Hydroxypropyl-Sulfobutyl-β-Cyclodextrin Improves the Oral Bioavailability of Edaravone by Modulating Drug Efflux Pump of Enterocytes

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ABSTRACT: The objective of the study was to evaluate the effect of hydroxypropyl-sulfobutyl-β-cyclodextrin (HP-SBE-βCD) on the bioavailability and intestinal absorption of edaravone, and identify its mechanism of action. We devised HP-SBE-βCD as a carrier and modulator of P-glycoprotein (Pgp) efflux pump, and edaravone as a model drug, and prepared edaravone/HP-SBE-βCD inclusion complex. HP-SBE-βCD improved the water solubility and enhanced the bioavailability of edaravone by 10.3-fold in rats. Then, *in situ* single-pass intestinal perfusion showed that HP-SBE-βCD had an effect of improving the permeability and inhibiting the efflux of edaravone. Furthermore, the effects of HP-SBE-βCD on Pgp were achieved through interfering with the lipid raft and depleting the cholesterol of enterocytes membrane. From the results, we presented the novel mechanisms. First, edaravone/HP-SBE-βCD had a lower release from the inclusion compound to protect edaravone from the low pH of the stomach. Then, HP-SBE-βCD modulated the membrane microenvironment of intestinal absorption epithelial cells. At last, the result was that HP-SBE-βCD enhanced the absorption of edaravone by interfering with Pgp. In conclusion, HP-SBE-βCD improves the bioavailability of drug not only because of its enhancing water solubility of the drug, but also because it modulates the Pgp-mediated efflux from enterocytes. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:730–742, 2014

Keywords: cyclodextrins; edaravone; bioavailability; permeability; P-glycoprotein; lipid raft; Caco-2 cells; pharamcokinetics; solubility

INTRODUCTION

Oral administration is the most preferred route regarding the conventional drug delivery system (DDS), and it also ameliorates patients' compliance and comfort, especially for the treatment of chronic diseases. It is the first strategy found to augment the oral bioavailability of poorly absorbed drugs. Successful oral drug delivery system (ODDS) is required to protect drugs from the damage of the highly acidic environment in the stomach, improve the solubility and stability of drugs in the gastrointestinal tract, and increase the adherence and permeation of drugs in the gastrointestinal mucosa.[1] The affecting factors of oral bioavailability include the physicochemical properties of drugs and the biological barrier of the body, such as solubility, acid dissociation constant (pK_a), permeability, P-glycoprotein (Pgp) efflux and first-pass metabolism, and so on.[2,3]

P-glycoprotein is an energy-dependent transmembrane protein that belongs to the family of ABC transporters. It is extensively distributed in the intestinal epithelium, the hepatocytes, and the renal proximal tubular cells in normal organs.[4,5] Pgp also has an overexpression in some tumor cells and is often responsible for multidrug resistance (MDR) in anticancer treatment. It is in charge of decreasing exogenous substance accumulation in cells by efflux function. Pgp can affect the pharmacokinetics of Pgp substrates by means of reducing the absorption, restraining the oral bioavailability, restricting distribution, and increasing excretion. The drug delivery of Pgp substrate coadministrated with Pgp inhibitor can improve the bioavailability of the substrate in ODDS via influencing Pgp in the enterocytes.[6] The approach of DDS for anticancer treatment has a role in MDR reversal via influencing Pgp overexpression in cancer cells. Nevertheless, some insufficiency accompanying with pharmacological effects of the Pgp inhibitors has been found. The excipients with modulating Pgp efflux pump attract the attention of pharmacists.

Extensive efforts are being focused on resolving the issue of poor bioavailability of clinically important drugs employing various pharmaceutical approaches. The choice of carrier in ODDS is very important, because it significantly affects the pharmacokinetics and pharmacodynamics of the drugs. A number of biomaterials, such as chitosan and cyclodextrins (CDs), have been used as the absorption enhancers to improve the oral bioavailability of drugs.[7,8] CDs are the truncated-cone polysaccharides, mainly composed of six to eight D-glucose monomers linked by α-1, 4-glucosidic bonds. They have a hydrophobic central cavity and a hydrophilic outer surface, and can encapsulate

Abbreviations used: CDs, cyclodextrins; HP-SBE-βCD, hydroxypropyl-sulfobutyl-β-cyclodextrin; HP-βCD, hydroxypropyl-β-cyclodextrin; SBE-βCD, sulfobutyl-β-cyclodextrin; Pgp, P-glycoprotein; DDS, drug delivery system; ODDS, oral drug delivery system; MDR, multidrug resistance; SPIP, *in situ* single-pass intestinal perfusion.

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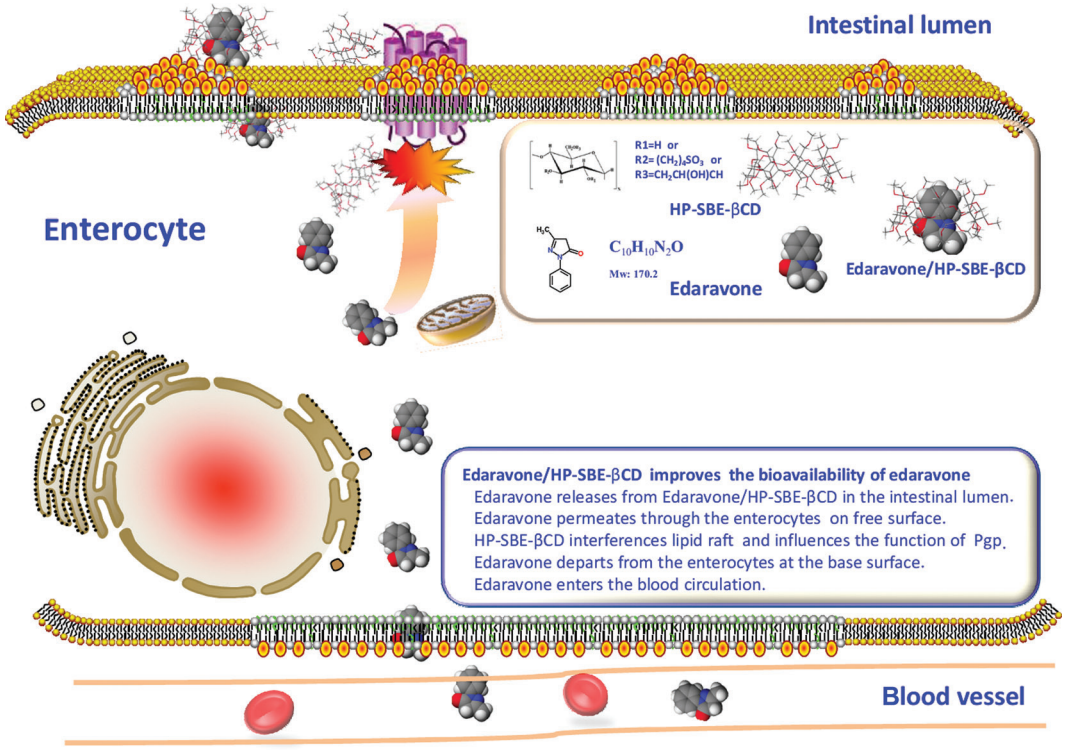

Figure 1. The chemical structures of edaravone and HP-SBE-βCD, and the schematic illustration of the novel mechanisms on edaravone/HP-SBE-βCD improving the bioavailability of edaravone.

some model substrates to form the host–guest complexes or supermolecular species. This usually affects the physicochemical properties of the guest drug, such as enhancing its solubility in water. Methyl-β-cyclodextrin (MβCD) is an excipient to improve the nasal and intestinal epithelial absorption of drugs.[9,10] MβCD is also usually employed as a control drug in research to disrupt lipid rafts by removing cholesterol from cell membranes. However, the cholesterol depletion effect of MβCD may be too strong, and will cause the cell toxicity. Recently, we developed a novel derivative of βCDs, hydroxypropyl-sulfobutyl-β-cyclodextrin (HP-SBE-βCD) (Fig. 1), as a carrier for drug delivery. HP-SBE-βCD is substituted by hydroxypropyl groups and sulfobutyl groups: n-(2,3,6-O-2-hydroxypropyl)-m-(2,3,6-O-sulfobutyl)-β-cyclodextrin.[11] Our previous investigations indicated that HP-SBE-βCD had higher water solubility, stronger inclusion capability, lower hemolytic, and lower toxicity than βCD, HP-βCD, and SBE_7-βCD. Additionally, of the various HP-SBE-βCD products, we found that HP_2-SBE_3-βCD appeared to be effective pharmaceutic adjuvant.[12]

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186, Fig. 1) is a free radical scavenger[13] and currently only used for an intravenous infusion formulation as commercial Radicut® (Mitsubishi Pharma, Osaka, Japan) or Bicun® (Simcere Pharma, Nanjing, China), which is applied to the emergency treatment of patients with acute brain infarction. However, edaravone can ameliorate the regional blood flow after cerebral infarction by attenuating the brain edema as well as inhibiting the oxidative damage of vascular endothelial cells and brain nervous cells.[14] It has shown that edaravone had the preventive effects on myocardial injury following ischemia and reperfusion in the rat,[15,16] and also may be a candidate for the treatment of retinal diseases.[17] Edaravone has been reported in pharmaceutical studies in different administration routes, including skin permeability,[18] intestinal mucosa absorption,[19] and intravitreal injection.[20] The clinical application of edaravone with extravascular administration has been limited owing to its poor bioavailability. In terms of physicochemical characteristics, edaravone is a slightly soluble compound (3 mg/mL in aqueous solutions).[21] Recently, Sato et al.[18,19] reported that edaravone/HP-βCD inclusion complex improved the skin permeability and intragastric mucosa absorption of edaravone. Zeng et al.[22] reported that HP-βCD enhanced edaravone's apparent solubility, dissolution rate, and stability. However, the above-mentioned research has not discussed the mechanism of those CDs improving the bioavailability of edaravone.

On the basis of these factors, to investigate the possible mechanisms of CDs enhancing the bioavailability of edaravone, we prepared the inclusion complex of edaravone with HP-SBE-βCD, named edaravone/HP-SBE-βCD, researched its physicochemical characteristics, and evaluated its pharmacokinetic parameters and oral bioavailability in rats. Even more importantly, we studied the novel mechanisms of improving the oral bioavailability using *in situ* single-pass intestinal permeability and Caco-2 cell model *in vitro*.

MATERIALS AND METHODS

Materials

Edaravone ($C_{10}H_{10}N_2O$, Mw 174.2 Da, purity >99%) was purchased from Adamas-beta (Shanghai, China). Bicun® (a commercial injection of edaravone) was obtained from Simcere Pharmaceutical (Nanjing, China). HP_2-SBE_3-βCD (the following abbreviated as HP-SBE-βCD) and SBE_7-βCD were provided