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Novel oral formulation approach for poorly water-soluble drug using lipocalin-type prostaglandin D synthase

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ABSTRACT

Lipocalin-type prostaglandin D synthase (L-PGDS), a member of the lipocalin superfamily, possesses the function of forming complexes together with various small lipophilic molecules. In this study, we chose telmisartan as a model drug due to its pH dependent poor water solubility, and developed and characterized a novel solubilized formulation of telmisartan using a complex formulation with L-PGDS. The solid state of the complex formulation was prepared using a spray-drying process. The spray-dried formulation of telmisartan/L-PGDS powder showed a typical spray-dried particle without any change in the secondary and tertiary structures of L-PGDS. Furthermore, the complex formulation showed a high rate and level of drug release in pH 1.2, 5.0, and 6.8 solutions in comparison with the active pharmaceutical ingredient (API) and commercial product. To validate the potential for oral administration of the telmisartan/L-PGDS complex *in vivo*, the pharmacokinetic and pharmacodynamic profiles were assessed in spontaneous hypertensive rats. An animal study revealed that the complex formulation led to a significant improvement of AUC and C_{max} as compared with API, and the prolonged pharmacologic effect on blood pressure reduction was comparable with the commercial product. These results, taken together, demonstrate that this novel approach is feasible for the solubilized solid oral formulation and it can be applied to poorly water-soluble drugs to enhance oral bioavailability.

1. Introduction

Insolubility or poor solubility in water of drug candidate is one of the most serious problems in pharmaceutical development. When the absorption of a drug is limited by its solubility, marked differences in oral bioavailability due to formulation factors can be observed. However, new drug entities tend to have a lower solubility using the current drug discovery approach (i.e., high-throughput screening, combinatorial chemistry) (Lipinski, 2000; Lipinski et al., 2001). These active pharmaceutical ingredients (APIs) belong to class II and IV drugs of the biopharmaceutical classification system (BCS) that are insoluble in water (Amidon et al., 1995), and about 65% of new drug candidates are classified in this class (Ku and Dulin, 2012). The development of drugs which include poorly soluble compounds is a serious bottleneck for the pharmaceutical industry. Telmisartan (Fig. 1A) is an antagonist of the angiotensin II type-1 receptor that is indicated for the treatment of hypertension (Wienen et al., 2000). Telmisartan is categorized as a BCS class II compound, and its solubility is quite low within the physiological gastrointestinal pH range (Tran et al., 2008).

As solutions to improve the low solubility of compounds, various technologies have been investigated from several aspects

Abbreviations: L-PGDS, lipocalin-type prostaglandin D synthase; API, active pharmaceutical ingredient; BCS, biopharmaceutical classification system; SEM, scanning electron microscope; SE-HPLC, size exclusion high-performance liquid chromatography; CD, circular dichroism; SGF, simulated gastric fluid; FaSSGF, fasted state-simulated gastric fluid; FaSSIF, fasted state-simulated intestinal fluid; SIF, simulated intestinal fluid; SHR, spontaneously hypertensive rat; SBP, systolic blood pressure.

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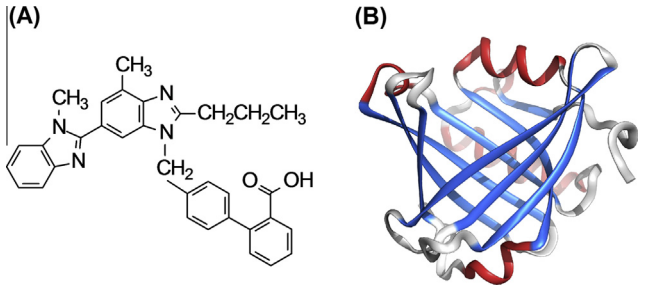

Fig. 1. Structures of telmisartan and L-PGDS [molecular mass: 18777.7]. (A) Chemical structure of telmisartan [relative molecular mass: 514.6]. (B) Three-dimensional structure of human L-PGDS (PDB code: 3O2Y).

(Kawabata et al., 2011; Fahr and Liu, 2007). The addition of solubilizers such as organic solvents, surfactants, lipids, cyclodextrin, and pH modifiers is considered as a beneficial approach to solubility improvement (Gao and Shi, 2012; Loftsson and Brewster, 2012). However, formulations including solubilizers need the quantitative and qualitative optimization of compounds to overcome the stability problem (Strickley, 2004).

Lipocalin-type prostaglandin D synthase (L-PGDS, Fig. 1B) is a multi-functional protein which plays the role of a catalyzer for the isomerization of prostaglandin H_2, a scavenger for reactive oxygen species, and an extracellular transporter for small lipophilic molecules as a member of the lipocalin superfamily (Nagata et al., 1991; Toh et al., 1996; Fukuhara et al., 2012). Recently, we showed that L-PGDS acted as a scavenger of biliverdin, a metabolite of hemoglobin that accumulates in the cerebrospinal fluid of aneurysmal subarachnoid hemorrhage patients (Inui et al., 2014). The structure of L-PGDS exhibited the typical lipocalin fold, consisting of an eight-stranded, antiparallel β-barrel and a long α-helix associated with the outer surface of the barrel. The interior of the barrel formed a hydrophobic cavity opening into the upper end of the barrel, the size of which was larger than those of other lipocalins (Shimamoto et al., 2007; Miyamoto et al., 2010; Zhou et al., 2010). We demonstrated that L-PGDS could bind to a large variety of lipophilic molecules such as heme metabolites, retinoids, thyroids, steroids, flavonoids, and saturated fatty acids in the hydrophobic cavity (Kume et al., 2012; Inui et al., 2003). Using the function of L-PGDS, we have already evaluated the feasibility of L-PGDS as a novel drug-delivery carrier to improve the solubility of the poorly water-soluble molecules mentioned in the previous article (Fukuhara et al., 2012).

Proteins in liquid formulations are generally at a greater risk of chemical and physical instability (Wang, 1999; Mahler et al., 2005). Therefore, L-PGDS needs to be prepared in dry form in order to increase the stability and apply it to multiple dosage forms. The common process to produce dried protein is lyophilization and spray drying. Lyophilization has less of a thermal influence on protein than other drying processes. However, the lyophilization process is energy-intensive. Furthermore, the process involves time-consuming steps such as freezing followed by drying under low pressure, and there are high production costs involved (Wang, 2000). On the other hand, the spray-drying process utilizes heat to evaporate micro-dispersed droplets created by atomization of a continuous liquid feed. Therefore, the one-step drying process leads to a significantly shorter operation time and cost-effective dehydration (Cal and Sollohub, 2010).

In the present study, we show the application of L-PGDS as a novel drug-solubilizing carrier for a solid oral formulation using telmisartan as a model compound. The solid state of the telmisartan and L-PGDS complex formulation was produced using the spray-drying technique, and the physicochemical properties of the produced particle were characterized. Finally, the *in vitro* and *in vivo* performance of the developed formulation was assessed.

2. Materials and methods

2.1. Materials

Telmisartan and the commercial product (Micardis®) were supplied by Boehringer Ingelheim GmbH & Co. KG (Ingelheim, Germany). Jet-milling of telmisartan was carried out by A–O jet mill (Seishin Enterprise Co., Ltd, Osaka, Japan). The injector air pressure and the grinding air pressure were 7.5 bar, and powder feed rate was set to 3 g min^{-1} at room temperature. All other chemicals were of analytical grade.

2.2. Purification of recombinant human L-PGDS

The recombinant human C65A/C167A (ε_{280} = 25,900 M^{-1} cm^{-1})-substituted L-PGDS mutant, in which a catalytic residue of cysteine was substituted to alanine to get rid of an enzymatic activity of L-PGDS was used in this study. And the 22 N-terminal amino acid residues corresponding to the putative secretion signal peptide of L-PGDS were truncated. C65A/C167A-substituted L-PGDS was expressed in *Escherichia coli* BL21 (DE3) (TOYOBO, Osaka, Japan) (Kume et al., 2012). Site-directed mutagenesis was performed using the QuikChange™ site-directed mutagenesis kit (Stratagene California, La Jolla, California, USA). The mutated L-PGDS was expressed as a glutathione *S*-transferase fusion protein. The fusion protein was bound to a glutathione–Sepharose 4B column (GE Healthcare Bio-Sciences, Little Chalfont, UK) and incubated overnight with thrombin (16.5 units mL^{-1} column bed volume) to release L-PGDS at room temperature. The protein was further purified by gel filtration chromatography with HiLoad 26/600 Superdex 75 (GE Healthcare Bio-Sciences) in 5 mM Tris–HCl (pH 8.0). The purified L-PGDS of 100–200 mg was routinely obtained from 1 L of culture.

2.3. Solubility study

An excess amount of telmisartan was weighed in a 2-mL microtube with 1 mL of aqueous solution containing several kinds of buffer medium in the presence of L-PGDS or without L-PGDS. Sealed microtubes were shaken with a Rotator RT-50 (TAITEC, Saitama, Japan) for 2 h at 37 °C, followed by filtration through a 0.45-μm filter (EMD Millipore, Billerica, Massachusetts, USA). After incubation, the sample solution was diluted with methanol and centrifuged at 3000 rpm for 10 min. Then, 20 μL of filtered supernatant (0.45-μm filter) was injected into a high-performance liquid chromatography (HPLC, Waters Corporation, Milford, Massachusetts, USA) equipped a YMC Pack ODS-AM column (150 × 4.6 mm I.D., 5 μm, YMC Co., Ltd., Kyoto, Japan) to analyze the amount of telmisartan. The mobile phase, a mixture of methanol and 2% (w/v) ammonium phosphate (pH 3.0) (70:30, v/v), was eluted at a flow rate of 1 mL min^{-1}. The chromatogram was monitored at 297 nm.

2.4. Spray drying of telmisartan/L-PGDS complex solution

Based on the solubility study, the complex of L-PGDS and telmisartan was prepared as a 1:1 M ratio. The complex solution was filtered with a 0.45-μm filter (EMD Millipore) before the spray-drying process. Dried L-PGDS or the telmisartan/L-PGDS complex was prepared with Mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland). Solution was delivered to the water-cooled nozzle (0.7-mm liquid orifice internal diameter)