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OATP1B1/1B3 activity in plated primary human hepatocytes over time in culture

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ABSTRACT

Primary human hepatocytes are widely used as an *in vitro* model for evaluation of drug metabolism and transport. However, it has been shown that the gene expression of many drug-metabolizing enzymes and transporters change in culture. The aim of the present study was to evaluate the activity of organic anion-transporting polypeptide 1B1 (OATP1B1) and 1B3 (OATP1B3) in plated primary human hepatocytes over time in culture. The uptake kinetics of the OATP1B1/1B3 substrate [^{3}H]-estradiol-17β-D-glucuronide was determined in cells from five donors. An extensive and variable decrease in OATP1B1/1B3 activity and/or increase in passive diffusion was observed over time. Already after 6 h in culture, the OATP1B1/1B3 activity was not possible to determine in liver cells from one donor, while after 24 h, the uptake activity was not measurable in one additional donor. In the other three, the decrease in CL_{int} (V_{max}/K_m) values ranged from 15% to 86% after 24 h in culture compared to the values measured at 2 h. Visual examination of OATP1B1 protein expression by confocal microscopy showed localization to the plasma membrane as expected, and an extensive decrease in OATP1B1 expression over time in culture supported the decline in activity. The significant reduction in *SLCO1B1* and *SLCO1B3* gene expression over time determined by RT-PCR also supported the loss of OATP1B1/1B3 activity. In conclusion, plated primary human hepatocytes are useful as an *in vitro* model for OATP1B1/1B3-mediated uptake studies, but the culture time may substantially change the uptake kinetics.

1. Introduction

The propensity of new drugs to exhibit large variation in pharmacokinetic profiles and to be sensitive to drug–drug interactions are major concerns when developing new drugs. Therefore, the factors determining the pharmacokinetic profile of a compound should be described as early as possible in the drug discovery process. Membrane transporters are today recognized as a major determinant of pharmacokinetic variability of certain drugs, in addition to drug metabolizing enzymes [1]. Therefore, in the drug discovery and development process of new chemical entities, both uptake and efflux processes should be characterized in parallel with potential metabolism of drug candidates. Such studies require robust and human relevant *in vitro* models for reliable predictions of hepatic uptake, distribution, and excretion of drug candidates during preclinical testing.

The hepatic uptake transporter organic anion-transporting polypeptide 1B1 (OATP1B1) and 1B3 (OATP1B3) are expressed predominantly in the basolateral membrane of human hepatocytes [2–4] and serve as bidirectional facilitated diffusion transporters [5]. The OATP-mediated substrate uptake is pH dependent and generally accompanied by bicarbonate efflux [6]. OATP1B1 and OATP1B3 have an overlapping substrate spectrum. They are carriers of a variety of endogenous substances, including bile salts, hormone conjugates and steroids [7,8], and play a key role in the hepatic uptake of many drugs, e.g. HMG-CoA reductase inhibitors (statins), angiotensin II receptor antagonists, angiotensin-converting enzyme inhibitor and anti-cancer agents [9–14].

Several OATP1B1-mediated drug–drug interactions involving the agents mentioned above have been reported [15–19], e.g. substantially increased statin plasma levels during co-administration of the OATP1B1 inhibitor cyclosporine A [20,21]. Furthermore, several single nucleotide polymorphisms (SNPs) and haplotypes of *SLCO1B1*, the gene encoding OATP1B1 [22], have shown to affect drug disposition and drug response of OATP1B1 substrates in individuals carrying specific variants of *SLCO1B1* [23–25]. Polymorphisms in the *SLCO1B3* gene encoding OATP1B3 have also been

Abbreviations: OATP, organic anion-transporting polypeptide; SNP, single nucleotide polymorphism; E17βG, estradiol-17β-D-glucuronide; FBS, fetal bovine serum; HBSS, Hanks' balanced salt solution; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; Ct, cycle threshold; OCT1, organic cation transporter 1; OAT2, organic anion transporter 2; NTCP, sodium-taurocholate cotransporting polypeptide.[2]

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reported [26–28], but the knowledge on functional consequences of genetic variants in the *SLCO1B3* gene is limited [29].

Transfected (over-expressed) cell systems offers a convenient model for identification of OATP1B1 and OATP1B3-mediated transport, but *in vitro–in vivo* clearance extrapolation is challenging. Primary hepatocytes, plated or in suspension, represent a common *in vitro* model for evaluation of human hepatic drug transport, metabolism and clearance [30]. Primary hepatocytes express a complete set of enzymes and transporters involved in hepatic drug clearance. This might be favorable compared to transfected cell systems in terms of ability to predict relevant *in vivo* clearance parameters. Freshly isolated primary human hepatocytes have been extensively characterized with respect to expression and activity of drug-metabolizing enzymes and canalicular transporters [31–36]. However, the expression, localization and function of sinusoidal transporters, such as OATP1B1 and OATP1B3, have been less studied, especially with respect to a potential altered transport activity with time in culture. The aim of the present investigation was therefore to study uptake kinetics and expression of OATP1B1/1B3 in plated primary human hepatocytes over time in culture.

2. Materials and methods

2.1. Chemicals and reagents

[^{3}H]-estradiol-17β-D-glucuronide ([^{3}H]-E17βG) was obtained from Perkin Elmer (Boston, MA). Unlabeled estradiol-17β-D-glucuronide, $CaCl_2$, collagenase, DNase, EGTA, fetal bovine serum (FBS), L-glutamine (200 mM), $NaHCO_3$, saponin and trypsine inhibitor were all obtained from Sigma–Aldrich (Steinheim, Germany). Alexa fluor 488 conjugated goat anti-rabbit secondary antibody, Hanks' balanced salt solution (HBSS), HBSS without Ca^{2+}, Mg^{2+} and phenol red, HEPES, phosphate-buffered saline (PBS) and Williams' Medium E were obtained from Invitrogen (Carlsbad, CA). HCl and NaOH were obtained from Merck (Darmstadt, Germany). Sodium chloride was obtained from Fresenius Kabi (Bad Homburg, Germany). Rat tail collagen type I was obtained from BD Biosciences (Bedford, MA). Rabbit polyclonal antibody against human OATP1B1 was a kind gift from Dr. Bruno Stieger (University Hospital Zurich, Zurich). High-purity water was obtained from an ELGA purification system (ELGA, High Wycombe, UK). All other chemicals and reagents were of analytical grade and were available from commercial sources.

2.2. Human hepatocyte isolation

Adult human liver biopsies were obtained from Sahlgrenska Hospital (Göteborg, Sweden) and originated from patients undergoing partial hepatectomy for primary or secondary tumours. All tissues were obtained by qualified medical staff, with donor informed consent and the approval of the Local Ethics Committee at Sahlgrenska University Hospital. Donor information regarding age, sex, disease, size of resection, cell viability, yield of cells and genotype is summarized in Table 1. All donors were Caucasian.

The resections were flushed with ice cold physiological NaCl solution immediately after surgery and kept in Williams' Medium E (supplemented with 25 mM HEPES and 2 mM L-glutamine), pH 7.4 over night (max 18 h). Hepatocytes were isolated by a two-step collagenase perfusion through the existing vasculature [37–39]. The general buffer consisted of HBSS without Ca^{2+}, Mg^{2+} and phenol red supplemented with HEPES (10 mM) and $NaHCO_3$ (3.75 mM), pH 7.4. Washing buffer I contained general buffer supplemented with EGTA (0.5 mM). Digestion buffer contained general buffer supplemented with $CaCl_2$ (5 mM), collagenase (157 Units/ml) and trypsin inhibitor (68 mg/L). Washing buffer III contained general buffer supplemented with DNase (28 kunitz Units/ml). Collagenase, trypsin inhibitor and DNase were added to its respective buffer solutions directly before use. Washing buffer II and IV were the same as the general buffer.

Four cannulae were inserted into present vasculature, and the cut surfaces of the resection as well as the point of entry of the cannulae were coated with glue (Loctite 401 Prism Cyanoacrylate Adhesive, Henkel Corporation, Rocky Hill, CT). The tissue was perfused (7 ml/min/cannulae) with washing buffer I for 10 min, with washing buffer II for another 15 min, and finally with digestion buffer for 15 min. All steps were conducted at 37 °C. The digested tissue was transferred to washing buffer III, and cell dissociation was obtained by breaking the capsule with a sharp instrument and gently squeezing the tissue. After filtering the residual tissue and cell suspension through a fine nylon mesh, the cell suspension was centrifuged at 100 × *g* for 3 min. The cell pellet was resuspended in washing buffer III and centrifuged at 100 × *g* for 3 min. The cell pellet was resuspended in washing buffer IV and centrifuged at 100 × *g* for 3 min. The final cell pellet was resuspended in Williams' Medium E containing 25 mM HEPES and 2 mM L-glutamine, pH 7.4. Initial cell viabilities were determined using trypan blue exclusion. If the cells did not fully dissociate after collagenase perfusion, direct digestion incubation of the residual liver tissue was performed. The residual liver was placed in a tube with digestion buffer and incubated for 20 min at 37 °C in water bath. The content was filtered through a nylon mesh, and the suspension was centrifuged at 100 × *g* for 3 min. The cell pellet was resuspended in washing buffer III and further treated as the cell suspension from the collagenase perfusion.

Primary human hepatocytes were suspended in *InVitro*GRO™ CP Medium supplemented with *Torpedo*™ Antibiotic Mix (Celsis In Vitro Technologies, Baltimore, MD), and plated in 24-well plates pre-coated with rat tail collagen type I and in collagen type I coated 4-well culture slides (BD Biosciences) at a density of 0.25×10^6 cells/well. Based on a pilot experiment where cells were cultured for up to 48 h, the experimental set-up was chosen to allow cells to be cultured for 2, 6 and 24 h at 37 °C, 5% CO_2, and 90–95% humidity. At each time point OATP1B1 transport activity was determined and separate hepatocytes were harvested in RNAprotect Cell Reagent

Table 1
Human donor demographics.

Donor ID	Age (years)	Sex (F/M)	Weight (kg)	Disease	Size of resection (g)	Yield (viable hepatocytes/g)	Viability (%)	*SLCO1B1 521T>C* polymorphism
Donor # 1	66	M	83	Metastasis (GIST[a])	N.A.	N.A.	67	*T/T*
Donor # 2	60	M	89	Metastasis (colon)	26.5	7.6×10^6	88	*C/C*
Donor # 3	42	F	78	Metastasis (GIST[a])	39.0	7.2×10^6	85	*C/T*
Donor # 4	63	M	65	Metastasis (rectal)	30.6	14.7×10^6	85	*C/C*
Donor # 5	59	M	76	Metastasis (colon)	20.2	6.4×10^6	80	*C/T*

N.A., not applicable; M, male; F, female.
[a] Gastrointestinal stromal tumor.