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OATP and MRP2-mediated hepatic uptake and biliary excretion of eprosartan in rat and human

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ABSTRACT

Background: Eprosartan is an angiotensin II receptor antagonist, used in the treatment of hypertension and heart failure in clinical patients. The objective of this study was to clarify the mechanism underlying hepatic uptake and biliary excretion of eprosartan in rats and humans.
Methods: Perfused rat liver *in situ*, rat liver slices, isolated rat hepatocytes and human organic anion-transporting polypeptide (OATP)-transfected cells *in vitro* were used in this study.
Results: Extraction ratio of eprosartan was decreased by rifampicin in perfused rat livers. Uptake of eprosartan in rat liver slices and isolated rat hepatocytes was significantly inhibited by Oatp modulators such as ibuprofen, digoxin, rifampicin and cyclosporine A, but not by tetraethyl ammonium or *p*-aminohippurate. Uptake of eprosartan in rat hepatocytes indicated a saturable process. Although uptake of eprosartan in OATP1B3-human embryonic kidney cells (HEK) 293 cells was not observed, significant differences in cellular accumulations of eprosartan between vector- and OATP1B1-Madin–Darby canine kidney strain (MDCK) II cells were found in transcellular transport study. Moreover, cumulative biliary excretion rate of eprosartan in the presence of probenecid (Multidrug resistance-associated protein 2 (Mrp2) inhibitor) was significantly decreased in perfused rat livers. Vectorial basal-to-apical transport of eprosartan was also observed in OATP1B1/MRP2 double transfectants.
Conclusions: Eprosartan was transported by multiple Oatps (at least Oatp1a1 and Oatp1a4)/Mrp2 in rat and OATP1B1/MRP2, at least, in human.
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Introduction

Eprosartan is an angiotensin II receptor antagonist (ARA-II) which has a high affinity for the angiotensin II type 1 receptor (AT1), used in treatment of hypertension and heart failure in clinical patients. Therefore, they have been proposed as an alternative to the traditional angiotensin-converting enzyme inhibitors [25]. Orally administered eprosartan has an absolute bioavailability of 13%, with peak plasma concentrations achieved 1–2 h after administration. The mean terminal elimination half-life of eprosartan is 20 h after multiple-dose administration. Eprosartan is not metabolized by the cytochrome P450 enzyme system and primarily eliminated as unchanged drug *via* biliary and renal excretion [33].

The hepatobiliary disposition of drugs in the liver is influenced by several processes, such as sinusoidal uptake into the hepatocytes, intracellular translocation and excretion into the bile, which may involve carrier-mediated mechanisms that are saturable and rate limiting. In liver, uptake transporters govern the transport of drugs from the basolateral side of the cell membrane (the blood side) into the hepatocyte, while the exit of drugs from inside hepatocytes was mediated either by a basolateral efflux transporter transported into the blood or by an apical (canalicular) efflux transporter transported into the bile [22,38,39,47]. Both sinusoidal and canalicular membrane transporters are important

Abbreviations: BCRP (Bcrp), breast cancer resistance protein; CE, collision energy; $E_2$17G, estradiol-17β-D-glucuronide; ES, estrone-3-sulfate; HEK293 cells, human embryonic kidney cells 293 cells; IS, internal standard; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MDCKII cells, Madin–Darby canine kidney strain II cells; MDR, multidrug resistance; MRP (Mrp), multidrug resistance-associated protein; OAT (Oat), organic anion transporter; OATP (Oatp), organic anion transporting polypeptide; OCT (Oct), organic cation transporter; PAH, *p*-aminohippurate; P-gp, P-glycoprotein; TEA, tetraethyl ammonium.

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for the hepatic clearance of drugs and may be concerned with potential of drug–drug interactions.

Various transporters are involved in the hepatobiliary transport process. Among them, OATP1B1 (OATP-C/OATP2/LST-1, organic anion-transporting polypeptide) [1] and OATP1B3 (OATP8/LST-2) [2], selectively expressed in the basolateral membrane of human hepatocytes with broad substrate specificities [2,11,12], are thought to be responsible for the hepatic uptake of various endogenous, such as neutral steroids [4,44] and bile salt [43], and xenobiotic substances, such as statins [13,14,18], sartans [15,29,46] and fexofenadine [36], in a Na+-independent manner. On the other hand, Na+-dependent taurocholate-cotransporting polypeptide (NTCP) is the most relevant Na+-dependent transporter identified as a hepatic uptake transporter and is thought to be responsible for the hepatic uptake of bile salts [43]. Conversely, transporters such as MDR1 (multidrug resistance 1/*ABCB1*), MRP2 (multidrug resistance-associated protein 2/*ABCC2*), and BCRP (breast cancer resistance protein/*ABCG2*) are involved in biliary excretion, which are predominantly expressed on canalicular membrane [5,6]. Moreover, the substrate specificity of each transporter is very broad and the transporters expressed in hepatocytes always share an overlapping substrate spectrum, suggesting that a substrate can be recognized by one transporter or multiple transporters.

However, the transporters involved in the hepatobiliary transport of eprosartan in human and rat have not been fully investigated yet. Therefore, the aim of this study is to show the involvement of transporters in the hepatic uptake and biliary excretion process of eprosartan. Investigation of the transporters involved in the hepatobiliary transport of eprosartan which is eliminated mostly by hepatic uptake and biliary excretion, is important for the prediction of potential eprosartan-related drug–drug interactions and may contribute to our understanding of the mechanisms of hepatobiliary excretion of sartans.

In present study, the perfused rat liver model, an ideal model for examining alterations in the hepatobiliary disposition of substrates without the influence of metabolism/excretion by other organ systems [3], was employed to investigate the possible involvement of hepatic uptake and efflux transporters in the hepatobiliary disposition of eprosartan. Furthermore, rat liver slices [8] and isolated hepatocytes [23] were used to evaluate the transporter-mediated hepatobiliary disposition of eprosartan. Also, bi-directional transport assays were conducted using OATP1B1/MRP2 double transfectants and OATP1B3 transfected cells [21,24,45] to identify which transporters are involved in the hepatic uptake and biliary excretion of eprosartan in human.

Materials and methods

Materials and animals

Eprosartan was purchased from Melone Pharmaceutical Co., Ltd. (Dalian, China). Tetraethyl ammonium (TEA) and digoxin were purchased from Hubei Saibo Chemical Co., Ltd. and Nanjing ZeLang Medical Technology Co., Ltd., respectively. Cyclosporine A and rifampicin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Probenecid was obtained commercially from Alexis Biochemicals (San Diego, CA, USA). Cilostazol (internal standard, IS), *p*-Aminohippurate (PAH), (*RS*)-2-(4-(2-methylpropyl) phenyl) propanoic acid (ibuprofen), novobiocin and verapamil were purchased from Sigma–Aldrich (St. Louis, MO, USA). The stable transfected OATP1B3-human embryonic kidney cells (HEK) 293 cells, vector-HEK293 cells, OATP1B1-Madin–Darby canine kidney strain (MDCK) II cells, OATP1B1-MRP2-MDCKII cells and vector-MDCKII cells were the generous gift from Professor Yuichi Sugiyama, Graduate School of Pharmaceutical Sciences, University of Tokyo (Tokyo, Japan). Cell culture reagents were purchased from GIBCO® (Grand Island, NY). All other chemicals were of analytical grade and were commercially available.

Male Wistar rats (220–250 g) obtained from the Experimental Animal Center of Dalian Medical University (Dalian, China; permit number SCXK 2008-0002) were allowed free access to water and kept under a chow diet but were fasted for 12 h (with water *ad libitum*) before the experiments. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Media and cell culture

HEK293 cells and MDCKII cells were grown in Dulbecco's modified Eagle medium (low-glucose; Invitrogen, Carlsbad, CA) with 10% (v/v) fetal bovine serum (Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin. All transfected cells were cultured at 37 °C with a 5% (v/v) CO_2 atmosphere and 95% relative humidity during cell culture.

Perfused rat liver experiments

Rat livers were prepared by standard techniques described by previous study [27]. Briefly, the bile duct was cannulated and the liver ($n = 3$) was perfused *in situ* through the portal vein with oxygenated Krebs-Henseleit buffer (118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5.0 mM glucose, 2.5 mM $CaCl_2$ adjusted to pH 7.4). After equilibrated with Krebs–Henseleit buffer for approximately 10 min, the liver was allowed to perfuse with oxygenated Krebs–Henseleit buffer containing 20% (v/v) washed bovine erythrocytes at a flow rate of 12 ml/min [20]. In order to collect the effluent perfusate, the superior vena cava was cannulated. Basis of initial bile flow (>2 μl/min) was determined as the standard of normal liver viability. Following equilibration, the liver was perfused for 45 min after addition of eprosartan 1 μM, eprosartan 1 μM + rifampicin 10 μM (final concentration, $n = 3$). On the other hand, following equilibration, the inhibitor of Mrp2, P-gp or Bcrp (20 μM of probenecid, verapamil or novobiocin; $n = 3$, respectively) was added to the perfusate buffer 5 min before the addition perfusate of eprosartan. Then the liver was perfused for 45 min after addition of eprosartan (1 μM). Effluent perfusate (500 μl) was collected at 1, 3, 5, 15, 25, 35, 45 min with the superior vena cava cannulation and bile samples were collected *in toto* at 15 min intervals with the bile duct cannulation. The samples were stored at −20 °C.

Eprosartan uptake in rat liver slices

Rat liver slices were prepared as described by Elferink et al. [8]. In brief, after the animals ($n = 3$) were anesthetized, livers were excised and immediately immersed in ice-cold buffer, saturated with 95% O_2–5% CO_2 (carbogen; pH 7.4), Then, livers were cut into slices (200–300 μm thickness; 10–14 mg wet weight) with a tissue slicer (ZQP-86; Zhixin Co., Ltd., Shanghai, China) and gassed with carbogen in 6-well culture plates with continuous shaking. After incubation for 3 min at 37 °C, liver slices were transferred to 24-well culture plates and contained with fresh carbogen-saturated eprosartan for further uptake study at 37 °C and 4 °C. Liver uptake of eprosartan (1 μM) was measured at 0, 1, 5, 15 and 30 min. Then, livers were rinsed with ice-cold Hanks' balanced salt solution (pH 7.5) for three times and dried on filter paper. Uptake of eprosartan at different time points was essentially linear, base on this, the overall rate of uptake time was chosen as 15 min. Also, this time was used to examine the effects of inhibitors (detailed specificities

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