Biochemical Pharmacology 86 (2013) 991–996

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

The anthraquinone drug rhein potently interferes with organic anion transporter-mediated renal elimination

Li Wang [1,*], Xiaolei Pan [1], Douglas H. Sweet

Department of Pharmaceutics, Virginia Commonwealth University, 410 N 12th Street, Richmond, VA 23298-0533, USA

ARTICLE INFO

Article history:
Received 23 July 2013
Accepted 13 August 2013
Available online 22 August 2013

Keywords:
Drug–drug interaction
Nephroprotective
Rhei rhizoma
SLC22A6
SLC22A8
Solute carrier

ABSTRACT

Rhein, a major metabolite of the prodrug diacerein and a major component of the medicinal herb *Rheum* sp., is used for its beneficial effects in a variety of clinical applications including the treatment of osteoarthritis and diabetic nephropathy. The physicochemical properties of rhein are consistent with those of known organic anion transporter (OAT) substrates and inhibitors. Therefore, the inhibitory effect of rhein on human (h) OAT1, hOAT3, hOAT4, and murine (m) Oat1 and mOat3 was examined in heterologous cell lines stably expressing each transporter in isolation. Rhein was shown to potently inhibit hOAT1 and hOAT3, with IC_{50} estimates in the low nanomolar range (IC_{50} = 77.1 ± 5.5 nM and 8.4 ± 2.5 nM, respectively), while poor affinity was observed for hOAT4 (IC_{50} > 100 μM). Marked species differences were observed with hOAT1 and hOAT3 exhibiting 3- and 28-fold higher affinity for rhein as compared to their murine orthologs. The estimated drug–drug interaction (DDI) indices (≫0.1) indicated a very strong potential for clinically relevant, rhein perpetrated DDIs mediated by inhibition of hOAT1 (DDI index = 5.0; 83% inhibition) and/or hOAT3 (DDI index = 46; 98% inhibition) transport activity. These results suggested that rhein, from herbal medicines and/or prodrug conversion, may significantly impact the dosing, efficacy and toxicity (*i.e.*, pharmacokinetics and pharmacodynamics) of co-administered hOAT1 and/or hOAT3 drug substrates.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Great insight has been gained from *in vitro* and *in vivo* studies on drug transporters regarding their role in physiology and biopharmaceutics [1]. These drug transporters are expressed in barrier organs and involved in the interchange (*e.g.* uptake or removal) of endogenous and exogenous substances between cells and biofluids. Among these transporters, organic anion transporters (OATs), which belong to the SLC22 family, interact with anionic compounds [2,3]. Three human (h) OAT paralogs, hOAT1, hOAT3, and hOAT4 have been identified as determinants for tubular secretion and reabsorption [2,3]. Human OAT1 and hOAT3, immunolocalized to the basolateral side of proximal tubules, mediate cellular uptake of negatively charged organic molecules from the blood [2,3]. Human OAT4, which is expressed on the apical side of proximal tubules, appears to mediate reabsorption of organic anions from tubular fluid [2,3]. Such OAT-mediated organic solute flux is vital for maintaining systemic homeostasis and normal renal elimination.

Numerous first-line therapeutics (*e.g.*, antibiotics, ACE inhibitors and NSAIDs) and components of herbal medicines have been identified as substrates and/or inhibitors for OATs [2,3]. These findings demonstrate the impact of OATs on the pharmacokinetic properties of these drugs and provide efficacy and safety information for their clinical application. Interestingly, Oat3 function has been linked to the regulation of blood pressure in mice. Oat3 knockout mice, and wild-type mice treated with potent Oat3 inhibitors, exhibited significantly reduced blood pressure compared to untreated wild-type animals [4]. For diabetic patients, blood pressure is a major determinant of the risk of developing nephropathy [5]. Thus, impairment of OAT function (either through genetics or pharmacology) may influence normal physiological status and result in unexpected drug–drug interactions (DDIs).

The anthraquinone compound rhein is a major component of the medicinal herb *Rheum* sp., which is widely used for its antidotal, anti-inflammatory, antipyretic and laxative properties in Asian countries including China, Korea, and Japan. This medicinal herb is also used to treat diabetic nephropathy [6]. Further, rhein was identified as a major metabolite of diacerein, a prodrug used in the treatment of osteoarthritis [7]. *In vivo*, diacerein is completely

* Corresponding author.
E-mail addresses: wangl4@vcu.edu (L. Wang), panx@vcu.edu (X. Pan), dsweet@vcu.edu (D.H. Sweet).
[1] L. Wang and X. Pan contributed equally to this work.

0006-2952/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2013.08.016

converted to rhein before entering the systemic circulation [8,9]. Rhein is highly protein bound in human plasma (99%) and, after oral administration, the majority of rhein is eliminated in urine as glucuronide conjugates (60%), followed by unchanged form (20%) and sulfate conjugates (20%) [8]. In clinical practice diacerein is utilized in polypharmacy therapies, *e.g.*, a fixed dose of diacerein (50 mg) and aceclofenac (100 mg) has been approved in India to treat osteoarthritis [10]. Recently, a phase II clinical trial was initiated in Thailand to investigate the safety and efficacy of the combined therapy of diacerein and methotrexate to treat early rheumatoid arthritis (http://clinicaltrials.gov/ct2/show/NCT01264211). As methotrexate and a number of NSAIDs are known inhibitors and substrates of OATs [2,3], the interaction of rhein with OAT family members needs to be investigated in order to meaningfully assess the potential for transporter-mediated adverse events.

Based on its chemical structure, rhein, which bears a carboxylic group, has the potential to be a substrate and/or inhibitor for OATs. Because OATs have broad substrate specificity, rhein may cause DDIs with co-administered therapeutics that are OAT substrates. This information may also be useful to elucidate the beneficial effects of *Rheum* sp. on diabetic nephropathy. In the present study, inhibition of hOAT1-mediated *p*-aminohippuric acid (PAH) transport and hOAT3- and hOAT4-mediated estrone sulfate (ES) transport by rhein was explored using stably transfected cell lines. Further kinetic studies were conducted to estimate the half maximal inhibitory concentration (IC_{50}) and inhibitory constant (K_i). In order to investigate potential species differences IC_{50} and K_i estimates also were determined in murine (m) Oat1 and mOat3 expressing cells. The results showed that rhein was a potent inhibitor for hOAT1, hOAT3, and hOAT4. The IC_{50} values for rhein on hOAT1 and hOAT3 were estimated as 77.1 ± 5.5 nM and 8.4 ± 2.5 nM, respectively. However, rhein failed to produce >50% inhibition on hOAT4 transport activity at 100 μM, indicating that the IC_{50} value on hOAT4 was higher than 100 μM. Comparison of estimated IC_{50} values with clinical unbound plasma concentrations indicated the potential for clinically relevant DDIs on hOAT1 and hOAT3 in the kidney. In addition, marked species differences appeared to exist in inhibitory potency, with hOAT1 and hOAT3 exhibiting 3- and 28-fold higher affinity with rhein as compared to their murine orthologs, respectively. Together, these findings suggested that rhein could interfere with hOAT1- and hOAT3-mediated renal elimination *in vivo*, leading to unintended changes in pharmacokinetics, pharmacodynamics, toxicity and the therapeutic effects of *Rheum* sp.

2. Materials and methods

2.1. Chemicals

Rhein (≥98% purity) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Tritiated *p*-aminohippuric acid ([^{3}H]PAH) and estrone sulfate ([^{3}H]ES) were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA) and unlabeled PAH, ES, and probenecid were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Tissue culture

Derivation of stably transfected Chinese hamster ovary (CHO) cells expressing hOAT1 (CHO-hOAT1), hOAT4 (CHO-hOAT4), mOat1 (CHO-mOat1), and mOat3 (CHO-mOat3) as well as stably transfected human embryonic kidney 293 (HEK) cells expressing hOAT3 (HEK-hOAT3), and their corresponding empty vector transfected background control cell lines, has been described previously [11–14]. All cells lines were maintained at 37 °C with 5% CO_2 in medium containing 10% FBS and 1% Pen/Strep. CHO-hOAT1 cells were cultured in phenol red-free RPMI 1640 media (Gibco-Invitrogen, Grand Island, NY) containing 1 mg/mL G418. CHO-hOAT4 cells were cultured in EMEM Alpha Modification media (Sigma–Aldrich (St. Louis, MO) containing 0.5 mg/mL G418. CHO-mOat1 and CHO-mOat3 cells were cultured in DMEM F-12 media (Mediatech, Inc., Herndon, VA) containing 125 μg/mL hygromycin B. HEK cell lines were cultured in DMEM high glucose media (Mediatech, Inc., Herndon, VA) containing 125 μg/mL hygromycin B.

2.3. Cellular uptake assay

The procedure for the cellular uptake assay was adapted from that previously published [15]. Two days before cellular uptake experiment, 2×10^5 cells/well were seeded in 24-well tissue culture plates and grown in the absence of antibiotics. On the day of the experiment, cells were equilibrated in transport buffer at room temperature (22–25 °C) for 10 min [500 μL of Hanks' balanced salt solution containing 10 mM HEPES, pH 7.4]. After equilibration, this solution was replaced with 500 μL of fresh transport buffer containing 1 μM [^{3}H]PAH (0.5 μCi/mL) or [^{3}H]ES (0.25 μCi/mL) with or without inhibitors. At the end of incubation, cells were quickly rinsed three times with ice-cold transport buffer. Then cells were lysed with 1 N NaOH, neutralized with 1 N HCl, and analyzed *via* liquid scintillation counting. The intracellular accumulation of tritiated substrates was reported as picomoles of substrate per milligram total protein. All uptake data were corrected for background accumulation in corresponding empty vector transfected control cells. Substrate concentrations and accumulation times used for kinetic analysis of hOAT1, hOAT3, mOat1, and mOat3 were determined previously [11,12,16,17]. Kinetic calculations were performed using GraphPad Prism Software version 5.0 (GraphPad Software Inc., San Diego, CA). The half maximal inhibitory concentrations (IC_{50}) and inhibitory constants (K_i) were calculated using nonlinear regression with the appropriate model. Results were confirmed by repeating all experiments at least three times with triplicate wells for each data point in every experiment.

2.4. Statistics

Data are reported as mean ± SD or mean ± SEM as indicated. Raw cell line accumulation data are reported as mean ± SEM. Statistical differences were assessed using one-way ANOVA followed by *post hoc* analysis with Dunnett's *t*-test ($\alpha = 0.05$) except for statistical differences between murine and human transporter K_i values which were assessed by two-tailed Student's unpaired *t*-test.

3. Results

3.1. Inhibitory effects of rhein on hOAT1-, hOAT3-, and hOAT4-mediated substrate uptake

Markedly increased cellular accumulation of PAH (7.9 ± 0.1 pmol mg protein^{-1} 10 min^{-1}) was observed in CHO-hOAT1 cells compared to that in the empty vector transfected background control cells (0.3 ± 0.1 pmol mg protein^{-1} 10 min^{-1}), and this hOAT1-mediated cellular uptake was inhibited by probenecid at 1 mM (Fig. 1A). Rhein was assessed for inhibitory effects on CHO-hOAT1 transport activity at 100 μM (Fig. 1A). Under this condition, rhein completely blocked PAH accumulation (>99% inhibition) in CHO-hOAT1 cells. Dose–response studies, applying increasing concentrations of rhein (10^{-9}–10^{-5} M), were performed to determine the IC_{50} value (Fig. 1B and Table 1). The IC_{50} value for rhein on hOAT1 was estimated as 77.1 ± 5.5 nM. Previous studies

Download English Version:

https://daneshyari.com/en/article/5823736

Download Persian Version:

https://daneshyari.com/article/5823736

Daneshyari.com