral bioavailability, CsA-loaded microspheres were prepared with varying ratios of HA/SLS/CsA using spray-drying technique (Choi and Kim, 2000; Lee et al., 2001). The effects of HA and SLS on the aqueous solubility and dissolution rate of CsA were investigated. The oral bioavailability of CsA-microsphere was then compared with CsA powder alone and commercial Sandimmun neoral sol® in rats.

2. Materials and methods

2.1. Materials

Cyclosporin A was supplied from Hanmi Pharm. Co. Ltd. (Hwaseong, South Korea). Sodium hyaluronate and sodium lauryl sulfate were purchased from Shandong Freda Biochem Co. Ltd. (Jinan, China) and Sigma Chemical Co. (St. Louis, USA), respectively. All other chemicals were of reagent grade and used without further purification.

Table 1
Composition of CsA-loaded microsphere

Ingredients	I	II	III	IV	V	VI	VII
Hyaluronic acid (HA)	4	4	4	4	8	2	0
Sodium lauryl sulfate (SLS)	0	0.5	2	4	0.5	0.5	0.5
Cyclosporin A (CsA)	1	1	1	1	1	1	1

2.2. Preparation of CsA-loaded microsphere

A Buchi 191 nozzle type mini spray-dryer (Model 191, Buchi, Flawil, Switzerland) was used for the preparation of CsA-loaded microsphere. HA, SLS and CsA were dissolved in the mixture of water–ethanol (1:1, v/v). CsA (1 g) and a varying amount of HA, SLS are shown in Table 1. The microspheres were obtained by spray drying through a standard nozzle with an inner diameter of 0.7 mm. The process parameters were set as follows: inlet temperature, 140 °C; outlet temperature, 70–75 °C; and feed flow rate, 4 ml/min; spray air, 3 kg/cm^2 and the flow rate of dry air, about 30 mbar. The direction of air flow was the similar as that of sprayed product (Kim et al., 1994; Lee et al., 1998; Lee et al., 2001).

2.3. Shapes and size of CsA-loaded microsphere

The surface morphology and shape of CsA-loaded microsphere were examined using a scanning electron microscopy (Hitachi S-4100, Tokyo, Japan). The samples were loaded on the specimen stub via double-side sticky tape and coated with gold (Hitachi Iron sputter, E-1030) for 5 min at 100–200 mTorr in a shutter coater before taking photograph at an accelerating voltage of 15 kV. The size of microsphere was also measured using a light scattering spectrophotometer (Nimcomp 370, Particle Sizing System Inc, Santa Barbara, CA, USA).

2.4. Determination of CsA contents in microspheres

For the determination of CsA contents in microspheres, exact amount of microsphere (5 mg) was added to 5 ml of 50% ethanol, shaken in water bath for 3 days and filtered through membrane filter (0.45 μm). The concentration of CsA in the resulting solution was analyzed by HPLC (Jasco PU-987, Japan) equipped with an Inertsil ODS-3 C_{18} column (GL science, 5 μm, 4.6 × 250 mm i.d.), UV detector (Jasco UV-975) and HPLC column temperature controller (Thermasphere™ TS-130, USA). The mobile phase consisted of acetonitrile/water (80:20, v/v) with a flow rate of 1.5 ml/min; the column was thermostated at 70 °C (Francis et al., 2003).

2.5. Solubility of CsA in microsphere

The aqueous solubility study of CsA was measured at 25 ± 1 °C. Excessive amount of each CsA-loaded microsphere or pure CsA was added to 5 ml of water, shaken in water-bath for 3 days. Triplicated samples were centrifuged at 3000 × *g* for 5 min using a centrifuge 5415C (Eppendorf, USA). After being filtered through a membrane filter (0.45 μm), the filtrate