Investigation of the Role of Transporters on the Hepatic Elimination of an LAT1 Selective Inhibitor JPH203

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ABSTRACT: JPH203 has been developed as an anticancer drug that inhibits L-type amino acid transporter 1-mediated essential amino acid uptake into tumor cells. This study sought to elucidate which drug transporters may be involved in JPH203 hepatic elimination, and to estimate human hepatic clearance. In Sprague–Dawley rats, JPH203 total body clearance approached blood flow rate. JPH203 biotransformation via phase II metabolism produces *N*-acetyl-JPH203 (NAc-JPH203). NAc-JPH203 accumulates in the bile, and NAc-JPH203 canalicular efflux was significantly decreased in Mrp2-deficient mutant rats (Eisai hyperbilirubinemic rats). JPH203 and NAc-JPH203 are organic anion transporters [organic anion transporting polypeptide (OATP)1B1, OATP1B3, OATP2B1, and OAT3] substrates. In human cryopreserved hepatocytes, JPH203 uptake was saturable and inhibited by rifampicin, a prototypical OATP inhibitor. JPH203 metabolic clearance was larger than influx clearance and eventually passive clearance; JPH203 uptake appears to be the rate-determining process in overall hepatic elimination. Furthermore, unlike rats, the human hepatic clearance was predicted to be intrinsic clearance rate limited. These results suggest that the hepatic uptake transporters are determinant factors to determine JPH203 systemic exposure. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3228–3238, 2013

Keywords: ADME; biliary excretion; bioavailability; cancer chemotherapy; hepatocytes; transporters

INTRODUCTION

L-type amino acid transporter 1 (LAT1) forms a heterodimer with 4F2hc chaperon glycol protein to induce system L-like amino acid transport activity in the plasma membrane.[1] In a Na^+-independent manner, LAT1 transports branched-chain (e.g., valine, isoleucine, and leucine) and aromatic (e.g., phenylalanine and tyrosine) amino acids into cells.[2] LAT1 has low expression in adult humans but is located in a few normal tissues (brain, placenta, and testis)[2–4]; however, LAT1 has been shown to be highly expressed in many tumor cells such as non-small-cell lung cancer, thymic carcinoma, prostate cancer, oral squamous cell carcinoma, and gastric carcinoma.[5–9] The fact that LAT1 expression level has been associated with cancer patient survival rates (e.g., astrocytoma, non-small-cell lung cancer, prostate cancer, and gastric carcinoma)[7,9–11] demonstrates that LAT1 may be a good molecular target for both therapy and diagnosis. In fact, [^{18}F]FMT—an LAT1 substrate—has been developed as a tumor diagnostic marker.[5,12]

Abbreviations used: EHBR, Eisai hyperbilirubinemic rat; [^{18}F]FMT, [^{18}F]fluoromethyl-D-tyrosine; HEK, human embryonic kidney; JPH203, 3-(4-((5-amino-2-phenylbenzo(d)oxazol-7-yl) methoxy)-3,5-dichlorophenyl)-2-aminopropanoic acid; LAT, L-type amino acid transporter; NAc-JPH203, 3-(4-((5-acetamido-2-phenylbenzo(d)oxazol-7-yl)methoxy)-3,5-dichlorophenyl)-2-amino-propanoic acid; NAT, N-acetyltransferase; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; PET, positron emission tomography; SD, Sprague–Dawley.

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Furthermore, an LAT inhibitor, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid has been shown to inhibit KB human oral cancer cell growth.[13,14] As LAT2 appears to be associated with normal tissues, a very selective LAT1 inhibitor may be expected to rarely show cytotoxicity in normal tissues. Synthetic chemistry efforts and *in vitro* screening have produced 3-(4-((5-amino-2-phenylbenzo(d)oxazol-7-yl) methoxy)-3,5-dichlorophenyl)-2-aminopropanoic acid (JPH203, previously known as KYT-0353), which has a profound and selective inhibition effect against human LAT1 *in vitro*; JPH203 also displays significant growth inhibition against HT-29 cells *in vitro*, and *in vivo* in nude mouse bearing HT-29 cells after intravenous administration of JPH203.[15] JPH203 undergoes *N*-acetylation to afford a metabolite 3-(4-((5-acetamide-2-phenylbenzo(d)oxazol-7-yl) methoxy)-3,5-dichlorophenyl)-2-aminopropanoic acid (NAc-JPH203),[16] which also shows an inhibitory effect against LAT1. Two N-acetyl transferase (NAT) isoforms are known, NAT1 and NAT2, with NAT2 is the more predominate isoform in the liver.[17] "CPathPred" is an *in silico* classification method to predict the major clearance pathways of drugs based on four physicochemical parameters [charge, molecular weight (MW), lipophilicity (log *D*), and plasma unbound fraction (f_p); URL http://www.bi.cs.titech.ac.jp/CPathPred/].[18] "CPathPred" analysis predicts that JPH203 will be an organic anion transporting polypeptide (OATP) substrate. The hepatic organic anion uptake system includes three isoforms: OATP1B1, OATP1B3, and OATP2B1. Of these isoforms, OATP1B1 and OATP1B3 are considered to be the most responsible for organic anion hepatic uptake in humans.[19,20] Although the impact of OATP2B1 in drug hepatic uptake remains unclear, it plays an important role to mediate intestinal drug absorption.[20]

The aims of the current study were: (1) to examine hepatic transporters and their role in JPH203 elimination, (2) to use the observed data to make predictions regarding JPH203 human hepatic clearance, and (3) to use the research findings to contribute to dose regimen design for patient clinical trials. JPH203 hepatic uptake comprises OATPs, and suggests that the uptake should be the rate-determining process in overall hepatic elimination. To our knowledge, this is the first study to illustrate OATPs and NAT cooperation in the hepatic elimination of a drug.

MATERIALS AND METHODS

Materials

[^{3}H]Estradiol-17β-glucuronide (E217βG; 50.1 Ci/mmol) and [^{3}H]estrone-3-sulfate (E1S; 54.3 Ci/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, Massachusetts). Unlabeled E217βG, E1S, and acetyl-coenzyme A were purchased from Sigma–Aldrich Chemical Company (St. Louis, Missouri). [^{14}C]JPH203 (0.053 Ci/mmol), unlabeled JPH203, and NAc-JPH203 were provided as a gift (J-Pharma Company, Ltd.; Tokyo, Japan). The cryopreserved human hepatocytes were purchased from KAC (Kyoto, Japan). All other chemicals used were of analytical grade and commercially available. NAT2-expressing cytosol was purchased from BD (lot #76873, Franklin Lakes, New Jersey).

Animals and Animal Studies

Male Sprague–Dawley (SD) rats and Eisai hyperbilirubinemia rats (EHBR) were purchased from Japan SLC (Shizuoka, Japan). All animals were maintained under standard conditions with a reverse dark–light cycle and acclimated at least 7 days before pharmacological experiments were conducted; rats were 8–9 weeks old. Food and water were provided *ad libitum*. The studies were conducted in accordance with the guidelines provided by our Institutional Animal Care Committee (Graduate School of Pharmaceutical Sciences, the University of Tokyo; Tokyo, Japan).

After anesthesia with isoflurane, rat urinary bladder and bile duct were catheterized. JPH203 [1.20 nmol/(min·kg)] was infused via the jugular vein and blood was collected (jugular vein; 30, 60 and 90 min after dosing), cooled on ice, and immediately centrifuged to obtain plasma. Urine samples were collected at 30–60 and 60–90 min. The animals were then immediately sacrificed and organs (kidneys and liver) were harvested. Samples and tissues were then processed and drug and metabolite concentrations determined via liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis.

In Vitro Transport Studies Using HEK293 Cells Expressing Transporters

HEK293 cells expressing OATP1B1, OATP1B3, OATP2B1, and OAT3 are previously established cell lines.[21,22] Cells were seeded (1.5×10^5 cells per well) 72 h before conducting the transport experiments in poly-L-lysine- and poly-L-ornithine-coated 12-well plates. Supplemented (sodium butyrate, 5.0 mM) cell culture medium was used 24 h before conducting the transport experiments, conditions to induce transporter protein expression. The transport experiments were conducted as previously described.[21] Briefly, uptake was initiated by substrate addition after the cells had been washed twice and preincubated with Krebs–Henseleit buffer (37°C; 15 min). The Krebs–Henseleit buffer contained NaCl (118.0 mM), $NaHCO_3$ (23.8 mM), KCl (4.8 mM), KH_2PO_4 (1.0 mM), $MgSO_4$ (1.2 mM), HEPES (12.5 mM), glucose (5.0 mM), and $CaCl_2$ (1.5 mM) and pH 7.4 was adjusted. After removing incubation buffer, uptake