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Dissolution and oral absorption of pranlukast nanosuspensions stabilized by hydroxypropylmethyl cellulose

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ABSTRACT

The objective of this study was to investigate the effect of particle size on the dissolution and oral absorption of pranlukast microsuspensions and nanosuspensions stabilized by hydroxypropylmethyl cellulose. Four pranlukast suspensions with different mean particle sizes (0.16, 0.89, 3.13, and 18.21 μm) were prepared by various top-down processes such as jet milling, high pressure homogenization, and bead milling. The dissolution rate and oral absorption of pranlukast suspensions were significantly affected by the particle size. The *in vivo* pharmacokinetic parameters of pranlukast suspensions were increased with decreasing mean particle size of suspensions. Especially, the $AUC_{0\rightarrow 24\,h}$ and C_{max} values of pranlukast nanosuspension with a particle size of 0.16 μm were approximately 3.5- and 6.3-fold greater, respectively, than that of pranlukast microsuspension with a particle size of 18.21 μm. Therefore, the preliminary results from our study suggest that a pranlukast nanosuspension with a mean particle size of about 0.16 μm may have significant potential for clinical application.

1. Introduction

Particle size reduction is a universal strategy used in the pharmaceutical industry to reduce particle size, and hence improve the dissolution rate and bioavailability of drugs with poor water solubility. Nanosuspensions of hydrophobic drugs typically consist of drugs and surface stabilizers, such as hydrophilic polymers and/or surfactants with mean particle size <1 μm, and can be produced by a bottom-up (precipitation) or top-down approach (high pressure homogenization and bead milling, etc.) [1–4]. Compared to other nanotechnology-based drug delivery systems, nanosuspensions can be applied to manufacture poorly water-soluble drugs such as BCS class II and IV drugs. Nanosuspenions have the potential to improve the dissolution and bioavailability of various poorly water-soluble drugs (e.g., aprepitant, cilostazol and nitrendipine, etc.) in a particle size-dependent oral absorption manner [5–7]. Recently, Sun et al. reported that 300 nm, 750 nm, and 5.5 μm itraconazole suspensions increased AUC values by 50.6-, 43.9-, and 6.5-fold, respectively, when compared with coarse suspensions, yet there was no significant difference in AUC values between 300 and 750 nm itraconazole suspensions in rats ($n = 3$, $P > 0.05$) [8]. However, for atorvastatin calcium, 179 nm nanoparticles showed higher oral absorption than 727 nm nanoparticles in rats ($n = 5$, $P < 0.05$) [9]. Thus, particle size reduction and determination of a critical particle size to improve *in vivo* performance of poorly water-soluble drugs are both very important for the development of new dosage forms.

In this study, we investigated the effect of particle size on the dissolution and oral absorption of pranlukast microsuspensions and nanosuspensions stabilized by hydroxypropylmethyl cellulose to develop a new dosage form containing pranlukast with high bioavailability. Pranlukast (*N*-[4-oxo-2-(1*H*-tetrazol-5-yl)-4*H*-chromen-7-yl]-4-(4-phenylbutoxy)benzamide) is a selective and competitive antagonist of cysteinyl leukotriene type 1 receptors that has been used to treat chronic bronchial asthma and to prevent exercise-induced asthma [10]. The solubility of pranlukast is approximately 1.03 μg/mL in water (37 °C) [11]. It was reported that the absolute bioavailability of pranlukast is approximately 4.3% after oral administration in rats using a specialized delivery device

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[12]. Minimally, 12.5% of a postprandial oral dose of pranlukast is absorbed based on the ratio of urinary and fecal excretion in healthy volunteers [10]. In this study, pranlukast microsuspensions and nanosuspensions stabilized by hydroxypropylmethyl cellulose and poloxamer 407 with four different particle sizes were prepared based on a top-down approach using different mills such as air-jet mill, high pressure homogenizer, and bead mill. The *in vitro* dissolution and *in vivo* oral absorption of pranlukast suspensions in rats were evaluated.

2. Materials and methods

2.1. Materials

Pranlukast was obtained from Yuhan Pharm. Co., Ltd. (Korea). Hydroxypropylmethyl cellulose (HPMC 2910) and montelukast were purchased from Shin-Etsu Chemical Co. (Japan) and Sigma Chemical Co. (USA), respectively. Poloxamer 407 was obtained from BASF Co. (Germany). All organic solvents were of HPLC grade.

2.2. Preparation of pranlukast suspensions

2.2.1. Microsuspension 1

All suspensions were 1.0% (w/v) pranlukast with 1.0% (w/v) HPMC and 0.5% (w/v) poloxamer 407 based on a preliminary study. First, HPMC and poloxamer 407 were dissolved in water. Without further particle size reduction, raw material was weighed into a 1 L beaker and dispersed in a mixed aqueous solution of HPMC and poloxamer 407 to form a microsuspension containing 1.0% (w/v) pranlukast with continuous mechanical mixing.

2.2.2. Microsuspension 2

The particle size reduction of raw material was performed using a jet mill (Micronizer, Sturtevant, USA) for preparation of microsuspension 2. Jet milled pranlukast was dispersed in a mixed aqueous solution of HPMC and poloxamer 407 to form a microsuspension containing 1.0% (w/v) pranlukast by continuous mechanical mixing with the same composition as microsuspension 1.

2.2.3. Nanosuspension 1

Nanosuspension 1 was prepared by particle size reduction of jet milled pranlukast using high-pressure homogenization. First, air-jet milled pranlukast was weighed in a beaker and the stabilizer solution of HPMC and poloxamer 407 was added. Then, suspensions were pre-milled using an Ultra-Turrax® T25 (IKA, Germany) at 20,000 rpm for 5 min. The suspensions were further processed through a microfluidizer model M-110P (Microfluidics, USA) by first performing 2 cycles at 20 MPa and 2 cycles at 50 MPa followed by 10 cycles at 100 MPa to obtain pranlukast nanosuspensions. An ice-water bath (0 °C) was used during the microfluidization process.

2.2.4. Nanosuspension 2

A laboratory bead mill (Model: Lab Star 1, Netzsch Mill, Germany) was used for preparing pranlukast nanosuspension 2. Briefly, obtained microsuspension 2 was charged into the milling chamber and milled for 2 h at 3500 rpm under controlled temperature conditions (i.e., below 25 °C). During the milling process, a high shear force was generated by the movement of yttrium-stabilized zirconium oxide grinding beads (0.2–0.4 mm).

2.3. Characterization of pranlukast suspensions

The particle size and distribution of pranlukast suspensions were measured by a Microtrac laser diffraction particle size analyzer (Microtrac, Inc., USA). The particle size of pranlukast nanosuspensions were also measured by dynamic light scattering (ELS-8000, Otsuka Electronics, Japan). Dissolution tests were conducted using a USP rotating paddle apparatus (VK 7000 Dissolution Testing Station, Vankel, USA) at 37 °C and 50 rpm. Accurately weighed suspensions containing the equivalent of 10 mg pranlukast were placed in 900 mL of pH 6.8 phosphate buffer with 0.05% (w/v) tween 80. Then, 5 mL aliquots were collected at different time intervals and filtered using a 0.05 μm syringe filter. The filtered samples were diluted with pH 6.8 phosphate buffer and the concentration of pranlukast was analyzed with a UV visible spectrophotometer (UVmini-1240, Shimadzu, Japan) by measuring the absorbance at 260 nm.

2.4. Pharmacokinetics in rats

Animal experiments were performed according to institutional guidelines for the care and use of laboratory animals, and were approved by the animal ethics committee of Kyungsung University. Twenty Male Sprague-Dawley rats weighing 240–260 g (Orient Bio, Inc., Korea) were housed in an animal facility at the College of Pharmacy, Kyungsung University. Prior to use in experiments, animals were kept under standard laboratory conditions for at least 1 week.

The study was approved by the Institutional Review Board of Kyungsung University. Rats were randomly divided into four groups in a parallel design. The rats were given pranlukast suspensions orally at a dose of 40 mg/kg. Blood samples (150 μL) were collected from the retro-orbital plexus of rats into microfuge tubes containing heparin as an anti-coagulant at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 h post-dosing. The blood samples were centrifuged at 10,000 rpm (13,416 × *g*) for 2 min and plasma was collected and stored at −70 °C until LC–MS/MS analysis. Pranlukast and the IS (montelukast) were extracted from the plasma matrix by liquid–liquid extraction according to a previously reported method [13]. Fifty microliters of plasma samples were mixed with 10 μL of the IS (0.5 μg/mL) and 400 μL extraction solvent consisting of ethyl acetate and methanol (2:8, v/v). After centrifugation at 13,000 rpm (22,673 × *g*) for 5 min, the supernatant was evaporated under a vacuum in a SpeedVac system at 55 °C for 60 min. The dried extract was reconstituted in 100 μL of 85% acetonitrile and then 10 μL volume of the extract was analyzed by LC–MS/MS. The plasma concentrations of pranlukast were determined using LC–MS/MS using an API 4000 LC–MS/MS system (Applied Biosystems, USA) equipped with an electrospray ionization (ESI) interface to generate negative ions $[M-H]^-$. The ion spray voltage and nebulizer gas were set at 5500 V. The collision energy (CE), declustering potentials (DP), and collision exit potential (CXP) were set at −24, −56, and −6 V for pranlukast and −28, −62, and −12 V for IS, respectively. Analytes were monitored via multiple reaction monitoring (MRM) employing the following precursor to product ion transitions: m/z 480.1 → 424.2 for pranlukast, m/z 584.2 → 472.2 for IS. An 1100-series LC system (Agilent Technologies, USA) consisted of a solvent degasser, binary pumps, and an autosampler. Pranlukast was separated on Phenomenex Kinetex C_{18} column (50 × 2.1 mm, 2.6 μm). The mobile phase was acetonitrile/water (85:15, v/v) and was pumped at a flow rate of 0.3 mL/min. The pharmacokinetic parameters were calculated with a noncompartmental method using the WinNonlin Standard Edition Software, version 5.3 (Pharsight Corp., USA). The $AUC_{0\rightarrow 24\,h}$ was calculated using a linear trapezoidal rule. The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) of pranlukast in the plasma were taken directly from the data. Statistical testing was performed by one-way ANOVA with Least Significant Difference and Student–Newman–Keuls tests using SPSS 19.0 software (IBM Corp., USA).