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Feasibility of the functional expression of the human organic anion transporting polypeptide 1B1 (OATP1B1) and its genetic variant 521T/C in the mouse liver

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ABSTRACT

The objective of this study was to examine the feasibility of functional expression of the human organic anion transporting polypeptide 1B1 (hOATP1B1) forms in the liver of the mouse. After the mouse received the gene of interest (i.e., luciferase as the reporter or hOATP1B1) via hydrodynamic gene delivery (HGD) method, the expression was found to be liver-specific while alterations in the serum biochemistry and hepatocyte histology were apparently transient and reversible. The reporter activity was also detected in the plasma, but not in the blood cell in mice that received HGD, suggesting that the protein is probably released due to transiently increased permeability in hepatocytes by HGD. Using this delivery condition, the expression of hOATP1B1 was readily detected in the liver, but not in other tissues, of the mice receiving HGD for the transporter gene. Compared with the sham control mice, the uptake of pravastatin into the liver increased significantly in mice receiving hOATP1B1 wild type; the uptake parameters decreased consistently in mice expressing the 521T>C variant compared with that of the wild type control. These observations suggest that the functional expression of human transporter gene in mice is feasible, further suggesting that this treatment is practically useful in the pharmacokinetic studies for hOATP1B1 substrates.

1. Introduction

It is known that the organic anion transporting polypeptide 1B1 (OATP1B1) is expressed in the basolateral membrane of human hepatocytes (Abe et al., 1999) and that it is responsible for the transport of certain clinically relevant substrates [e.g., 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase inhibitors; pravastatin, atorvastatin, and rosuvastatin] as well as endogenous compounds (e.g., estrone-3-sulfate and thyroxin) from the systemic circulation to the liver (Tirona and Kim, 2002). For the case of HMG-CoA reductase inhibitors, the anion transporter has not only pharmacokinetic implications, but it also has pharmacological relevance since the liver is the primary site for most pharmacological responses. Any change in the function, e.g., rendered by genetic variations of human OATP1B1 (hOATP1B1) or drug-drug interactions, is likely to lead to altered therapeutic responses for statins. Consistent with this statement, in clinical studies (Kameyama et al., 2005; Niemi et al., 2004; Zhang et al., 2007), a higher exposure of pravastatin was noted in the systemic circulation, but with a reduced therapeutic effect, in the individuals carrying OATP1B1 521T>C, which is a genetically variant form with reduced function (Tachibana-limori et al., 2004). Due to the association between the functional variation of the anion transporter and the pharmacokinetics/therapeutic effect, it has been recently proposed by the US Food and Drug Administration that drug-drug interactions involving an anion transporter be studied as if they were new drugs (U.S. FDA, 2012).

It has been reported that rodents do not produce an anion transporter that is significantly homologous to hOATP1B1 (Hagenbuch and Meier, 2004); Oatp transporters in rodents with the highest amino

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, the area under the plasma concentration-time curve; $CL_{app,\ hep}$, hepatic uptake clearance; hOATP1B1, human organic anion transporting polypeptide 1B1; HGD, hydrodynamic gene delivery; HMG-CoA, 3-hydroxy-3-methylglutaryl; IS, internal standard; LC-MS, liquid chromatography-mass spectrometry; mOATPs, mouse organic anion transporting polypeptides; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecyl sulfate; SIM, selected ion monitoring.

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acid sequence homology is seen in the mouse OATP1b2 which has an amino acid sequence identity to hOATP1B1 of only 64% (Hagenbuch and Meier, 2003). This observation suggests that rodents may not be a reasonable model for studies of the pharmacokinetics of OATP1B1 substrates in humans. A mouse strain expressing hOATP1B1, while lacking mOATP1b2, recently became commercially available (Higgins et al., 2014). However, it may be necessary to conduct a series of kinetic studies with the humanized animal to verify whether it is a valid model to resemble humans with respect to the pharmacokinetics for hOATP1B1 substrates. In addition, a parallel mouse model expressing hOATP1B1 521T/C, the reduced functional form of the anion transporter, is not currently available. As a result, an in vivo kinetic study with the reduced functional genotype is not possible.

Hydrodynamic gene delivery (HGD) is a relatively straightforward method for the delivery of a gene of interest to the liver in animals (Bonamassa et al., 2011). When a large volume of a solution containing plasmid DNA is injected via the tail vein of mice for a short period of time (e.g., 5 s), it is thought that the heart would be immediately congested and the solution would cause an accumulation in the inferior vena cava (Suda et al., 2007; Suda and Liu, 2007; Zhang et al., 2004). The increased intravascular pressure would then induce a reflux of the solution into the liver through the hepatic vein and force the blood contained in the liver out to the portal vein. As a result, it is believed that the plasmid DNA containing the gene of interest would avoid coming into direct contact with the nucleases in the blood. Consequently, the solution transported into the liver would then expand the sinusoid and enlarge the fenestrae, and subsequently, the relatively intact plasmid DNA would be delivered to the parenchyma of hepatocytes (Suda et al., 2007; Suda and Liu, 2007; Zhang et al., 2004). Since HGD would be generally applicable to any gene, particularly a gene that is primarily expressed in the liver (e.g., hOATP1B1), it may also be delivered to the mouse liver. It would be, then, possible that in vivo animal models expressing the wild type and variant forms of hOATP1B1 could be generated to study the pharmacokinetic relevance of the functional variations (e.g., drug-drug interaction and/or genetic polymorphisms) of the transporter. Considering that the impact of genetic variation on function is well known to be substrate specific for hOATP1B1 (Romaine et al., 2010), the delivery and expression of the genes may be a useful experimental tool in studying the human genetic variation of hOATP1B1. Unfortunately, however, such research has never been attempted to date, and such animal model is currently not available.

The objective of the current study, therefore, is to examine the feasibility of the functional expression of hOATP1B1 in the mouse liver in vivo by HGD. We were particularly interested in whether a hOATP1B1 521T>C variant could also be expressed in the mouse liver and in comparing its function with that of the wild-type. Herein, we report that the functional expression of both the wild type and the function reduced form of hOATP1B1, is feasible in the mouse liver.

2. Materials and methods

2.1. Materials

Three representative delivery vectors for HGD were selected (i.e., pcDNA3.1, pLIVE, and pGL3) based on a literature survey (Arad et al., 2005; Layzer et al., 2004; Marx et al., 2008; Pan et al., 2004; Yang et al., 2001; Yin et al., 2012) and were purchased from Invitrogen (for pcDMA3.1; Carlsbad, CA), Mirus Bio (pLIVE; Madison, WI), and Promega (pGL3; Madison, WI). Pravastatin and atorvastatin were obtained from Sigma–Aldrich (St. Louis, MO) and the assay kit for luciferase activity was purchased from Promega. All other chemicals were of reagent grade or better and were used without further purification. Human liver samples (as liver biopsy specimen), a gift from Professor Im-Sook Song of the Department of Pharmacology and Pharmacogenomics Research Center, Inje University College of Medicine, South Korea, was also used in this study.

2.2. Animals

Male ICR mice, weighing 20–25 g, were purchased from Orient Bio Inc. (Sungnam, Korea). The experimental protocols involving animals used in this study were reviewed by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University, in accordance with the National Institutes of Health Publication Number 85-23, revised 1985, in *Principles of Laboratory Animal Care*.

2.3. Plasmid DNAs

The pCMV-Luc, pLIVE-Luc, and pSV40-Luc plasmids, containing the gene for firefly luciferase [i.e., the luciferase gene was under the control of cytomegalovirus (CMV in the pCMV plasmid), mouse minimal albumin promoter (in the pLIVE plasmid), and simian vacuolating virus 40 (SV40 in the pSV40 plasmid) promoter, respectively] as a reporter gene, were used in this study. pCMV-Luc or pLIVE-Luc was constructed by subcloning the luciferase cDNA fragment obtained from pSV40-Luc (pGL3-control vector) into the multi-cloning sites of pcDNA3.1 or pLIVE vector, respectively.

When necessary, the protein coding region of hOATP1B1 was also cloned from a human mRNA library (BD Biosciences, San Jose, CA) by reverse transcription-polymerase chain reaction (RT-PCR) and inserted into pcDNA3.1 (called as pCMV-hOATP1B1). The wild type hOATP1B1 was selected by sequencing reaction and hOATP1B1 521T>C was generated using the QuickChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA). The purity of the plasmid was determined by measuring the absorbance ratio at 260/280 nm and 1% agarose-gel electrophoresis. The plasmid with the absorbance ratio at 260/280 nm > 1.9 was used only.

2.4. HGD in mice

A saline solution or a saline solution containing the naked luciferase-encoding plasmid (e.g., pCMV-Luc, pLIVE-Luc, or pSV40-Luc) was administered to the mice by the HGD method via a tail vein injection. In this study, each animal received amounts of from 10 to 100 μg of the plasmid per animal. Mice were injected with a volume of saline solution equivalent to 8% of their body weight in 5 s through the tail vein (Liu et al., 1999) and blood samples were obtained from the orbital venous plexus using a capillary glass tube at pre-determined intervals. The samples were then centrifuged at 12,000 rpm for 10 min, and the plasma fraction was collected. If necessary, tissue samples were collected, the wet weight determined and the samples were used in subsequent experiments.

When necessary, mice were also injected with a saline solution containing the hOATP1B1 gene (e.g., pCMV-hOATP1B1). In particular, the relevant question was whether hOATP1B1 gene delivery would lead to the expression of hOATP1B1 and whether the administration would affect the expression of endogenous mouse OATPs (mOATPs) in the liver. In this study, mice were injected with various amounts of the plasmid containing hOATP1B1 gene while 5 μg of pCMV-Luc was co-injected for normalization of the efficiency of the gene delivery. In this experiment, the total volume of the tail vein was limited to 8% of the body weight.

2.5. Assessment of liver toxicity and change in body weight

To examine the toxicological manifestations of HGD in the mice, a series of biochemical parameters for liver toxicity and histological changes in the liver after delivery were determined. Mice were injected with saline, as described above, and blood and liver samples were collected at 1, 2, 3, 5, and 7 days after the injection. In addition, saline solutions containing various amounts of pCMV-Luc (e.g., 10, 20, 30, 50, and 100 μg) were similarly given to the mice; blood and liver samples were collected at 1 and 3 days after the delivery in order to examine whether