Short Communication

Hepatocellular Disposition and Transporter Interactions with Tolvaptan and Metabolites in Sandwich-Cultured Human Hepatocytes

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ABSTRACT

Tolvaptan is a selective V2-receptor antagonist primarily metabolized by CYP 3A. The present study investigated the hepatocellular disposition of tolvaptan and the generated tolvaptan metabolites, DM-4103 and DM-4107, as well as the potential for drug-drug interactions (DDIs) with metabolic and transport proteins in sandwich-cultured human hepatocytes (SCHH). Tolvaptan was incubated with SCHH and quantified by liquid chromatography-tandem mass spectrometry. Pioglitazone, verapamil, MK-571, and elacridar were used as inhibitors to investigate mechanisms of transport and metabolism of tolvaptan and metabolites. Taurocholate (TCA), pravastatin, digoxin, and metformin were used as transporter probes to investigate which transport proteins were inhibited by tolvaptan and metabolites. Cellular accumulation of tolvaptan (0.15–50 μM), DM-4103, and DM-4107 in SCHH was concentration-dependent. Tolvaptan accumulation (15 μM) in SCHH was not altered markedly by 50 μM pioglitazone, verapamil, MK-571, or 10 μM elacridar. Coincubation of tolvaptan with pioglitazone, verapamil, MK-571, and elacridar reduced DM-4107 accumulation by 45.6, 79.8, 94.5, and 23.0%, respectively, relative to control. Coincubation with increasing tolvaptan concentrations (0.15–50 μM) decreased TCA (2.5 μM) cell bile accumulation and the TCA biliary excretion index (BEI; from 76% to 51%), consistent with inhibition of the bile salt export pump (BSEP). Tolvaptan (15 μM) had no effect on the cellular accumulation of 2.5 μM pravastatin or metformin. Digoxin cellular accumulation increased, and the BEI of digoxin decreased from 23.9 to 8.1% in the presence of 15 μM tolvaptan, consistent with inhibition of P-glycoprotein. In summary, SCHH studies revealed potential metabolic- and transporter-mediated DDIs involving tolvaptan and metabolites.

Introduction

Tolvaptan is an orally available selective V2-receptor antagonist used to treat hypervolemic and euvolemic hyponatremia in patients with heart failure and refractory ascites in cirrhosis (Berl et al., 2010; Sakaida, 2014). After oral administration, tolvaptan was absorbed readily from the gastrointestinal tract with an absolute bioavailability of ~50% after a 30-mg dose, and was metabolized extensively, with ~1% of the dose excreted in the urine unchanged (Shoaf et al., 2007, 2012a). CYP3A is the main enzyme involved in tolvaptan metabolism, primarily forming dehydrogenated and hydroxylated metabolites (Shoaf et al., 2012b). DM-4103 and DM-4107 are two major metabolites of tolvaptan primarily excreted in urine and feces, respectively (Tammara et al., 1999). When [14C]tolvaptan was administered orally to rats, biliary excretion was a predominant route of elimination for tolvaptan and metabolites (Furukawa et al., 2011). Hepatocyte cultures preserve whole cellular architecture and function and have been useful for understanding and estimating metabolic clearance and hepatocellular transport (Chiba et al., 2009). In particular, sandwich-cultured human hepatocytes (SCHH) have become a prominent tool to evaluate hepatobiliary drug disposition owing to their ability to retain in vivo-like metabolic and excretory properties, some of which are not preserved using conventional culture conditions (Swift et al., 2010). Transport proteins, including basolateral and biliary efflux transporters, have been shown to properly localize on the basolateral and canalicular domains of SCHH; this allows for accurate correlation between in vitro intrinsic biliary clearance and in vivo biliary clearance (Liu et al., 1999; Swift et al., 2010). Despite research characterizing the metabolism of tolvaptan, the hepatobiliary disposition and potential interactions with hepatic transporters have not been fully explored. Previous studies demonstrated that tolvaptan is a substrate and an inhibitor of P-glycoprotein (P-gp) (Shoaf et al., 2011). Our recent studies in membrane vesicles and transfected cells revealed that tolvaptan and metabolites could inhibit several transporters, including sodium taurocholate (TCA) cotransporting polypeptide (NTCP), the bile salt export pump (BSEP), and multidrug resistance-associated protein (MRP). The present study investigated the hepatocellular disposition of tolvaptan and the generated tolvaptan metabolites, DM-4103 and DM-4107, as well as the potential for drug-drug interactions (DDIs) with metabolic and transport proteins in sandwich-cultured human hepatocytes (SCHH). Tolvaptan was incubated with SCHH and quantified by liquid chromatography-tandem mass spectrometry. Pioglitazone, verapamil, MK-571, and elacridar were used as inhibitors to investigate mechanisms of transport and metabolism of tolvaptan and metabolites. Taurocholate (TCA), pravastatin, digoxin, and metformin were used as transporter probes to investigate which transport proteins were inhibited by tolvaptan and metabolites. Cellular accumulation of tolvaptan (0.15–50 μM), DM-4103, and DM-4107 in SCHH was concentration-dependent. Tolvaptan accumulation (15 μM) in SCHH was not altered markedly by 50 μM pioglitazone, verapamil, MK-571, or 10 μM elacridar. Coincubation of tolvaptan with pioglitazone, verapamil, MK-571, and elacridar reduced DM-4107 accumulation by 45.6, 79.8, 94.5, and 23.0%, respectively, relative to control. Coincubation with increasing tolvaptan concentrations (0.15–50 μM) decreased TCA (2.5 μM) cell bile accumulation and the TCA biliary excretion index (BEI; from 76% to 51%), consistent with inhibition of the bile salt export pump (BSEP). Tolvaptan (15 μM) had no effect on the cellular accumulation of 2.5 μM pravastatin or metformin. Digoxin cellular accumulation increased, and the BEI of digoxin decreased from 23.9 to 8.1% in the presence of 15 μM tolvaptan, consistent with inhibition of P-glycoprotein.
(BSEP), the multidrug resistance–associated protein 2 (MRP2), MRP3, and MRP4 (Slizgi et al., 2016). However, the role of hepatic transporters in the disposition of tolvaptan and metabolites has not been identified in hepatocytes under physiologic conditions. The ability to accurately predict the potential for transporter-mediated drug-drug interactions (DDIs) of tolvaptan and metabolites at the cellular level solely on the basis of membrane vesicle data remains to be determined. The purpose of this study was to investigate the hepatobiliary disposition of tolvaptan and generated metabolites, and possible DDIs with hepatic metabolic and transport proteins using SCHH.

Materials and Methods

Materials. Tolvaptan, DM-4103, and DM-4107 (purity > 99%) were provided by Otsuka Pharmaceutical Co. (Tokyo, Japan). Stably labeled TCA (d8-TCA) was purchased from Martex Inc. (Minnetonka, MN). Elacridar, digoxin, metformin, MK-571, and pravastatin were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals were of analytical grade.

Cell Culture and Transport Studies in SCHH. Transporter-Certified sandwich-cultured human hepatocytes (SCHH) were purchased from Triangle Research Laboratories (lot no. HUM4059; Caucasian female; age = 17 year; body mass index = 25.5; no tobacco, alcohol, or drug use; see Supplemental Data) and prepared by Qualyst Transporter Solutions (Durham, NC) using cryopreserved hepatocytes as described by Slizgi et al. (2016). On day 5 of culture, the media was aspirated and the SCHH were rinsed with 0.5 ml well of standard or Ca2+-free Hank’s balanced salt solution (HBSS). After the second rinse, HBSS buffers were aspirated completely and SCHH were incubated with 0.5 ml of standard or Ca2+-free HBSS buffers for 10 minutes. After 10 minutes, HBSS buffers were aspirated and the cells were incubated with standard HBSS buffer containing tolvaptan (0.15–50 µM) and 4% bovine serum albumin (BSA) for 10 minutes. For studies investigating possible DDIs with tolvaptan and drug-metabolizing enzymes and transporters, tolvaptan (15 µM) was incubated with pioglitazone (50 µM), verapamil (50 µM), MK-571 (50 µM), or elacridar (10 µM). For studies investigating possible DDIs of tolvaptan with hepatic transporters, tolvaptan (15 µM) was incubated with d8-TCA (2.5 µM), pravastatin (2.5 µM), digoxin (2.5 µM), or metformin (2.5 µM). Dimethyl sulfoxide was used to solubilize all compounds; the final dimethyl sulfoxide concentration was 0.2% v/v. After incubation, the HBSS buffer was aspirated from all wells and each well was rinsed three times with ice-cold standard HBSS buffer. Plates were sealed and stored at –80°C until analysis.

Sample Preparation and Analysis. Cell samples from each well were lysed with 500 µl 70% methanol containing internal standard, placed on a shaker for 15 minutes, filtered through a Whatman 96-well Unifilter 25-m filter plate and 5-mL70% methanol containing internal standard, placed on a shaker for 15 minutes. For studies investigating possible DDIs of tolvaptan with hepatic transporters, tolvaptan (15 µM) was incubated with d8-TCA (2.5 µM), pravastatin (2.5 µM), digoxin (2.5 µM), or metformin (2.5 µM). Dimethyl sulfoxide was used to solubilize all compounds; the final dimethyl sulfoxide concentration was 0.2% v/v. After incubation, the HBSS buffer was aspirated from all wells and each well was rinsed three times with ice-cold standard HBSS buffer. Plates were sealed and stored at –80°C until analysis.

Data Analysis. Protein content was determined by Pierce BCA protein assay kit according to the manufacturer’s protocol (ThermoScientific, Rockford, IL). Cellular accumulation determined in Ca2+-free HBSS (Accumulation standard HBSS − Accumulation Ca2+-free HBSS) represents the total mass of analyte in the hepatocyte at the end of the incubation time period. Total accumulation determined in standard HBSS (Accumulation standard HBSS) represents the total mass of compound taken up and excreted (cells+bile). The biliary excretion index (BEI) was calculated using the following equation as reported previously by our laboratory (Liu et al., 1999):

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\text{BEI} = \frac{\text{Accumulation standard HBSS} - \text{Accumulation Ca}^{2+}-\text{free HBSS}}{\text{Accumulation standard HBSS}} \times 100
\]

The intracellular concentration of each test compound was calculated by dividing the mass of compound in cells (Accumulation standard HBSS) by the intracellular fluid volume for human hepatocytes (7.6 µl/mg protein; Qualyst Transporter Solutions Technical Application Bulletin, 2011).

Results and Discussion

Accumulation of Tolvaptan and Metabolites in SCHH. The accumulation of tolvaptan, DM-4103, and DM-4107 was investigated in SCHH. Accumulation of tolvaptan was concentration-dependent after a 10-minute incubation over the tolvaptan concentration range of 0.15–50 µM (Fig. 1A). The total (cells+bile) accumulation ranged from ~13 to 3900 pmol/mg protein, whereas the biliary excretion of tolvaptan was negligible (Fig. 1A). Metabolism of tolvaptan to DM-4103 and DM-4107 was observed at tolvaptan incubation concentrations greater than 15 µM and 1.5 µM, respectively (Fig. 1B). Cellular concentrations of tolvaptan, DM-4103, and DM-4107 also showed a concentration-dependent increase after a 10-minute incubation of 0.15–50 µM tolvaptan (Fig. 1B). The total cellular concentration of tolvaptan ranged from ~2 µM to 500 µM and was greater than the respective incubation concentration (0.15–50 µM). DM-4103 could not be detected at low tolvaptan incubation concentrations (0.15–1.5 µM). At higher tolvaptan incubation concentrations (15–50 µM), cellular concentrations of DM-4103 ranged from ~0.16 to 0.19 µM. Concentrations of DM-4107 were higher than DM-4103 and
accumulation was not markedly altered by 50 μM tolvaptan (IC50 values: 12.3, 20, 11.3, and 4.9 μM, respectively) (Achira et al., 1999; Sahi et al., 2003; Englund et al., 2014). Tolvaptan and DM-4103 were evaluated in SCHH in the presence and absence of various inhibitors. For these studies, a tolvaptan concentration of 15 μM was incubated with probes for 10 minutes. Pioglitazone, verapamil, MK-571, and elacridar were selected as inhibitors of canalicular transporters (e.g., P-gp, MRP2, BCRP) (Achira et al., 1999; Slizgi et al., 2016). These compounds also inhibit CYP3A (IC50: 12.3, 20, 11.3, and 4.9 μM, respectively) (Achira et al., 1999; Sahi et al., 2003; Englund et al., 2014). Tolvaptan accumulation was not markedly altered by 50 μM pioglitazone, verapamil, MK-571, or 10 μM elacridar (data not shown), consistent with the finding that the biliary excretion of tolvaptan was negligible in the present study design. Pioglitazone, verapamil, MK-571, and elacridar reduced mean DM-4107 accumulation by 45.6, 79.8, 94.5, and 23.0% relative to control, respectively (Fig. 2), consistent with CYP3A inhibition. DM-4103 concentrations were below the limit of detection when tolvaptan was coincubated with pioglitazone, verapamil, MK-571 and elacridar, probably due to reduced tolvaptan metabolism.

Effect of Tolvaptan on the Disposition of Transporter Probes. The effects of tolvaptan coincubation for 10 minutes on the hepatobiliary disposition of select transporter substrates in SCHH were evaluated. TCA cell+bile accumulation and BEI were decreased as tolvaptan disposition of select transporter substrates in SCHH were increased (Fig. 3A). These data are consistent with recent findings that tolvaptan and DM-4103 can inhibit BSEP transport in membrane vesicles from Sf9 insect cells expressing human BSEP. Although the cellular accumulation of tolvaptan is high when 15 μM tolvaptan is incubated with SCHH for 10 minutes in the presence of 4% BSA in the incubation medium (Fig. 1), the cellular unbound concentration of tolvaptan should be less than the reported IC50 values for BSEP inhibition considering that the protein binding of tolvaptan is 98.5% in human plasma (Furukawa et al., 2011). On the basis of these data, tolvaptan-mediated BSEP inhibition should be low at typical clinical tolvaptan doses in patients with normal bile acid homeostasis. However, in patients with disease or polymorphisms that disrupt bile acid homeostasis, BSEP inhibition at very high clinical doses of tolvaptan may result in an adverse outcome.

Although tolvaptan can inhibit NTCP-mediated TCA uptake at high concentrations, unbound tolvaptan concentrations in the present studies ranged from ~0.15 to 4.26 μM at tolvaptan incubation concentrations of 1.5 to 50 μM, respectively, after a 10-minute incubation (Fig. 1B).

Effect of Inhibitors on the Disposition of Tolvaptan and Metabolites. The hepatobiliary disposition of tolvaptan, DM-4103, and DM-4107 was evaluated in SCHH in the presence and absence of various inhibitors. For these studies, a tolvaptan concentration of 15 μM and incubation time of 10 minutes were selected. Pioglitazone, verapamil, MK-571, and elacridar at the concentrations tested were selected as inhibitors of canalicular transporters (e.g., P-gp, MRP2, BCRP) (Achira et al., 1999; Englund et al., 2014). These compounds also inhibit CYP3A (IC50: 12.3, 20, 11.3, and 4.9 μM, respectively) (Achira et al., 1999; Sahi et al., 2003; Englund et al., 2014). Tolvaptan accumulation was not markedly altered by 50 μM pioglitazone, verapamil, MK-571, or 10 μM elacridar (data not shown), consistent with the finding that the biliary excretion of tolvaptan was negligible in the present study design. Pioglitazone, verapamil, MK-571, and elacridar reduced mean DM-4107 accumulation by 45.6, 79.8, 94.5, and 23.0% relative to control, respectively (Fig. 2), consistent with CYP3A inhibition. DM-4103 concentrations were below the limit of detection when tolvaptan was coincubated with pioglitazone, verapamil, MK-571 and elacridar, probably due to reduced tolvaptan metabolism.
Authorship Contributions

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References


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