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A new mechanism for increasing the oral bioavailability of scutellarin with Cremophor EL: Activation of MRP3 with concurrent inhibition of MRP2 and BCRP

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ABSTRACT

Efflux transporters are extensively distributed and expressed in the intestinal epithelium and contribute to the low oral bioavailability of flavonoids and flavonoid glucuronides by pumping these compounds back into intestinal lumen. Our previous study has shown the inhibitory effect of Cremophor EL, a non-ionic surfactant, on efflux transporter multidrug resistance-associated protein (MRP) 2. In the current study, by using membranes overexpressing several common ATP-binding cassette (ABC) transporters including P-glycoprotein (P-gp), MRP1, MRP2, MRP3 and breast cancer resistance protein (BCRP), scutellarin, a poorly water-soluble flavonoid, was identified as the substrate of MPR2, MRP3 and BCRP. The effects of Cremophor EL on the transmembrane transportation of scutellarin by MRP2, BCRP, and MRP3 were investigated with inside-out Sf9 vesicles. Results showed that at nontoxic concentrations, Cremophor EL enhanced the transportation of scutellarin by MRP3 and inhibited the efflux transportation of scutellarin by MRP2 and BCRP concurrently. The relations between Cremophor EL and these transporters were explored using MDCK II-MRP2, MDCK II-BCRP, and MDCK II-MRP3 cell models. Compared with the control group, 5 μg/ml Cremophor EL decreased the $Papp_{(BL-AP)}$ of scutellarin in MDCK II-MRP2 cell monolayers by >4 fold (from $13.57 \pm 0.76 \times 10^{-7}$ to $2.90 \pm 0.14 \times 10^{-7}$ cm/s), and the $Papp_{(BL-AP)}$ in MDCK II-BCRP cell monolayers decreased from $9.12 \pm 0.15 \times 10^{-7}$ to $6.34 \pm 0.08 \times 10^{-7}$ cm/s. On MDCK II-MRP3 cell monolayers, 5 μg/ml Cremophor EL increased the $Papp_{(AP-BL)}$ of scutellarin by 3.5 fold (from $7.88 \pm 0.43 \times 10^{-7}$ to $2.79 \pm 1.61 \times 10^{-6}$ cm/s), and caused an over 5-fold increase in $Papp_{(AP-BL)}/Papp_{(BL-AP)}$. These findings suggested that Cremophor EL possesses the potent ability of inhibiting MRP2 and BCRP, as well as activating MRP3 effectively. In vivo pharmacokinetic research in rats further confirmed the improvement of oral absorption of scutellarin by Cremophor EL. In summary, our present study has identified a new mechanism for increasing the oral absorption and bioavailability of poorly absorbed drugs in which Cremophor EL increased MRP3 mediated transport but reduced MRP2 and BCRP mediated efflux concurrently, thereby enhancing the entry of drugs from enterocytes into the blood circulation.

1. Introduction

The efficacy of many orally administered drugs depends on their ability of crossing the intestinal epithelium. Compared with lipophilic drugs that may diffuse freely across the plasma membrane, hydrophilic drugs normally require specific transport mechanisms to facilitate cellular uptake and transcellular transport (Hunter and Hirst, 1997). However, the extent of drugs accumulating within target tissues is generally limited by their tendency to leave cells instead of entering cells (Chan et al., 2004). Efflux proteins, located at the apical membrane, including P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) 2, breast cancer resistance protein (BCRP) in the small intestine, may drive compounds from inside the cell back into the intestinal lumen, preventing their absorption into blood (Fromm et al., 2000; Hunter et al., 1993; Murakami and Takano, 2008). In contrast, MRP3, which is located on the basolateral membrane of enterocytes, facilitates cellular drug efflux into the systemic circulation thereby benefiting oral bioavailability (Kitamura et al., 2008; Kitamura et al., 2010; Rost et al., 2002).

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Flavonoids are generally found at higher concentration in the outer layers of fruits and vegetables (Nemeth and Piskula, 2007), and show a variety of biological activities including anti-cancer, anti-inflammatory, anti-oxidant and anti-viral properties (Amin and Buratovich, 2007; Gonzalez-Gallego et al., 2007; Nijveldt et al., 2001). Typical flavonoids only have oral bioavailability of 10% or less (Hu, 2007; Manach et al., 2005) that is attributed to the two plasma membrane barriers of the intestinal epithelium at the apical and basolateral membranes (Passamonti et al., 2009). Flavonoids are substrates of efflux transporters which cause their poor absorption and diminished tissue distribution (Alvarez et al., 2010). To develop flavonoids as therapeutic agents for oral administration, it is necessary to solve an essential problem, i.e. improving their oral bioavailability by inhibiting efflux transporters in the intestinal epithelium. Scutellarin is an active flavonoid component extracted from *Erigeron breviscapus*, a Chinese species of flowering *Erigeron* plants from the family *Compositae*. It has been clinically used as therapeutic drug by oral, intramuscular and intravenous administration in China for the treatment of cerebral infarction and paralysis induced by cerebrovascular diseases (Gao et al., 2011). Unfortunately, the oral bioavailability of scutellarin is very low (Gao et al., 2011; Hao et al., 2005). Two possible reasons are that the solubility of scutellarin in water is only 14–20 μg/ml (Cao et al., 2006; Lu et al., 2010), and it is too polar to penetrate the intestinal membrane (Li et al., 2013b). Another important reason is its efflux transportation by intestinal transporters, which has been documented in some reports. For example, apical side transporters MRP2 and BCRP might efflux scutellarin absorbed into the epithelial cells back to the intestinal lumen (Cao et al., 2008; Li et al., 2013a). Therefore, the blockage of these transporters could be an effective way of enhancing the oral absorption of scutellarin. Further, MRP3 is extensively expressed on the basolateral membrane of enterocytes in human and rat intestine under physiological conditions. It may play an important role in transporting drugs with poor aqueous solubility and low lipophilicity into the blood circulation (Hirohashi et al., 2000). Therefore, we hypothesized that the activation of MRP3 with concurrent inhibition of MRP2 and BCRP may provide a promising solution for improving the oral absorption of scutellarin thereby increasing its oral bioavailability.

After oral administration, scutellarin is largely hydrolyzed to aglycone which is absorbable in the intestinal tract and then extensively glucuronidated into scutellarin and its other conjugates. In situ rat intestinal infusion with scutellarin or aglycone showed that scutellarin dominated in mesenteric blood with approximately 15-fold concentration higher than its isomeric metabolite iso-scutellarin (scutellariein-6-o-glucuronide). These findings showed that hydrolyzation may enhance entry of scutellarin into the cell since its aglycone permeates easier and thus benefits for intestinal absorption. However, scutellarin in blood decreased sharply from hepatic first-pass elimination which means that minimizing hepatic metabolism is also very important for improving oral bioavailability of scutellarin (Gao et al., 2011).

Many studies have demonstrated that sufactants are versatile tools capable of improving the absorption of transporter substrates by inhibiting efflux transporters, such as P-gp, MRP2 and BCRP (Hanke et al., 2010; Lin et al., 2007; Yamagata et al., 2007). The advantage of using sufactants to improve oral bioavailability is that they are non-absorbable and usually inhibit transporters in the intestinal epithelial cells and will not inhibit other organs expressing transporters (Hanke et al., 2010; Lin et al., 2007). Therefore, sufactants have better safety profiles than those of pharmacological transporter inhibitors. In our previous study, Cremophor EL exhibited the most potent inhibitory effect on MRP2 activity among the excipients studied (Li et al., 2014). It was therefore selected in the current study for assessing its enhancement of the oral bioavailability of scutellarin and underlying mechanisms of this action.

In the present study, changes in ATPase activity of P-gp, MRP1, MPR2, MPR3 and BCRP under different scutellarin concentrations were measured. Further, the effects of Cremophor EL on MRP2, MRP3 and BCRP by both the vesicles transport assay of MRP2, MRP3 and BCRP and the transport study on MDCK II-WT, MDCKII-MRP2, MDCKII-MRP3 and MDCKII-BCRP cell models were investigated. Meanwhile, pharmacokinetic experiments were conducted in rats to verify the ability of Cremophor EL to increase the oral bioavailability of scutellarin.

2. Materials and methods

2.1. Materials

Cremophor® EL was supplied by BASF Wyandotte Corp. (Parsippany, NJ. USA). MK-571 sodium salt hydrate (MK-571) and Ko-143 hydrate (Ko-143) were purchased from Sigma Chemical Corp. (St. Louis, MO, USA) and TOCRIS Bioscience Co. (Ellisville, MO, USA), respectively. Indomethacin (purity > 98%) and scutellarin (purity > 98%, Scu) were obtained from Zizhu Pharmaceutical Corp. (Beijing, China) and the National Institute for Food and Drug Control (Beijing, China), respectively. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), Penicillin-Streptomycin-Glutamine (10,000 IU/ml penicillin and 10,000 μg/ml streptomycin) and 0.25% trypsin-EDTA solution were purchased from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Human P-gp, MRP1, MRP2, MRP3 and BCRP membranes and human MRP2, MRP3 and BCRP vesicles were obtained from BD Bioscience (Mountain View, CA, USA). High performance liquid chromatography (HPLC) grade acetonitrile and formic acid were obtained from Merck Co. Ltd. (Darmstadt, Germany). Transwell permeable supports (12 mm inserts fit in 12 well plates, 3.0 μm polycarbonate membrane) for MDCK II cells monolayer culture were purchased from Corning Costar Corp. (Cambridge, MA, USA). 96-well filter plates (pore size 0.7 μm in Polyethylene Terephthalate filter) for vesicle transport inhibition assay of MRP2 were obtained from Millipore Corp. (Bedford, MA, USA).

2.2. ATPase assay

The purpose of this assay was to measure changes in basal ATPase activity in the presence and absence of scutellarin. The modified assay protocol of Becton Dickinson & Co. was followed as previously described (Li et al., 2014; Wahajuddin et al., 2014) for identifying transporters involved in the transport of scutellarin. All reagents and buffers were supplied by BD Gentest™ ATPase Assay Kit. Eight different concentrations (from 0 to 2.00×10^3 μM) of scutellarin were used as substrates, and the major intestinal transporters, i.e. P-gp, MRP1, MRP2, MRP3 and BCRP membranes were studied. Each assay preincubated in 90 μl assay buffer (Tris-Mes buffer, pH 6.8) consisting of 20 μg membrane and 2.67 mM Mg-ATP with or without scutellarin for 5 min at 37 °C. The reaction was initiated by adding 20 μl of Mg ATP solution (12 mM) and terminated at the designated incubation time (20 min for P-gp, 60 min for MRP1, 40 min for MRP2, 60 min for MRP3 and 10 min for BCRP) with the addition of 30 μl of 10% sodium dodecyl sulfate. To each reaction mixture, 200 μl color reagent (freshly prepared) containing 1.25% ammonium molybdate, 1.25% ascorbic acid and 3.75 mM zinc acetate was used. For each reaction, identical incubations containing 1.4 mM Na_3O_4V (ATPase inhibitor) served as the baseline ATPase activity. The vanadate-sensitive ATP hydrolysis was determined by subtracting the value obtained with the Na_3O_4V co-incubated membrane from Na_3O_4V-free membrane. The amount of released inorganic phosphate in reaction was quantified by the measurement of absorbance at 800 nm using a microplate UV/VIS spectrophotometer (Infinite M200 pro, Tecan Group Ltd., Männedorf, Switzerland). The standard curve of phosphoric acid and the ATPase activity was established. Each sample was analyzed for three times in parallel, and data obtained in ATPase assay were repeated for three times.