Note

More Relevant Prediction for In Vivo Drug Interaction of Candesartan Cilexetil on Hepatic Bile Acid Transporter BSEP Using Sandwich-cultured Hepatocytes

Hajime Fukuda, Takeo Nakanishi and Ikumi Tamai*

Department of Membrane Transport and Biopharmaceutics, Faculty of Pharmaceutical Sciences, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan

Summary: Bile salt export pump (BSEP) plays a major role in biliary secretion of bile salts; therefore, drug-induced cholestasis could occur because of BSEP inhibition by drugs. Drug interaction on hepatic bile canalicular transporters such as BSEP with prodrugs that are rapidly metabolized has not been evaluated well. In the present study, candesartan cilexetil (CIL) was used as a model compound and its inhibitory potential against BSEP was determined in sandwich-cultured human hepatocytes (hSCH) as well as in BSEP-expressing membrane vesicles. CIL exhibited potent BSEP inhibition with an IC50 value of 6.2 µM in the transport assay using membrane vesicles. In contrast, BSEP inhibition by CIL was not observed in hSCH after 120 min exposure. This discordance is possibly explained by metabolic elimination of CIL in hSCH because BSEP inhibition became reversely pronounced under the conditions where CIL metabolism was suppressed by diisopropyl fluorophosphates. The results observed in hSCH are consistent with the fact that liver dysfunction or jaundice occurs with low frequency in clinical use of CIL, which may not be obtained by membrane vesicle study on the effect of CIL on BSEP.

Keywords: BSEP inhibition; cholestasis; sandwich-cultured human hepatocytes; candesartan cilexetil; biliary excretion index; vesicular transport assay

Introduction

Bile salt export pump (BSEP/ABCB11) is an efflux transporter expressed in the bile canalicular membranes of hepatocytes, and plays an important role in secretion of conjugated/unconjugated bile salts. Loss-of-function mutations in BSEP are associated with progressive familial intrahepatic cholestasis type 2 that likely progresses to cirrhosis; therefore, impaired BSEP function by drugs may involve accumulation of cytoxic bile salts in cholestatic liver injury. Indeed, troglitazone and nefazodone, which inhibit BSEP-mediated transport, have been withdrawn from the market due to severe liver injury. Thus, it becomes widely accepted that BSEP is a pharmacologically significant target to avoid drug-induced liver injury for drug candidates. To date, several experimental methods have been established to evaluate interaction of drugs with BSEP. Among them, transport assay using membrane vesicles prepared from BSEP over-expressing cells is a common technique; however, this method does not take account of biological processes such as drug metabolism and the sinusoidal membrane transport process. For instance, since troglitazone sulfate was reported to be a more potent BSEP inhibitor than its parent form, a drug inhibition profile predicting for BSEP-mediated bile salt transport can lead to a high false negative rate. From this point of view, we recently developed a preferable assay system designated as the quantitative time lapse imaging (QTLI) method using sandwich-cultured hepatocytes (SCHs), where both metabolic enzyme and sinusoidal uptake transporter activities are reflected to transporter-based drug-drug interaction at the bile canalicular membrane.

In the present study, candesartan cilexetil (CIL) was selected as a test compound that is rapidly hydrolyzed to candesartan (CAN) by esterases in the gastrointestinal tract or liver, and their inhibitory potential against BSEP in human SCH (hSCHs) as well as BSEP-expressing membrane vesicles can be determined in order to predict the effect of CIL metabolism on cholestatic liver disease.

Materials and Methods

Chemicals: [3H]Taurocholic acid ([3H]TA, 50 Ci/mmol) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). InVitroGro® CP media, CM4000, Matrigel®, diisopropyl
uptake was determined by quantifying radioactivity. The IC50 values of 0.25 mg/mL Matrigel protein content and the reported cell volume (7.69 µL/mg protein). In Intracellular concentration of the test compound was normalized by increased to 90% over 15 min at a by volume) and then the proportion of acetonitrile was linearly UV detector (G1315B, Agilent Technologies). The mobile phase gies, Palo Alto, CA) with a Symmetry C18 column (2.1 microsomes. CIL and CAN were separated by high perform- Liquid chromatography (HP1100 system, Agilent Technolo- microsomes,8) correspondingly. The CIL metabolism was almost completely metabolized in hSCHs. In order to determine the influence of the metabolism of CIL, the following experiments were carried out at 120 min. Since DFP, an irreversible cholinesterase inhibitor, was reported to inhibit metabolism of CIL in human liver microsomes,5 its effect on the intracellular amount of CIL and CAN was evaluated in hSCHs (Fig. 1B). Intracellular CIL increased depend- ing on DFP concentration for 120 min, whereas CAN decreased correspondingly. The CIL metabolism was almost completely inhibited by 10 µM of DFP as reported previously in human liver microsomes.5) Effect of CIL exposure on [3H]TA accumulation in bile canaliculari in hSCHs: To assess effect of CIL on BSEP-mediated [3H]TA transport in hepatocytes, hSCHs were exposed to CIL (10 µM) with or without DFP (1 or 10 µM) for 120 min before the initiation of uptake of [3H]TA. The BEI of [3H]TA in a CIL-treated hepatocytes was 62.7%, which is comparable to that in vehicle- treated hepatocytes (66.5%), indicating that BSEP inhibition by CIL was not observed in hSCHs even after 120 min exposure despite its inhibitory potential against BSEP, verified by the membrane vesicle assay (Fig. 2). This discordance is possibly explained.

**Results and Discussion**

Effect of CIL and CAN on BSEP-mediated [3H]TA transport: CIL and CAN were evaluated for their inhibitory effect on BSEP-mediated [3H]TA transport in hBSEP-expressing membrane vesicles (Supplemental Fig. S1). Both CIL and CAN inhibited the ATP-dependent uptake of [3H]TA in concentration-dependent manners with IC50 values of 6.2 ± 1.2 µM and 70.5 ± 2.4 µM, respectively (means ± S.E.M.), demonstrating that CIL is ten times more potent than CAN.

**Metabolic profiles of CIL in hSCHs:** The total amount of CIL recovered from cells and medium is shown in Figure 1A. The amount time-dependently decreased to approximately 10% of the initial amount at 120 min, suggesting that most of CIL was metabolized in hSCHs. In order to determine the influence of the metabolism of CIL, the following experiments were carried out at 120 min. Since DFP, an irreversible cholinesterase inhibitor, was reported to inhibit metabolism of CIL in human liver microsomes,5 its effect on the intracellular amount of CIL and CAN was evaluated in hSCHs (Fig. 1B). Intracellular CIL increased depend- ing on DFP concentration for 120 min, whereas CAN decreased correspondingly. The CIL metabolism was almost completely inhibited by 10 µM of DFP as reported previously in human liver microsomes.5)

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**Data analysis:** Biliary excretion was quantitatively assessed as a biliary excretion index (BEI; %). The BEI was calculated by using B-CLEAR® technology (Qualyst, Inc., Research Triangle Park, NC) based on the following equation:

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\text{BEI} = \frac{\text{uptake (Ca}^{2+}/\text{Mg}^{2+} \text{ presence) − uptake (Ca}^{2+}/\text{Mg}^{2+} \text{ absence)}}{\text{uptake (Ca}^{2+}/\text{Mg}^{2+} \text{ presence)}} \times 100
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**Fig. 1. Metabolic profiles of CIL in hSCHs**

(A) Time profiles of a total amount of CIL (cells + medium) in hSCHs. Thirty µM of CIL was incubated with hSCHs up to 240 min. The amount was shown as percent of initial. (B) Effect of DFP on intracellular accumulation of CIL (black bars) and CAN (white bars) in hSCHs. Thirty µM of CIL was incubated with hSCHs in the presence of DFP (0.1, 1, 10 µM) for 120 min. Data represent means of duplicate determinations from one donor (lot. HC2-8) as a representative result of two donors (lot. HC2-8 and 582).
by the metabolic elimination of CIL in hSCHs. Next, the effect of CIL on the BEI was evaluated under the conditions where CIL metabolism was inhibited by DFP. The BEI was significantly decreased in a combination use of CIL with DFP, compared with CIL alone. Besides, the observed decrease was dependent on DFP concentrations. Since no influence of DFP was detected in the BEI of [3H]TA, impaired metabolic conversion of CIL to CAN may result in significant inhibition of BSEP. Estimated intracellular concentration of CIL was much higher than its IC50 value for BSEP-mediated TA transport measured in the transport assay using membrane vesicles, whereas CAN concentration was reasonably lower than the IC50 value. However, we were unable to estimate intracellular concentration of free CIL because tissue binding of CIL was not determined in the present study. The extent of binding to hepatocytes or intracellular microsomes of drugs is highly correlated with their lipophilicity, and the calculated log D7.4 (ACD/Log D Suite, Advanced Chemistry Development, Inc., Toronto, Canada) of CIL is as high as 5.82, implying that intracellular concentration of free CIL would be much lower than the concentration actually measured in hSCHs. For a more accurate and quantitative analysis of relationship between intracellular CIL and BSEP-mediated transport, free concentration of CIL should be determined in a future study. In the uptake study, sinusoidal uptake of [3H]TA decreased in the presence of CIL alone, and was further reduced in the presence of both CIL and DFP, while the reduction was not observed in the presence of DFP alone. Additionally, sinusoidal uptake of [3H]TA was almost completely inhibited in the presence of 30 µM of CIL and DFP (data not shown). These results suggest that increased CIL by DFP is associated with inhibition of uptake transports for [3H]TA such as Na+-taurocholate cotransporting polypeptide (NTCP). The potent inhibition by test compounds on sinusoidal uptake of a probe substrate may not be the best choice, because it narrows the detection range of uptake amount, and consequently can make an estimation of BEI difficult. Accordingly, a QTLI method with a hydrophobic BSEP probe substrate, which is independent of sinusoidal uptake transporters in its entry hepatocytes, should be more suitable for this purpose.

In summary, BSEP inhibition by CIL was observed in the transport assay using membrane vesicles, but was not observed in the hSCHs. Product information for BLOPRESS states that liver dysfunction or jaundice occur with low frequency and are not critical issues for CIL in clinical use. The present study indicates that the clinical impact of CIL on BSEP-based cholestasis and subsequent liver injury can be predicted by hSCHs, but not by the membrane vesicle study due to the effect of metabolic enzymes for conversion of CIL to CAN. Additionally, it is also suggested that adverse effects of CIL could be enhanced in a combination of metabolic enzyme inhibitors in clinical use, or among poor metabolizers. It is concluded that hSCHs maintaining metabolic activities is a preferable model to predict the clinical impact of BSEP inhibition affected by metabolism, and the assay system will provide us with more accurate information to avoid false positive or negative reactions. This experimental system is applicable to predict BSEP inhibition caused by drug metabolites such as troglitazone sulfate, which exhibits more potent BSEP inhibition than its parent form.3,7

References