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Potential role of ATP-binding cassette transporters in the intestinal transport of rhein

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ABSTRACT

Rhein, a lipophilic anthraquinone, exhibits anti-inflammatory and anti-tumor activities; however, it is hepatotoxic. ATP-binding cassette transporters, including P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance-associated protein 2 (MRP2), can pump toxicants from gut epithelial cells back into the intestinal lumen to prevent poisoning. We investigated their roles in rhein transport using a rat intestinal perfusion model and Caco-2, MDCKII-MDR1 (high expression of P-gp), MDCKII-BCRP (high expression of BCRP) and MDCKII-MRP2 (high expression of MRP2) cell models. The permeability of rhein in the duodenum significantly increased with increasing perfused concentration of rhein in the rat model, suggesting that efflux transporters were involved in rhein transport. In the Caco-2 cells, the permeability of rhein from the basolateral (B) to the apical (A) was significantly higher than that from A to B. In the presence of BCRP or MRP2 inhibitor, the permeability of rhein significantly decreased from B to A direction. In the MDCKII-BCRP cells, rhein was more permeable in B to A side than that in the opposite side. However, no significant differences of rhein permeability were observed in two directions in both MDCKII-MDR1 and MDCKII-MRP2 cells. Taken together, these results suggested that only BCRP was involved in rhein transport.

1. Introduction

Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid), a lipophilic anthraquinone, is one of the most important active components of rhubarb (*Rheum officinale*), which is widely used as a medicinal herb in traditional Chinese medicine. Various studies have demonstrated its numerous pharmacological effects, such as anti-diabetic and anti-inflammatory activities (Bironaite and Öllinger, 1997; Liu et al., 2004). In addition, rhein has been shown to exert anti-tumor activity (Fei et al., 2007; Lin et al., 2003; Sun et al., 2009; Wang, 2008) and to induce apoptosis in human colonic adenocarcinoma monolayer cells by generating nitric oxide *in vitro* as well as in human promyelocytic leukemia cells (HL-60) via an reactive oxygen species – independent mitochondrial death pathway (Lin et al., 2003). Moreover, rhein reportedly can enhance insulin-stimulated glucose uptake in 3T3-L1 adipocytes and decrease triglyceride accumulation (Li et al., 2007).

Nevertheless, the toxicity of rhein has been reported to be associated with redox cycling and addition reactions with nucleophiles (Ollinger and Brunmark, 1991; Powis, 1989). Rhein has been shown to inhibit mitochondrial NADPH dehydrogenase and mitochondrial transhydrogenase (Kean et al., 1971). Research has also shown that rhein involved in the nucleophilic addition of thiols might lead to a depletion of reduced glutathione (Bellomo et al., 1987). The hepatotoxicity of rhein was initially expressed as redox cycling as well as subsequent oxidation of intracellular thiols and increase in intracellular free Ca^{2+} in primary cultures of rat hepatocytes (Bironaite and Ollinger, 1997).

The intestinal transport of rhein is the key to understanding its toxicity and effectiveness. Rhein is the major compound absorbed by the body as determined by human plasma analysis after oral administration of the water extract of Rhei Undulati Rhizoma (167.0 ± 20.5 μg/g rhein). The area under the time–concentration curve and half-life of rhein have been determined to be 9746.68 ± 1011.72 ng h/ml and 3.38 ± 0.35 h, respectively (Lee et al., 2003). ATP-binding cassette (ABC) transporters including

Abbreviations: ABC, ATP-binding cassettet; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; MRP2, multidrug resistance-associated protein 2; B, basolateral; A, apical; MDCKII, Mardin–Darby canine kidney II.

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P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein 2 (MRP2) abundantly located in the apical membrane of the intestinal epithelium, are crucial to limiting toxicant absorption to prevent poisoning. These efflux transporters could pump toxicants from gut epithelial cells back into the intestinal lumen (Hoffmaster et al., 2004; Kim and Benet, 2004). We have previously demonstrated that P-gp, BCRP, and MRP2 are involved in the transport of highly toxic *aconitum* alkaloids. Thus, we deemed it meaningful to determine the potential role of ABC transporters in the intestinal transport of rhein.

Rat intestinal perfusion models and human Caco-2 cell models have been widely used to study intestinal transport (Hu and Borchardt, 1992; Jeong et al., 2005; Liu and Hu, 2002; Nozawa and Imai, 2011). The Mardin–Darby canine kidney II (MDCKII) cell is a dog renal epithelial cell line. In the late 1990s, MDCKII-MDR1, MDCKII-BCRP and MDCKII-MRP2 cell lines have been reported to express high levels of P-gp, BCRP and MRP2 on the apical side of the polarized cell monolayer, respectively. Investigation on these cells could confirm the roles of P-gp, BCRP and MRP2 in the transport of a drug.

Therefore, the aim of this study was to investigate the potential role of ABC transporters in the intestinal transport of rhein using an *in situ* rat intestinal perfusion model and an *in vitro* Caco-2, MDCKII-MDR1, MDCKII-BCRP and MDCKII-MRP2 cell models.

2. Materials and methods

2.1. Materials

Cloned Caco-2 cells (TC7) were a kind gift from Dr. Ming Hu (Department of Pharmaceutical Sciences, College of Pharmacy, University of Houston, USA). Parental MDCKII, MDCKII-MDR1 (high expression of P-gp), MDCKII-BCRP (high expression of BCRP) and MDCKII-MRP2 (high expression of MRP2) cells were provided by The Netherlands Cancer Institute (Amsterdam, The Netherlands). Rhein (purity, ⩾98%; HPLC grade; confirmed by LC/MS) was purchased from Chengdu Mansite Pharmaceutical Company. Chrysazin (purity, ⩾98%; used as an internal standard) was purchased from J&K Scientific Company. MK-571, verapamil, and Ko143 were purchased from Sigma–Aldrich (St. Louis, MO), Zelang Company (Nanjing, China), and Santa Cruz Biotechnology (California, USA), respectively. All other chemicals used were of analytical reagent grade or better and used as received.

2.2. Animals

Male Sprague–Dawley rats (70–110 days old) weighing between 260 and 350 g were obtained from Southern Medical University (Guangzhou, China). The animal experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* by the National Institutes of Health, and the procedures were approved by the ethics committee of Southern Medical University. Rats were individually kept in a controlled environment at 22 °C under a 12-h light/12-h dark cycle. All rats had free access to water and standard diet (from the Experimental Animal Center of Southern Medical University) for 1 week for acclimatization and were fasted with free access to water for at least 12 h before the experiments were performed.

2.2.1. Transport of rhein in the rat intestinal perfusion model

The experiments were performed as in our previous research (Chen et al., 2003; Hu et al., 2003; Jia et al., 2004). Rats were anesthetized with urethane at a dose of 1.5 g/kg by intraperitoneal injection. Four segments of the intestine (i.e., duodenum, jejunum, ileum, and colon; 5–10 cm each) were simultaneously cannulated. The main tube was attached to a syringe driven by an infusion pump (Model PHD2000 Harvard Apparatus, Cambridge, Massachusetts). The inlet cannula was insulated and kept warm in a 37 °C circulating water bath to maintain the temperature of the perfusate. After the surgery, the four segments were perfused with rhein (10 or 40 μM) at the flow rate of 0.17 ml/min. After a washout period, perfusate samples from the four perfused intestinal segments and the control syringe were collected every 30 min (30, 60, 90, 120, and 150 min). After perfusion, the length of each segment was measured and each sample-containing tube was weighed as described previously (Liu and Hu, 2002). The outlet concentrations of rhein in the perfusate were determined by UPLC.

2.3. Cell culture

Caco-2 TC7 cells were routinely maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (HyClone, Logan, UT), 1% nonessential amino acids, 1% L-glutamine (Sigma–Aldrich), and 1% antibiotics (penicillin and streptomycin). Cells were grown in an atmosphere of 5% CO_2 and 90% relative humidity at 37 °C. The cells were seeded on 3-μm porous six-well polycarbonate cell culture inserts (Nalge Nunc International, Naperville, IL) at the seeding density of 400,000 cells/well (4.2 cm^2/monolayer). The medium was routinely replaced with a fresh one every other day. The cells were ready for experimentation 19–22 days after seeding (Hu et al., 2003; Liu and Hu, 2002).

Parental MDCKII, MDCKII-MDR1, MDCKII-BCRP and MDCKII-MRP2 cells were seeded at 2.0 × 106 cells/well and fed daily. The cell monolayers were ready for transport experiments (4–5) day post-seeding.

2.3.1. Transport of rhein in the Caco-2 cell and transfected MDCKII cell models

Transport experiments were performed as described previously (Liu and Hu, 2002). Briefly, prior to experimentation, the integrity of the cell layer was evaluated by measuring its transepithelial electrical resistance (Millicell-ERS), and those with values less than 460 Ω/cm^2 were discarded. Various concentrations (2.5–20 μM) of rhein were loaded on the apical (A) or basolateral (B) side of the Caco-2 cell or transfected MDCKII cell monolayer. Samples (500 μl) were collected from both sides of each transwell at the proper time points (0, 30, 60, 90, and 120 min). The same volume of testing solution or blank HBSS was immediately added to replace the samples obtained. In total, 250 μl of an internal standard solution (50 μM chrysazin) was immediately added to each sample for stabilization. Intracellular concentrations of rhein were determined when the transport experiments were completed. Briefly, the cell membranes were carefully washed thrice with ice-cold blank HBSS (pH 7.4) at the end of the experiments (Hu et al., 2003). Cells attached to the polycarbonate membranes were cut off from the inserts, immersed in blank HBSS (1 ml), and sonicated for 30 min in an ice bath (4 °C). All samples were centrifuged at 13,000 rpm for 30 min before analysis. The concentrations of rhein were measured by UPLC.

2.3.2. Inhibition experiments in Caco-2 cells

The inhibition experiments on Caco-2 permeability were performed as described above but with minor modifications. Ko143 is a highly specific inhibitor of BCRP (Allen et al., 2002), MK-571 is used as a selective inhibitor of MRP (Leier et al., 2000), and verapamil is extensively used as a P-gp inhibitor (Qadir et al., 2005). In the present study, Ko143 (10 μM), MK-571 (50 μM), and verapamil (50 μM) were used to determine the effects of BCRP, MRP2, and P-gp, respectively, on the bidirectional transport of rhein (10 μM). All these inhibitors were added to the A side.

2.4. UPLC analysis of rhein

The conditions used to analyze rhein were as follows: systems, Waters Acquity UPLC (Premier XE, Waters Corp., Milford, MA, USA) with a photodiode array detector and Empower software; column, BEH C18, 1.7 μm, 2.1 × 50 mm; mobile phase B, 100% acetonitrile; mobile phase A, 0.1% formic acid (pH 2.5); flow rate, 0.4 ml/min; gradient, 80% A for 0–0.5 min, 80–50% A for 0.5–2.0 min, 50–20% A for 2.0–3.0 min, and 20–80% A for 3.0–3.5 min; wavelength, 254 nm for rhein and chrysazin; and injection volume, 10 μl.

2.5. Statistical analysis

Data were expressed as mean ± SD. One-way ANOVA with or without Tukey–Kramer multiple comparison (post hoc) was used to evaluate statistical differences. $P < 0.05$ was considered statistically significant.

In the Caco-2 cell and transfected MDCKII cell models, the apparent permeability (P_{app}) of rhein across a cellular membrane was calculated using the following equation (Liu and Hu, 2002):

$$P_{app} = \frac{dQ/dt}{AC_0} \quad (1)$$

where dQ/dt is the steady-state appearance rate of the drug in the receiver side, C_0 is the initial concentration of the drug in the donor side, and A is the monolayer growth surface area (4.2 cm^2).In the intestinal model, effective permeability (P^*_{eff}) was calculated as follows (Liu and Hu, 2002):

$$P^*_{eff} = \frac{1 - C_m/C_o}{4\,Gz} \quad (2)$$

where C_o and C_m are the inlet and outlet concentrations, respectively. $Gz = \pi DL/2Q$ is a scaling factor that incorporates the flow rate (Q), intestinal length (L), and diffusion coefficient (D) to make the permeability dimensionless.