Enhanced Boosting of Oral Absorption of Lopinavir Through Electrospray Coencapsulation with Ritonavir

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Received 21 February 2015; revised 18 April 2015; accepted 21 April 2015

Published online 18 May 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24492

ABSTRACT: *In vivo* activities of absorption enhancers coencapsulated with poorly absorptive drugs in the same enteric-coated particles were evaluated. Lopinavir [a substrate of cytochrome P450 3A (CYP3A)] and ritonavir (an inhibitor of CYP3A-mediatd metabolism) were used as a model drug and a model absorption enhancer, respectively. Lopinavir and ritonavir were encapsulated into enteric-coated particles as amorphous forms using coaxial electrospray deposition. The electrospray treatment resulted in dramatic improvement of dissolution profiles of both compounds, probably because of complete amorphization and superior dispersion efficiency of the particles. Poor absorption of lopinavir in rats was observed after oral administration of enteric-coated particles containing lopinavir alone. When the particles were coadministered with enteric-coated particles containing ritonavir alone, lopinavir absorption was boosted. The boosting effect was further enhanced when ritonavir was coencapsulated with lopinavir into the same enteric-coated particles. A significant increase in area under the plasma concentration–time curve reflected an extension of mean residence time rather than an elevation of C_{max}. Lopinavir absorption was improved presumably because lopinavir was always accompanied by a practical amount of ritonavir required for the boosting during the gastrointestinal transit of the particles. Not only did the electrospray coencapsulation technique improve drug absorption, but also increased trough concentration that might result in the reduction of the number of doses. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2977–2985, 2015

Keywords: boosting; oral absorption improvement; coaxial electrospray deposition; enteric-coated particle; lopinavir; ritonavir

INTRODUCTION

Intestinal epithelial cells possess diverse abilities. Influx transporters at the apical side of the intestinal membranes are essential for the absorption of nutrients through molecular recognition.[1–3] Prodrugs with chemical structures that are recognized by such transporters are commercially available.[4–6] However, most oral medicines are passively absorbed through the intestinal membranes on the basis of their lipophilicity and molecular weights.[7–9] Intestinal epithelial cells often protect bodies from exposure to harmful substances. Cytosolic enzymes such as cytochrome P450 (CYP) 3A in the epithelial cells play an important role in inactivating drugs, as do hepatic cells before drugs are delivered into the systemic circulation.[10,11] Drugs taken up into the cells are often excreted to the luminal side via efflux transporters.[12–14] Drug absorption is also affected by the solubility and dissolution rate from dosage forms.[15–17] Drug–drug and drug–food interactions sometimes induce dynamic changes of the absorption properties.[18–20] Oral bioavailability of drugs results from the integration of these physiological and physicochemical properties.

Lopinavir, a human immunodeficiency virus (HIV) protease inhibitor, is used for the treatment of HIV infection. Low bioavailability and fast elimination are observed when lopinavir alone is orally administered; however, coadministration with ritonavir dramatically improves poor pharmacokinetic properties of lopinavir.[21] Numerous studies demonstrated that ritonavir enhanced the transport of lopinavir from the intestinal lumen to the systemic circulation through inhibition of presystemic and systemic metabolism of lopinavir, which is a substrate of CYP3A.[21–25] The findings form rationales for the therapy of HIV infection by lopinavir with a subtherapeutic dose of ritonavir, which results in increased and sustained plasma levels of lopinavir. Liquid and solid formulations are clinically used for the combination of lopinavir and ritonavir (the brand name: Kaletra). HIV patients take either formulation once (800 mg of lopinavir/200 mg of ritonavir) or twice (400 mg of lopinavir/100 mg of ritonavir × 2) a day. Water-miscible alcohols and surfactants are included in the liquid formulation to improve water solubility of lopinavir and ritonavir. An alternative formulation is a tablet containing both compounds dispersed in water-soluble polymer/surfactant matrices as amorphous forms with superior solubility profiles.

We have been investigating the potential of electrospray deposition (ESD) as an advanced pharmaceutical technology.[15,16] ESD enables micro/nanofabrication of formulations without the application of serious stresses such as high temperature and strong share force. Besides the advantage, ESD is one of the techniques for manufacturing amorphous solid dispersions. ESD is therefore a promising technology for improving the dissolution behavior of poorly water-soluble drugs through a couple of mechanisms: size reduction and amorphization. In our previous study,[15,16] we used a coaxial nozzle-equipped ESD, which provides particles with core-shell structure, and prepared enteric-coated particles containing poorly water-soluble drugs such as griseofulvin and fenofibrate in amorphous forms.

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Journal of Pharmaceutical Sciences, Vol. 104, 2977–2985 (2015)

Rat experiments revealed that oral absorption of poorly water-soluble drugs encapsulated into enteric-coated particles was superior to that of crystalline drugs.

Lopinavir and ritonavir are released in the stomach when patients take the commercial Kaletra. The intestinal absorption is followed by gastric emptying of the compounds dissolved in the stomach, irrespective of the formulation type. However, transit of the compounds through the gastrointestinal tract are not interfered each other. As the solubility of ritonavir in acidic media is more than 100 times greater than that of lopinavir, independent transit is perhaps observed in their molecular levels. Dilution in digestive fluids is also observed during the transit. These phenomena often result in a reduction of *in vivo* activities of absorption enhancers formulated with drugs.[9,26] When lopinavir is coencapsulated with ritonavir into the same enteric-coated particles, it is expected that lopinavir is accompanied by formulated ritonavir during the gastrointestinal transit of the particles. The commercial formulations indicate that both compounds need to be encapsulated in the amorphous form to improve their dissolution profiles. Coaxial ESD is a technique capable of creating formulations that meet this requirement. The present study validated our strategy through *in vitro* and *in vivo* experiments.

MATERIALS AND METHODS

Materials

Lopinavir and ritonavir were obtained from LKT Laboratories, Inc. (St. Paul, Minnesota). Poly(methacrylic acid-co-methyl methacrylate) (Eudragit L100) was supplied from Rohm Pharma GmbH (Darmstadt, Germany). Propylene glycol and poly(vinylpyrrolidone) [PVP; K12–17 (average molecular weight: 10 kDa)] were purchased from Wako Pure Chemical Industries Company, Ltd. (Osaka, Japan) and Sigma–Aldrich Chemical Company (St. Louis, Missouri), respectively. All other chemicals were commercial products of reagent grade. All materials were used without further purification.

Solubility of Crystalline Bulk Substances

Solubility of lopinavir and ritonavir in the first fluid of the Japanese Pharmacopoeia 16 (JP-1 solution, pH 1.2) and 50 mM phosphate-buffered solution (pH 7.0) was measured. Each compound (~2 mg) was loaded in a test tube ($n = 3$). When the solubility was measured in the presence of the counterpart, both compounds (2 mg of each) were weighed in the same tube. After addition of the solvent (5 mL), tubes were shaken in a water bath at 37°C for 3 h (JP-1 solution) or 24 h (phosphate-buffered solution). Each solution was filtrated using a syringe filter with a pore size of 0.45 μm. All items used for filtration were incubated in an oven at 37°C prior to their use. The filtrate was diluted with an appropriate amount of an ethanol–water mixture (1/1, v/v). A HPLC system with a column of 150 × 2.0 mm filled with octadecylsilyl silica (ODS) gel of 5 μm mean particle size (YMC-Pack Pro C18; YMC, Kyoto, Japan) was used for assay. Water was mixed with an equivalent volume of acetonitrile to prepare a mobile phase. The injection volume was 2 μL, the flow rate was 0.2 mL/min, and the column temperature was 30°C. Both compounds were assayed by measuring the UV absorption at 210 nm. Measurements were made in the concentration range from 0.25 to 20 μg/mL with a good correlation coefficient ($r^2 > 0.999$).

Preparation of Enteric-Coated Particles

Enteric-coated particles were prepared by coaxial ESD.[15,16] Experimental conditions are summarized in Table 1. Lopinavir and ritonavir were dissolved in ethanol at concentrations of 1.6% (w/v) and 0.4% (w/v), respectively, when enteric-coated particles containing both compounds were prepared. PVP was also dissolved in the drug-containing ethanol at a concentration of 1% (w/v). Eudragit L100 was separately dissolved in ethanol at a concentration of 1.5% (w/v). Drug and enteric coating agent solutions were used as inner and outer solutions, respectively. They were supplied by syringe pumps to the coaxial nozzle at a flow rate (mL/h) of either 0.4/0.6 (inner/outer solutions) or 0.2/0.8. Inner and outer diameters of the nozzle were 0.4 and 0.8 mm, respectively. Positive (15 kV) and negative voltages (−15 kV) were applied to the nozzle and the steel-plate target, respectively. The plate was placed perpendicular to the nozzle at a distance of 12 cm. The electrospray was conducted in an acrylic chamber at ambient temperature. The humidity was set to below 20% RH by flowing nitrogen gas. As a reference, enteric-coated particles containing either lopinavir or ritonavir were prepared by means of the same procedure as described above, except that the concentration of each compound in ethanol was set to 2% (w/v).

Physical Characterization of Enteric-Coated Particles

Particle Morphology

Particle morphology was evaluated by scanning electron microscopy (SEM) (S4800; Hitachi, Tokyo, Japan) with an accelerating voltage of 1 kV. Samples were sputter-coated using a platinum coater (E-1030 ion sputter; Hitachi) prior to the measurement. Sizes of individual particles were determined by the SEM image analysis using Mac View ver. 4.0 (Mountech, Tokyo, Japan). The average Heywood diameter was calculated from 300 particles selected randomly from the SEM image.

Crystallinity

Crystallinity of lopinavir and ritonavir in enteric-coated particles was evaluated through X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC). A Rigaku RINT Ultima X-ray Diffraction System (Rigaku Denki, Tokyo, Japan) with CuKα radiation was used for XRPD measurements. The voltage and current were set to 40 kV and 40 mA, respectively. Data were collected between 5° and 40° (2θ) at 0.02° intervals with a scan speed of 2°/min. DSC Q2000 (TA Instruments, New Castle, Delaware) calibrated periodically using indium and sapphire was used for DSC measurements. The sample (~5 mg) was placed in an aluminum pan and the pan was sealed by a cover with a pinhole. Dry nitrogen was used as inert gas at a flow rate of 50 mL/min. Data were collected at a heating rate of 10°C/min.

Dissolution Test

Paddle Method. SR8 Plus (Hanson Research, Chatsworth, California) was used manually as a dissolution apparatus. Solid samples (bulk substances and enteric-coated particles) were dispersed in 500 mL of the second fluid of the Japanese Pharmacopoeia 16 (JP-2 solution, pH 6.8) at 37°C under stirring at 100 rpm. The sample weight was adjusted to 2 mg as a lopinavir equivalent. When the test was performed for ritonavir, its weight was adjusted to 0.5 mg as a ritonavir equivalent.