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Self-micellizing solid dispersion of cyclosporine A for pulmonary delivery: Physicochemical, pharmacokinetic and safety assessments

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ABSTRACT

The present study aimed to develop an inhalable self-micellizing solid dispersion of cyclosporine A (SMSD/CsA) for the direct delivery to the respiratory system with improved therapeutic efficacy and minimized systemic exposure. SMSD/CsA was obtained by wet-milling, and then jet-milled SMSD/CsA was blended with lactose carrier, producing a respirable powder of SMSD/CsA (SMSD/CsA-RP). The physicochemical, pharmacological, and pharmacokinetic properties of SMSD/CsA-RP were characterized, and the hepatotoxic and nephrotoxic potentials were investigated by biomarker analysis. Cascade impactor analysis demonstrated that SMSD/CsA-RP had high *in vitro* inhalation performance, with a fine particle fraction of 36%. In simulated lung fluid, the SMSD/CsA exhibited better dissolution behavior than amorphous CsA. Pretreatment with SMSD/CsA-RP resulted in significant suppression of antigen-evoked inflammatory events in rats. After intratracheal administration of SMSD/CsA-RP at a pharmacologically effective dose (100 μg-CsA/rat), the AUC_{0-24} value was <1% of that after oral administration of Neoral® at a toxic dose (10 mg-CsA/kg). Compared with oral Neoral®, insufflated SMSD/CsA-RP showed 99% reductions of CsA concentrations in both liver and kidney. No significant increases of biomarker levels in plasma were observed even after repeated intratracheal administration of SMSD/CsA-RP for 7 days. From these findings, SMSD/CsA-RP might be a favorable dosage form for effective and safe inhalation therapy of CsA.

1. Introduction

Cyclosporine A (CsA), a neutral lipophilic cyclic undecapeptide, inhibits calcium-dependent signal transduction pathways in T lymphocytes, leading to reduction in activity of the immune system (Borel et al., 1994; Underwood et al., 2001). CsA has been widely used to prevent transplant rejection (Kamar et al., 2004) and to treat psoriasis, rheumatoid arthritis, nephrotic syndrome, and atopic dermatitis (Czogalla, 2009). In addition to these inflammatory disorders, CsA has been shown to be effective in asthma treatment and has attracted much attention as a therapeutic option in patients with steroid-dependent asthma (Coren et al., 1997; Nizankowska et al., 1995). However, excessive systemic exposure to CsA can cause systemic adverse effects such as hepatotoxicity (Kwak and Mun, 2000) and nephrotoxicity (Burdmann et al., 2003; Sereno et al., 2014). Therefore, development of a suitable dosage form might lead to efficacious treatment of asthma by CsA with a wide safety margin.

Pulmonary drug delivery provides unique advantages for the treatment of airway diseases due to achievement in a high pulmonary concentration of the drug with low systemic exposure (Chan and Chew, 2003). The total lung surface fluid volume in humans is generally estimated to be 15–70 mL (Rennard et al., 1986). Sufficient dissolution of CsA in such a small fluid volume may be challenging because of its low solubility (Ismailos et al., 1991), suggesting limited therapeutic efficacy. Thus, improving the dissolution behavior of CsA is essential for the development of a respirable powder (RP) formulation of CsA. A self-micellizing solid dispersion of CsA (SMSD/CsA) was developed in the previous study, employing poly[MPC-*co*-BMA] to improve dissolution behavior of CsA (Onoue et al., 2014). The SMSD/CsA have characterized in terms of surface morphology, crystallinity, and micellizng potency, and re-suspension of the SMSD/CsA in aqueous medium led to formation of fine micelles and therefore much better dissolution behavior than amorphous CsA. The combination use of pulmonary drug delivery system and SMSD formulation might be a promising strategy for treating inflammatory airway diseases with CsA. However, the feasibility has not been fully clarified.

Abbreviations: AUC, area under the curve of blood concentration versus time curve; BALF, bronchoalveolar lavage fluid; BUN, blood urea nitrogen; C_{max}, maximum concentration; CsA, cyclosporine A; CsA-RP, amorphous cyclosporine A-based respirable powder; FPD, fine particle dose; FPF, fine particle fraction; HPMC, hydroxypropyl methylcellulose; MPO, myeloperoxidase; OVA, ovalbumin; OVA-RP, ovalbumin-based respirable powder; PBS, phosphate buffer solution; RP, respirable powder; SMSD/CsA-RP, self-micellizing solid dispersion of cyclosporine A-based respirable powder; SEM, scanning electron microscopy; SLF, simulated lung fluid; SMSD/CsA, self-micellizing solid dispersion of cyclosporine A; T_{max}, time to maximum plasma concentration.

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The present study aimed to develop a new RP formulation of CsA, employing the SMSD approach, for the safe treatment of asthma and other airway inflammatory diseases. SMSD/CsA was prepared with a wet-milling system, and SMSD/CsA-based RP (SMSD/CsA-RP) was produced by blending jet-milled SMSD/CsA with a lactose carrier. The CsA formulations were physicochemically characterized in terms of morphology, *in vitro* inhalation performance, and dissolution behavior in simulated lung fluid (SLF). The anti-inflammatory effects of SMSD/CsA-RP were assessed in ovalbumin (OVA)-sensitized rat model of asthma. The CsA concentrations in plasma, kidney, and liver were determined after intratracheal administration of SMSD/CsA-RP at a pharmacologically effective dose (100 μg-CsA/rat), and safety assessment on continuously insufflated SMSD/CsA-RP was also conducted by biomarker analyses to predict the potential for hepatotoxicity and nephrotoxicity.

2. Materials and methods

2.1. Chemicals

Amorphous CsA was supplied by ILS Inc. (Ibaraki, Japan), and poly[MPC-*co*-BMA] (PUREBRIGHT® MB-37-50T) was provided by NOF Corporation (Tokyo, Japan). Zirconia (zirconium oxide) balls with a diameter of 0.1 mm were purchased from Nikkato Company, Ltd. (Osaka, Japan). Respitose® SV-003 and erythritol were supplied by DMV (Veghel, The Netherlands) and Nikken Chemicals (Tokyo, Japan), respectively. OVA and Neoral® capsules (10 mg) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Novartis Pharma (Tokyo, Japan), respectively. All other chemicals were obtained from various commercial sources.

2.2. Preparation of CsA formulations

SMSD/CsA was prepared by wet-milling in accordance with a previous report (Onoue et al., 2014). Briefly, CsA (30 mg) was weighed and added into the vessel of a rotation/revolution mixer (NP-100, Thinky Co. Ltd., Tokyo, Japan). Poly[MPC-*co*-BMA] was dissolved in water (20 mg/mL), and the solution (0.5 mL) was added into the vessel. The CsA suspension was wet-milled with zirconia balls (2.5 g) at 2000 rpm for 2 min, and further pulverized at 400 rpm for 2 min with 8.0 mL of poly[MPC-*co*-BMA] solution. The pulverized CsA suspension containing 15% (*w*/*w*) of CsA was collected in a 30 mL vial and freeze-dried using an FD-81 freeze-dryer (Tokyo Rikakikai, Tokyo, Japan). The amount of CsA in the obtained formulation was determined by an absolute calibration curve method using a Waters Acquity UPLC system (Waters, Milford, Massachusetts). The UPLC system consisted of a binary solvent manager, sample manager, column compartment, and SQD connected with the MassLynx software, and the analysis was performed according to the following conditions: An Acquity UPLC BEH C 18 column (particle size: 1.7 μm, column size: 2.1 mm × 50 mm; Waters); column temperature at 65 °C; and gradient mobile phase consisting of acetonitrile (A) and 5 mM ammonium acetate (B) with a flow rate of 0.25 mL/min. The gradient condition was as follows: 0–1.0 min (50% A); 1.0–2.5 min (80–95% A); 2.5–3.0 min (95% A); and 3.0–3.5 min (50% A). Mass spectrum was recorded using selected ion recording mode for specific *m*/*z* 1203 for CsA $[M + H]^+$.

For preparation of SMSD/CsA-RP, SMSD/CsA and erythritol were blended with a pestle and mortar, followed by micronization of the powder using an A-O JET MILL (Seishin Enterprise, Tokyo, Japan) at a pusher nozzle pressure and grinding nozzle pressure of 0.60 and 0.55 MPa, respectively. The ratio of the drug to excipient was 1:1 (w/w). The micronized mixture was added to lactose carrier (Respitose® SV-003) at a ratio of 1:3 (w/w). The prepared SMSD/CsA-RP with the drug loading of ca. 2.0% (w/w) was stored in a vacuum until testing. The amount of CsA in the RP formulation was determined by UPLC analysis as described above.

2.3. Scanning electron microscopy (SEM)

The SMSD/CsA-RP was observed with SEM, Miniscope® TM3030 (Hitachi, Tokyo, Japan), at 15 kV. The sample was coated with platinum using a magnetron sputtering device, MSP-1S (Vacuum Device Inc., Ibaraki, Japan) and was fixed on an aluminum sample holder with double-sided carbon tape.

2.4. Cascade impactor

Cascade impactor analysis was conducted using an AN-200 system (Shibata Scientific Technology, Tokyo, Japan) to assess *in vitro* inhalation performance of SMSD/CsA-RP. The AN-200 system consisted of a vacuum pump, a mass flow meter, and an eight-stage Andersen cascade impactor. The cascade impactor analysis was conducted according to USP 29 < 601 > AEROSOLS. Briefly, 30 mg of SMSD/CsA-RP was filled into a JP No. 2 hard capsule of hydroxypropyl methylcellulose (HPMC), and the capsule was installed in a JetHaler® (Hitachi Unisia, Kanagawa, Japan) powder inhaler. The formulation in each capsule was dispersed through the device at 28.3 L/min for 10 s × 3 times, and the amount of CsA in each stage (stages 0–7) and the capsule was determined by UPLC as described in Section 2.2. The fine particle dose (FPD) was defined as the total mass of drug particles deposited at stage 2 and lower, and the fine particle fraction (FPF) was calculated as the ratio of FPD to the total loaded dose.

2.5. Dissolution test

Dissolution testing was carried out in SLF to predict the dissolution behavior of CsA in the respiratory system. In this study, SLF was prepared based on a previous report with some modifications (Drysdale et al., 2012). The SLF contained $MgCl_2$ (0.20 g/L), NaCl (6.02 g/L), KCl (0.30 g/L), Na_2SO_4 (0.07 g/L), $CaCl_2 \cdot 2H_2O$ (0.37 g/L), $CH_3COONa \cdot 3H_2O$ (0.95 g/L), $NaHCO_3$ (2.60 g/L), $C_6H_5Na_3O_7 \cdot 2H_2O$ (0.10 g/L), $NaH_2PO_4 \cdot H_2O$ (0.14 g/L), and dipalmitoylphosphatidylcholine (0.02%w/v). The pH of SLF was adjusted to 7.1 with HCl to mimic the airway condition of asthmatics (Kodric et al., 2007). The testing was performed in 50 mL of SLF with constant stirring at 50 rpm using a magnetic stirrer SST-66 (Shimadzu, Kyoto, Japan) at 37 °C. CsA samples were weighed to be ca. 5 mg of CsA in the dissolution vessel. Each sample (400 μL) was collected and centrifuged at 10,000 rpm for 5 min. After each supernatant was diluted with a 2-fold volume of methanol, the concentration of CsA was determined by UPLC as described in Section 2.2.

2.6. Animals and drug administration

Male Sprague–Dawley rats at 8–9 weeks of age (250–350 g, body weight) were purchased from Japan SLC Inc. (Shizuoka, Japan) and were housed three per cage in a laboratory with free access to food and water and were maintained on a 12-h dark/light cycle in a room with controlled temperature (24 ± 1 °C) and humidity (55 ± 5%). According to a previously reported procedure (Misaka et al., 2009), OVA, a major egg white protein, with aluminum hydroxide gel (100 μg-OVA/rat) was injected in rats on days 0, 7, and 14. They were anesthetized with intraperitoneally-injected sodium pentobarbital (50 mg/kg), and OVA-based RP (OVA-RP) consisting of jet-milled OVA powders and lactose carrier was intratracheally administered (100 μg-OVA/rat) at 24 h after the last OVA sensitization to produce experimental rat model of asthma. Pretreatment of amorphous CsA-based RP (CsA-RP) and SMSD/CsA-RP (100 μg-CsA/rat) was conducted at 1 h before the OVA challenge. The dosage amount of drugs- and OVA-RP formulations was ca. 5 mg, and a PennCentury insufflation powder delivery device (DP-4, INA Research Inc., Nagano, Japan) was employed with pulse of air at 2 mL for the intratracheal administration. All procedures used in the present study were carried out according to guidelines approved