Drug Metabolism and Pharmacokinetics xxx (2018) 1–9

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular Article

Total hepatocellular disposition profiling of rosuvastatin and pitavastatin in sandwich-cultured human hepatocytes

Katsuhiro Kanda [a, *], Ryosuke Takahashi [b], Takashi Yoshikado [c], Yuichi Sugiyama [c]

[a] Bio Systems Design Dept., Bio Analytical Systems Product Div., Hitachi High-Technologies Corporation, Ibaraki, Japan
[b] Center for Technology Innovation – Healthcare, Hitachi, Ltd., Tokyo, Japan
[c] Sugiyama Laboratory, RIKEN Innovation Center, RIKEN, Kanagawa, Japan

ARTICLE INFO

Article history:
Received 13 December 2017
Received in revised form
14 March 2018
Accepted 3 April 2018
Available online xxx

Keywords:
Drug disposition
Efflux transporters
Biliary excretion
Basolateral efflux
Sandwich cultured human hepatocyte
Liquid chromatography mass spectrometry
Membrane vesicle
Ko143

ABSTRACT

This study describes the total disposition profiling of rosuvastatin (RSV) and pitavastatin (PTV) using a single systematic procedure called D-PREX (Disposition Profile Exploration) in sandwich-cultured human hepatocytes (SCHH). The biliary excretion fractions of both statins were clearly observed, which were significantly decreased dependent on the concentration of Ko143, an inhibitor for breast cancer resistance protein (BCRP). Ko143 also decreased the basolateral efflux fraction of RSV, whereas that of PTV was not significantly affected. To understand these phenomena, effects of Ko143 on biliary excretion (BCRP and multidrug resistance-associated protein (MRP) 2) and basolateral efflux (MRP3 and MRP4) transporters were examined using transporter-expressing membrane vesicles. BCRP, MRP3 and MRP4-mediated transport of RSV was observed, and Ko143 inhibited these transporters except MRP3. BCRP and MRP4 also mediated the transport of PTV, but the Ko143-mediated inhibition was only clear for BCRP. These results might explain the Ko143-mediated complete and partial inhibition of the biliary excretion and the basolateral efflux of RSV, respectively, in SCHH. In conclusion, D-PREX with sequential sampling of supernatants prior to cell lysis enables the evaluation of total drug disposition profiles resulting from complex interplays of intracellular pathways, which would provide high-throughput evaluation of drug disposition during drug discovery.

1. Introduction

Clarification of the hepatic pharmacokinetics and disposition of xenobiotics, including various types of drugs, mediated by a number of transport proteins is of great interest for the efficient development of new drugs. The sandwich culture system was developed in the late 1980s as a method to mimic *in vivo* biliary excretion [1,2], and has been effectively utilized to investigate the pharmacokinetics of drugs *in vitro* and to predict their disposition under clinically relevant conditions [3–5]. Sandwich-cultured hepatocytes exhibit structural polarity, including a bile canalicular (BC) network and the appropriate localization of hepatocyte-specific transporters, as well as liver-specific functions such as albumin secretion over several days of culture [1,6,7].

Recently, we developed the D-PREX (Disposition Profile Exploration) methodology, a procedure for multiple assessments of hepatocellular drug disposition using sandwich-cultured rat hepatocytes (SCRH) [8]. The advantage of D-PREX is that it is a single systematic procedure that can simultaneously evaluate overall hepatocellular disposition profiles, allowing high-throughput evaluation of multiple endpoints including drug uptake, biliary excretion (BilEx), basolateral efflux (BasEf), which is further subdivided into a transporter-mediated component (BasEf-TP) and a passive-diffusion component (BasEf-PD), and intracellular residue (IcRes). In the previous D-PREX evaluation, SCRH were treated with fluorescent reagents (5(6)-carboxy-2′,7′-dichlorofluorescein and rhodamine 123) and eventually D-PREX demonstrated the overall hepatocellular disposition characteristics of these compounds.

HMG-CoA reductase inhibitors rosuvastatin (RSV) and pitavastatin (PTV) have been widely used for the studies of biliary excretion in SCHH and SCRH [5,10,14,15]. One of the critical transporters for the biliary excretion of RSV is breast cancer resistance protein (BCRP). The biliary clearance (CL_{Bile}) of RSV in SCRH decreased after treatment with Ko143 (BCRP inhibitor) and

* Corresponding author. Bio Systems Design Dept., Bio Analytical Systems Product Div., Hitachi High-Technologies Corporation, 882 Ichige, Hitachi-naka, Ibaraki, 312-8504, Japan.
E-mail address: katsuhiro.kanda.pv@hitachi-hightech.com (K. Kanda).

https://doi.org/10.1016/j.dmpk.2018.04.001

Please cite this article in press as: Kanda K, et al., Total hepatocellular disposition profiling of rosuvastatin and pitavastatin in sandwich-cultured human hepatocytes, Drug Metabolism and Pharmacokinetics (2018), https://doi.org/10.1016/j.dmpk.2018.04.001

Abbreviations

ABC	ATP-binding cassette
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BasEf	Basolateral efflux
BasEf-PD	Passive-diffusion portion of basolateral efflux
BasEf-TP	Transporter-mediated portion of basolateral efflux
BC	Bile canaliculi
BCRP	Breast cancer resistance protein
BilEx	Biliary excretion
BSA	Bovine serum albumin
CE	Conditioning efflux
CL_{Bile}	Biliary clearance
CV	Coefficient of variation
DDU	Directly-measured drug uptake
DILI	Drug-induced liver injury
D-PREX	Disposition Profile Exploration
EA	Efflux assay
EGTA	Ethylene glycol tetraacetic acid
HBSS	Hanks' Balanced Salt Solution
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HPLC	High-performance liquid chromatography
IA	Intracellular assay
IcRes	Intracellular residue
Ki	inhibition constant
K_m	Michaelis constant
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
MRP	Multidrug resistance-associated protein
OATP	Organic anion transporting polypeptide
P-gp	P-glycoprotein
PTV	Pitavastatin
RSV	Rosuvastatin
RT	Retention time
SCHH	Sandwich-cultured human hepatocytes
SCRH	Sandwich-cultured rat hepatocytes
Std	Standard
TP	Transporter

elacridar (BCRP and P-glycoprotein inhibitor) [5,10]. RSV accumulated in Caco-2 cell monolayers via the inhibition of BCRP by Ko143 [11]. Furthermore, membrane vesicles isolated from mammalian cells expressing human BCRP transported RSV at a greater rate than control vesicles [12,13]. As for PTV, its biliary excretion in SCRH was reduced by knockdown of Bcrp [15]. In addition, significant basal-to-apical transport of PTV has been observed in human OATP1B1/BCRP double-transfected Madin–Darby canine kidney II cells [16], and the uptake of PTV into membrane vesicles isolated from human BCRP-transfected HEK293 cells is markedly higher than that observed in vesicles isolated from GFP-transfected control cells [16]. These reports show that BCRP is primarily responsible for the biliary excretion of RSV and PTV. On the other hand, limited information is available for the involvement of multidrug resistance-associated protein 2 (MRP2) in the biliary excretion of RSV and PTV. In regard to the studies using rat hepatocytes, Mrp2-deficient (TR^-) rat sandwich-cultured hepatocytes showed smaller biliary excretion of RSV than that of wild-type [5], and MRP2 inhibitors (benzbromarone, probenecid, and sulfasalazine) decreased the biliary excretion of RSV in SCRH [10].

In terms of hepatic basolateral efflux, MRP3 [17,18] and MRP4 [19,20] play some roles in the excretion of endogenous substrates, drugs and metabolites. The transport of RSV into MRP3- and MPR4-expressing membrane vesicles was markedly higher than that into control vesicles [5]. The other report showed the MRP3-mediated transport of PTV using membrane vesicles, whereas MRP4 did not transport PTV to a measurable extent [14].

In this study, the total disposition profiles (BilEx, BasEf, and IcRes) of the medicinal drugs (RSV and PTV) in SCHH were investigated in the presence and absence of Ko143 using D-PREX methodology. We further examined the mechanism of their altered disposition after Ko143 treatment using human ATP-binding cassette (ABC) efflux transporter-expressing membrane vesicles.

2. Materials and methods

2.1. Chemicals and reagents

RSV calcium salt and PTV calcium were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Rosuvastatin-d6 sodium salt and PTV-d5 sodium salt were purchased from Toronto Research Chemicals, Inc. (Ontario, Canada). Ko143 hydrate was purchased from Sigma-Aldrich (St. Louis, MO). Matrigel was purchased from BD Biosciences (San Jose, CA). Cryopreserved human hepatocytes (HMCPTS, Lot #: HU1437) were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA). All other chemicals and reagents were of analytical grade and readily available from commercial sources.

2.2. Seeding and culturing of SCHH

Cryopreserved human hepatocytes were thawed at 37 °C and placed into hepatocyte thawing medium (CM7500, Thermo Fisher Scientific, Inc.). The hepatocytes were centrifuged at 100 × *g* for 10 min at room temperature and resuspended in plating medium (Thermo Fisher Scientific, Inc.) consisting of Williams' E medium containing 5% fetal bovine serum supplemented with 1 μM dexamethasone, 4 μg/mL human insulin, 2 mM GlutaMAX, and 15 mM HEPES. The cells were seeded at a density of 7.5×10^5 cells/mL in a 24-well culture plate coated with Type I collagen (AGC Techno Glass Co., Ltd., Chiba, Japan). The seeded hepatocytes were incubated in a humidified chamber with 5% CO_2 at 37 °C for 24 h postseeding. The plating medium was replaced with incubation medium (Thermo Fisher Scientific, Inc.) consisting of serum-free Williams' E medium supplemented with 1 μM dexamethasone, 6.25 μg/mL human insulin, 6.25 μg/mL human transferrin, 6.25 μg/mL selenous acid, 1.25 mg/mL BSA, 5.35 μg/mL linoleic acid, 2 mM GlutaMAX, and 15 mM HEPES plus 250 μg/mL Matrigel (BD Biosciences, San Jose, CA). Subsequently, the incubation medium (without Matrigel) was changed daily. The SCHH were cultured for a total of four days.

2.3. Preparation of samples for total disposition profiling by D-PREX

The overall procedure for the D-PREX methodology used in this study is outlined in Fig. 1, with reference to a previous report [8]. On day 5 after seeding, the SCHH were rinsed and preincubated with 400 μL/well standard Hanks' Balanced Salt Solution containing Ca^{2+} and Mg^{2+} (Std HBSS) for 10 min in a humidified 37 °C incubator under 5% CO_2. After discarding the preincubation buffer, the SCHH were then incubated with 400 μL/well dosing solution containing a cocktail of 10 μM RSV and 10 μM PTV in Std HBSS, and placed in a 5%

Please cite this article in press as: Kanda K, et al., Total hepatocellular disposition profiling of rosuvastatin and pitavastatin in sandwich-cultured human hepatocytes, Drug Metabolism and Pharmacokinetics (2018), https://doi.org/10.1016/j.dmpk.2018.04.001