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Bezafibrate–mizoribine interaction: Involvement of organic anion transporters OAT1 and OAT3 in rats

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ABSTRACT

A patient with rheumatoid arthritis developed rhabdomyolysis while undergoing treatment with mizoribine concomitantly with bezafibrate. The symptoms rapidly disappeared and laboratory test results normalized when she discontinued the two drugs. The purpose of the present study was to elucidate the transporter-mediated molecular pharmacokinetic mechanisms of drug–drug interactions between bezafibrate and mizoribine. Comparing bezafibrate–mizoribine group with bezafibrate group, the T_{max} and C_{max} of bezafibrate were essentially unchanged in rats. The AUC of bezafibrate was significantly increased and $t_{1/2\beta}$ was prolonged markedly with an obviously reduction in plasma clearance and cumulative urinary excretion. The changes were similar to oral studies following intravenous co-administration. In rat kidney slices, the uptake of bezafibrate was markedly inhibited by p-aminohippurate, benzylpenicillin and probenecid but not by tetraethyl ammonium. Mizoribine not only decreased the uptake of bezafibrate, but also inhibited the uptake of p-aminohippurate and benzylpenicillin. The uptakes of bezafibrate and mizoribine were significantly higher compared to vector-HEK293 cells. The uptakes of bezafibrate and mizoribine in highest concentration were increased 1.63 and 1.46 folds in hOAT1-transfected cells, 1.43 and 1.24 folds in hOAT3-transfected cells, respectively. The K_m values of bezafibrate uptake by hOAT1/3hOAT1-/hOAT3-HEK293 K293 cells were increased 1.68 fold in hOAT1-HEK293 cell and 2.12 fold in hOAT3-HEK293 cell in the presence of mizoribine with no change of V_{max}. It indicated that mizoribine could inhibit the uptake of bezafibrate by hOAT1/3-HEK293 cells in a competitive way. In conclusion, OAT1 and OAT3 are the target transporters of drug–drug interactions between bezafibrate and mizoribine in pharmacokinetic aspects.

1. Introduction

Mizoribine (MZR, Fig. 1B), an imidazole nucleoside, inhibits the synthesis of purine (Mizuno et al., 1974; Turka et al., 1991). It is an orally available immunosuppressive agent widely used to prevent the rejection of organ allografts (Cho et al., 2001), as well as for the treatment of rheumatoid arthritis (Takei, 2002), lupus nephritis (Abe et al., 2004), nephrotic syndrome and immunoglobulin A nephropathy (Shibasaki et al., 2004). Bezafibrate (BZF, Fig. 1A), a representative fibric acid analogue, is relatively well tolerated at the usual dosage with a wide application in the treatment of hypertriglycemia. However, a patient with rheumatoid arthritis and angina pectoris resulted in rhabdomyolysis while treated with mizoribine and bezafibrate together. It indicated that the co-administration of bezafibrate and mizoribine might lead to the drug–drug interactions (DDIs) during the therapy (Morimoto et al., 2005). The symptoms rapidly resolved and laboratory test results normalized when she discontinued these two drugs.

Rheumatoid arthritis is a chronic inflammatory disease primarily targeting the synovial membrane of the joints (Feely et al., 2009). Aged patients with rheumatoid arthritis are at risk of DDIs because of the multiple co-medications that might be taken as a consequence of the co-morbid conditions (Feely et al., 2009; van Roon et al., 2009). Despite some beneficial effects of the interactions (Cundy et al., 1995), accumulating evidences suggested that the DDIs could lead to more deleterious consequences such as toxicity or lack of efficacy for the substrate (Alsheikh-Ali et al., 2004; Endres et al., 2006). Recently, the assessment of carrier-mediated transport of compounds has become increasingly important to help predict the potential relevance of transporters mediated drug disposition and DDIs (Yamazaki et al., 2005).

Mizoribine, a water soluble hydrophilic compound, is a renal excretion-type drug that needs to penetrate lipoidal biomembranes

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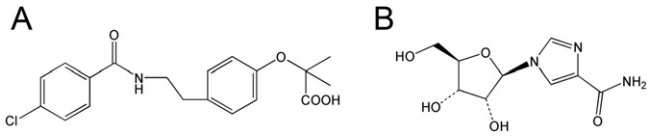

Fig. 1. Chemical structures of bezafibrate (A) and mizoribine (B).

via some specific transport system (Honda et al., 2006). Mizoribine did not appear to be hepatically metabolized (Akiyama et al., 1982; Takada et al., 1983). Most of the oral doses were excreted unchanged in the urine in both the single- and multiple-dose studies. It has been reported that the secretion of MZR is influenced by probenecid which is a uricosuric drug in clinical (Utsunomiya et al., 2010). Probenecid, a potent inhibitor of organic anion transporters (OATs), has been used to characterize the urinary excretion mechanisms of drugs because of its potential to inhibit the renal tubular secretion of concomitantly administered anionic agents by inhibiting organic anion transport system (Takeda et al., 2001).

OATs are uptake transporters that locate in proximal tubular basolateral membrane in kidney. They play crucial roles in mediating the urinary excretion of various endogenous compounds, toxins and clinically important drugs into urine for excretion such as β-Lactam antibiotics, antivirals, antihypertensive drugs and uricosuric drugs (Huo et al., 2014; Wang et al., 2014; Xu et al., 2013; You, 2004). OAT1 and OAT3 are considered to be the major transporters among OATs for their broad substrate specificities (Maeda et al., 2014). The expression level of hOAT3 mRNA is the highest among the organic ion transporter family, followed by that of hOAT1 mRNA (Motohashi et al., 2002). Previous reports demonstrated that the uptake process mediated by OATs was the rate-determining process in overall tubular secretion of anionic drugs in the kidney (Watanabe et al., 2009). Moreover, OATs can be the site of DDIs during the competition of two or more drugs for the same transporter, and mediate cell damage by transporting cytotoxic compounds (Rizwan and Burckhardt, 2007).

In addition to all of the above, it has been reported that bezafibrate could inhibit the uptake of PAH by hOAT1-expressing cells (Sugawara et al., 2005) and showed similar effect on hOAT3 (Munns, 2009). However, whether it is a substrate or just an inhibitor which could interact with OATs has not been further discussed. Based on these data, we assumed that bezafibrate and mizoribine were involved in the urinary secretion system via OATs and the excretion of bezafibrate could be altered in the kidneys by co-administration of mizoribine. On the other hand, it is widely known that the expression and function of transporters can be modulated by many factors including kinase signaling pathways and sex hormones (Burckhardt and Burckhardt, 2011; Kittayaruksakul et al., 2012; Soodvilai et al., 2004). The change of transporter activity may influence renal clearance of both toxic and therapeutic xenobiotics (Soodvilai et al., 2004). It has been reported that fenofibrate could decrease the activity of OCT2 by reducing the number of functional transporters on the membrane, and DDI needs to be monitored when patients receive polypharmacy containing fenofibrate (Asavapanumas et al., 2012). Therefore, another important goal of this research is to explore whether bezafibrate has the similar effect on the expression of OAT1/3. Taken together, the present study aims to elucidate the transporter-mediated molecular pharmacokinetic mechanisms of DDIs between bezafibrate and mizoribine.

2. Material and methods

2.1. Chemicals

Bezafibrate and mizoribine were purchased from Dalian Meilun Biology Technology Co., Ltd., China. p-Aminohippurate (PAH), benzylpenicillin (PCG), probenecid, and tetraethyl ammonium (TEA) were purchased from Sigma-Aldrich (St. Louis, MO). Clofibric acid and thiamphenicol were purchased by Guangzhou Baiyunshan Pharmaceutical Co., Ltd., China. Methanol and formic acid (Tedia, Fairfield, OH, USA) of HPLC grade were used throughout the study. All other chemicals and reagents are of analytical grade and are commercially available.

2.2. Animals

Male Wistar rats weighing about 200 to 220 g were purchased from the Experimental Animal Center of Dalian Medical University (Dalian, China; permit number SCXK 2008-0002) for pharmacokinetic studies. Rats were housed in a temperature- and humidity-controlled room with free access to water and standard rat chow. Rats were fasted overnight with water available before surgery and anesthetized with pentobarbital (60 mg/kg, intraperitoneal) at the beginning of each experiment. All animal experiments were performed in strict adherence to local institutional guidelines.

2.3. Pharmacokinetic interaction in rats

Rats were divided randomly into two groups in the pharmacokinetic interaction studies: control group (bezafibrate, 20 mg/kg) and experimental group (bezafibrate, 20 mg/kg + mizoribine, 15 mg/kg). There were 4 rats in each group.

In the absorption studies, rats received a p.o. administration of bezafibrate (20 mg/kg) and/or mizoribine (15 mg/kg) dissolved in 75 mM NaOH solution from a gavage needle. Serial blood samples (200 μL) were collected from the jugular vein with heparinized syringes at designated time points (0, 15, 30, 60, 90, 120, 150, 180, 210, 240, 360, 480, 600, 720 and 1440 min).

In the renal excretion studies, rats received an i.v. administration of bezafibrate (20 mg/kg) and/or mizoribine (15 mg/kg) dissolved in 75 mM NaOH solution via the jugular vein (Agrawal et al., 1998). Serial blood samples (200 μL) were collected from the jugular vein with heparinized syringes at designated time points (0, 1, 5, 10, 20, 30, 60, 120, 240, 360, 480, 600 and 720 min).

Bladders were cannulated with polyethylene tubing for urine collection at 2, 4, 6, 8, 10, 12, and 24 h following administration. The blood samples were transferred to heparin-coated polypropylene tubes immediately after collecting and centrifuged (1000 g, 4 °C) for 10 min to obtain plasma. Then isotonic saline solution (200 μL) was injected to each blood sample collection. Plasma and urine samples were stored at −20 °C until analytical determination as described below.

2.4. Renal slice preparation and uptake study

This study protocol was approved by the Ethics Review Boards at Dalian Medical University, Dalian, China. All participants provided written informed consent. Intact human renal cortical tissues were obtained from surgically nephrectomized patients with renal cell carcinoma at the Second Hospital of Dalian Medical University.

Rats' and human's kidney cortical tissues were cut into slices as previously described (Wang et al., 2014; Zhang et al., 2010). The slices were kept in oxygenated (O_2/CO_2, 95%:5%) ice-cold Krebs-bicarbonate slicing buffer before use. After a pre-incubation for 3 min with oxygenated buffer at 37 °C in 6-well culture plates, the kidney slices were immediately transferred to 24-well culture plates containing 1 mL fresh oxygenated buffer with bezafibrate (20 μM) or bezafibrate and mizoribine (10 μM) for a further incubation at 37 °C or 4 °C with gentle shaking. In the inhibition assay, probenecid (100 μM), PAH (100 μM), PCG (100 μM) and TEA (100 μM) were added into buffer at the same time. The same procedures were performed to evaluate the effect of mizoribine on the uptake of PAH and PCG. After incubating for the designated times, the uptake was terminated by removal of the incubation buffer. The kidney slices were washed three times with 1 mL of ice-cold Hanks' balanced salt solution (HBSS) (pH 7.5), then blotted on filter