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Pharmacokinetic evaluation and modeling of formulated levodopa intranasal delivery systems

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ABSTRACT

Levodopa (L-dopa), the metabolic precursor of dopamine, has primarily been used for the treatment of Parkinson's disease (PD) in combination with carbidopa (C-dopa). This study aims to investigate the feasibility of L-dopa nasal delivery systems prepared using maleic acid solution containing 2-hydroxypropyl-β-cyclodextrin, and to develop pharmacokinetic models. Following oral or intravenous administration of L-dopa plus C-dopa and intranasal dosing of L-dopa in the presence and absence of C-dopa to the rat, the concentrations of L-dopa in plasma and brain were determined using HPLC. The pharmacokinetic profiles were analyzed using non-compartmental and compartmental modeling approaches. Simultaneous nonlinear regression was performed to improve the identifiability of model parameters. L-Dopa was rapidly absorbed into blood and brain. The absolute bioavailabilities of oral and nasal preparations containing C-dopa were 17.7 and 45.4%, respectively. C-dopa caused a 1.2-fold decrease in the elimination rate of L-dopa, indicating decreased metabolism. Although the half-life after nasal administration was relatively short (less than 30 min) in both blood and brain regardless of C-dopa addition, the systemic exposure was promising due to rapid absorption. In conclusion, the L-dopa nasal delivery system could be used as a good rescue therapy for PD patients who experience symptom fluctuation with oral L-dopa administration.

1. Introduction

Parkinson's disease (PD) is a debilitating motor neuron disease that affects the dopaminergic neurons within the nigral-striatal and surrounding pathways. Pathologically, the cause of the disease is a dopamine (DA) deficiency in the basal ganglia of the brain. The clinical manifestations begin to appear when dopaminergic neuron loss is up to 70–80% (Chen et al., 2008).

Levodopa (L-dopa), the metabolic precursor of DA, has been regarded as the standard for treating PD (Cotzias et al., 1967). L-Dopa works by replacing the DA that would be normally released by the substantia nigra DA pathway. It can cross the blood brain barrier (BBB) via a saturable transporter, *i.e.*, a large neutral amino acid active carrier system. In the brain, it is converted to DA by L-aromatic amino acid decarboxylase (AAAD, also known as dopa decarboxylase), primarily within the presynaptic terminals of dopaminergic neurons in the striatum (Chen et al., 2008; Okereke, 2002). However, L-dopa is converted to DA outside the CNS as well, so that relatively little unchanged drug reaches the cerebral circulation (probably less than 1%) when L-dopa is administered alone (Durso et al., 2000). Moreover, the DA released into the circulation by peripheral conversion of L-dopa produces undesirable effects (Furlanut et al., 2001).

Carbidopa (C-dopa) is a competitive peripheral AAAD inhibitor with little or no pharmacological activity when given alone in usual doses to PD patients. Unlike L-dopa, it does not cross the BBB. It contributes to the production of effective brain concentrations of DA from lower doses of L-dopa by inhibiting the peripheral decarboxylation of L-dopa to DA. In addition, reduced peripheral formation of DA decreases the peripheral side effects such as nausea, vomiting and cardiac arrhythmia. Furthermore, the combination of L-dopa and C-dopa leads to better symptom control and improvements in the 'wearing-off' phenomenon in PD patients (Contin et al., 1996).

Currently, the oral route is the most popular clinically available route for L-dopa administration in PD patients. However, oral administration of L-dopa causes variable and unreliable clinical responses. Since L-dopa is immediately absorbed in the proximal duodenum by an active transport system, *i.e.*, a saturable large neutral amino acid (LNAA) transport system, its plasma concentrations

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rapidly rise and fall. The rate and extent of L-dopa absorption may be affected by diet and other gastric factors. The LNAAs such as leucine, valine, phenylalanine, tyrosine and methionine contained in food may compete with L-dopa for intestinal absorption (Simon et al., 2004; Müller et al., 2006). Thus, high-protein diets reduce the absorption of L-dopa from the intestines.

In addition, dysphagia is a well-recognized manifestation of PD; the prevalence of dysphagia is uncertain but may be as high as 52% in this disease. Patients experiencing this manifestation cannot be expected to comply with oral administration or to obtain optimal bioavailability of L-dopa (Johnston et al., 1995; Nyholm, 2006).

A nasal delivery system has been considered a viable option as an alternative dosage form to oral delivery. The nasal cavity is easily accessible, extensively vascularized and highly permeable. The large surface area of the nasal mucosa affords rapid absorption, fast onset of therapeutic effect, and higher bioavailability. Compounds administered via this route are absorbed directly into the systemic circulation, avoiding the hepatic first-pass effect. In this respect, it could be an ideal route for non-invasive administration with reduced infectious disease transmission, which may maximize convenience and self-medication and improve patient compliance. Therefore, intranasal administration is regarded as a good alternative to invasive injections or oral delivery (Costantino et al., 2007; Illum, 2000; Dahlin et al., 2001).

The objectives of this study were to characterize the pharmacokinetics of L-dopa in rat plasma and brain following intranasal administration using a compartmental modeling approach, and to investigate the feasibility of L-dopa nasal delivery systems.

2. Materials and methods

2.1. Materials

Levodopa (L-dopa), carbidopa (C-dopa) and α-methyldopa were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Xylazine (Rompun®, Bayer Korea, Ansan, Korea), tiletamine-xolazepam (Zoleti®50, Virbac Korea Medicine, Seoul, Korea), 2-hydroxypropyl-β-cyclodextrin (2-HPβCD, Cargill Inc., Minneapolis, MN, USA), ether (Daejung Chemicals and Metals, Siheung, Korea), heptanesulphonate (HAS), disodium ethylenediamine tetraacetic acid ($EDTANa_2$), citric acid, sodium citrate, perchloric acid, ortho-phosphoric acid, maleic acid and triethanolamine (Duksan Pure Chemical, Ansan, Korea) were also obtained. Methanol was used for HPLC analysis. Other reagents were of analytical grade.

2.2. Formulation of nasal delivery systems

Two nasal compositions were formulated. One formulation consisted of maleic acid, 2-HPβCD, triethanolamine, L-dopa and C-dopa (8:77:5:8:2, w/w). L-Dopa and C-dopa were dissolved in 0.1 mol/L maleic acid, and the pH was adjusted to 2.7 with triethanolamine. The other formulation had the same composition but lacked C-dopa. The products were hermetically sealed in an aluminum pan, frozen to −60 °C, and then heated at 1 °C/min from −60 to 0 °C. The primary and secondary drying were performed at −39 to −35 °C and at 40 °C for 4 h, respectively.

2.3. Pharmacokinetic studies

All animal experiments adhered to the Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences. Male Sprague–Dawley (SD) rats weighing 250–260 g were obtained from Samtako Bio Co., Ltd. (Osan, Korea). All surgical procedures were performed under anesthesia with ether, and an intraperitoneal injection of 0.2 mL/kg xylazine with 0.2 mL/kg tiletamine-xolazepam was given for deeper anesthesia and immobilization. Rats were divided into three groups – nasal, intravenous and oral groups – and each group comprised six samples for each time point. Blood samples were collected from the caudal vein. Blood collection was terminated by decapitation. The brain was removed quickly and weighed. The brain (1 g) sample was snap-frozen on liquid nitrogen (LN_2, −196 °C) and stored at −70 °C until analyzed.

For intravenous (IV) administration, L-dopa (20 mg)/C-dopa (5 mg) were dissolved in 9 mL of normal saline and filtered through a 0.2-μm polyvinylidene fluoride (PVDF) filter; the jugular vein was cannulated using a polyethylene tube (0.76 mm i.d. × 1.22 mm o.d.; Becton Dickinson, Franklin Lakes, NJ, USA).

Prior to oral administration, the animals were fasted overnight and kept under fasted conditions until 4 h after oral administration of a single dose of L-dopa/C-dopa; they were allowed water *ad libitum*. The dosages for IV and oral administration were 80 mg/kg of L-dopa and 20 mg/kg of C-dopa. Blood and brain samples were collected at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24 and 36 h after IV and oral administration.

The nasal absorption study of L-dopa/C-dopa was conducted using an *in vivo* experimental technique described by Hussain et al. (1980). The reconstituted solution of L-dopa (2.5 mg/kg)/C-dopa (0.63 mg/kg) and L-dopa alone (2.5 mg/kg) was administered into one nostril using a microsyringe. Blood and brain were sampled at 0.08, 0.16, 0.25, 0.5 and 1 h.

Total 288 rats were used in this study because each rat was assigned only one time point due to decapitation.

2.4. Chromatographic conditions

Samples from pharmacokinetic study were analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (PU-2080, Jasco, Tokyo, Japan) with an electrochemical detector (CouloChem III, ESA, Muskegon, MI, USA). The first electrode in the analytical cell was set at −400 mV (range 100 μA) and the second one at 450 mV (range 100 nA) with a flow-rate of 1 mL/min. A Gemini 5μ C18 column (4.6 mm × 150 mm, 5 μm bead size, Phenomenex, Torrance, CA, USA) was used. The mobile phase was composed of 16.5 g of potassium phosphate, 1.0 mL of 0.1 M $EDTANa_2$, 1.2 mL of 0.5 mM HAS, and 19.5 mL of methanol with pH adjusted to 3.4 with phosphoric acid. The mobile phase was filtered through a 0.2-μm cellulose membrane (Whatman, Maidstone, England).

2.5. Plasma and brain preparations

Working stock solutions of L-dopa and methyldopa (internal standard, IS) were prepared in water at a concentration of 1 mg/mL. Prior to use, the stock solutions of L-dopa and IS were further diluted with water to obtain working solutions. An appropriate dilution of the working solution with drug-free plasma or brain gave an L-dopa concentration between 50 and 1000 ng/mL.

Two-hundred microliters of the IS (10 ng/mL aqueous solution) and 100 μL of 4 M perchloric acid were added to 1 mL of plasma, then vortex-mixed for 2 min and centrifuged at 9000 rpm for 20 min. A 300-μL aliquot of the supernatant was added to 200 μL of 2 M potassium citrate buffer (pH 3.8) to precipitate the perchlorate. Each tube was vortexed for 1 min and then centrifuged as above. A 20-μL aliquot of the clear supernatant was injected onto the HPLC for analysis.

For the preparation of brain standard solutions or samples, the frozen brain was ground using mortar and pestle. The prepared standard brain or sample brain from the pharmacokinetic study was spiked with 200 μL of IS and 300 μL of 4 M perchloric acid,