Original Article

Differential expression of several drug transporter genes in HepG2 and Huh-7 cell lines

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Abstract

Background: Cell culture techniques have many advantages for investigation of drug transport to target organ like liver. HepG2 and Huh-7 are two cell lines available from hepatoma that can be used as a model for hepatic drug transport. The present study is aimed to analyze the expression level of several drug transporter genes in two hepatoma cell lines, HepG2 and Huh-7 and their response to inhibitors.

Materials and Methods: This is an *in vitro* study using HepG2 and Huh-7 cells. The expression level of the following drug transporter genes was quantified: P-glycoprotein/multidrug resistance protein 1, Organic Anionic Transporter Protein 1B1 (OATP1B1) and Organic Cationic Transporter-1 (OCT1). Ribonucleic acid was extracted from the cells using Tripure isolation reagent, then gene expression level of the transporters is quantified using Applied Biosystems quantitative reverse transcriptase polymerase chain reaction. Verapamil (P-glycoprotein inhibitor), nelfinavir (OATP1B1 inhibitor), quinidine (OCT1 inhibitor) were used to differentiate the inhibitory properties of these agents to the transporter expressions in HepG2 and Huh-7 cells.

Results: Huh-7 shows a higher level of P-glycoprotein, OATP1B1 and OCT1 expressions compared with those of HepG2. Verapamil reduces the expressions of P-glycoprotein in HepG2 and Huh-7; nelfinavir reduces the expression of OATP1B1 in HepG2 and Huh-7; while quinidine reduces the OCT1 gene expressions in HepG2, but not in Huh-7 cells.

Conclusion: This study indicates that HepG2 might be a more suitable *in vitro* model than Huh-7 to study drug transport in hepatocytes involving drug transporters.

Key Words: HepG2, Huh-7, *in vitro* model, transporters

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INTRODUCTION

In the field of pharmacokinetics, the importance of drug transporters as factors in determining drug efficacy and tissue distribution and elimination has recently been recognized.[1,2] Drug elimination in the liver consists of the following process: (1) Hepatic uptake; (2) metabolism and/or (3) biliary excretion and (4) sinusoidal efflux from the inside of the cell to the blood. Among these process, drug transporters are

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involved in the uptake, sinusoidal efflux and biliary excretion.[3] It should be noted that hepatic uptake and biliary excretion determine the drug concentration in the liver.[4] Thus, action of drug transporters is also determinant of pharmacological effects of drugs whose target is in the liver.[3]

Until now, primary human hepatocytes are still gold standard to study human drug metabolism and transport, but their availability is limited.[2,5] Therefore, hepatoma cell lines can serve as valuable alternatives to study transport of drugs and xenobiotic to the liver. The use of cell lines has many advantages for investigation of drug transport to target organs like liver. The major advantage of cell lines is immediate availability, standardized culture conditions and unlimited life span.[6]

HepG2 and Huh-7 are two cell lines available from hepatoma that can be used as a model for hepatic drug transport. HepG2 is widely used human hepatocellular carcinomas that are highly differentiated and display many of the genotypic features of the normal liver cells.[7] HepG2 is a standard *in vitro* model for drug metabolism and transport study, despite the low expression levels of drug metabolizing enzymes.[8,9] Recently, Huh-7, a human hepatoma cell line, frequently used as *in vitro* system to study hepatotoxicity, hepatitis C virus infection and gene regulation, has been used as an alternative to HepG2 cell line for drug metabolism and transport study.[10]

The present study is aimed to analyze the expressions of several drug transporters in two hepatoma cell lines, HepG2 and Huh-7 and their response to inhibitors. Tissue specific messenger ribonucleic acid (mRNA) expression profiles proved to be important information to study the mechanism of drug disposition. The information gained from this study provides gene expression profiles of HepG2 and Huh-7 cell lines for the use of future research using *in vitro* model for drug transports in the liver.

MATERIALS AND METHODS

Cell culture

HepG2 cells were obtained from BPPT Serpong while Huh-7 was a kind gift from Dr. Chie Aoki, Kobe University.

The human hepatoma HepG2 cell line was cultured in Roswell Park Memorial Institute 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin and 1% fungizone.

The human hepatoma Huh-7 cell line was grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin and 100 μg/mL streptomycin, 1% Fungizone and 1% non-essential amino acids. Medium was routinely changed every 2 days. The cells were sub-cultured when reaching 90% of confluence. All the cell culture plates were purchased from NUNC Thermo Fisher Scientific and culture media and supplements from Invitrogen.

RNA extraction

Total RNA was extracted using Tripure Isolation Reagents (Roche) according to the manufacturer's protocol. Quantity and purity of the RNA were determined by measuring absorbance in 260/280 nm wavelength using NanoDrop spectrophotometer. RNA was then subjected to quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

qRT-PCR

The mRNA expression levels of the following drug transporters were determined: P-glycoprotein/ABCB1, Organic Anionic Transporter Protein 1B1 (OATP1B1) and Organic Cationic Transporter-1 (OCT1).

qRT-PCR was done using SYBR Green methodology from KAPA Biosystem, USA and ABI Prism 7500 detector (Applied Biosystems). Primers for transporters are as described previously:

ABCB1, ABCB1_F: AAAGCGACTGAATGTTCAGTGG; ABCB_R: TTCACCTTCCCAAAGACCAC.[11] OATP1B1, OATP1B1_F: GAATGCCCAAGAGATGATGCTT; OATP1B1_R: AATCCAGTGCAAGTGATTTCAAT.[12] OCT1, OCT1_F: CGCCGAGAACCTTGGGAGAAA; OCT1_R: AATAGATGCCTTTCTGTGCCAG.[13] Cyclophilin A was used as housekeeping gene. Primers for cyclophilin A: cyclophilinA_F AGCATACAGGTCCTGGCATC; cyclophilinA_R: TTCACCTTCCCAAAGACCAC.[14]

PCR reaction mixture was prepared using the following reagents: 10 μL KAPA SYBR Fast qPCR master mix, 200 nM forward primer, 200 nM reverse primer, 200 μM dUTP, 0,4 μL KAPA RT mix, 500 ng RNA template and RNAse free water up to 20 μL.

Samples were incubated in qRT-PCR machine with the following conditions: Complementary deoxyribonucleic acid synthesis at 50°C for 2 min, RT enzymes inactivation at 95°C for 10 min, 40 cycles consists of 15 s at 95°C for denaturation step; 1 min at 60° for annealing step and 30 s for extension step. Melting curve analysis was performed for 80 cycles of: 1 min at 95°C; 1 min at

60°C and 15 s at 95°C. Ct was generated automatically by ABI Prism™ software (Applied Biosystems Inc). Level of mRNA transporter expressions in untreated HepG2 and Huh-7 cells were calculated using the equation of 2^-d(Ct),[15,16] while the relative mRNA transporter expressions in cells treated with inhibitors were calculated using Livak and Schmittgen method.[17]

Treatment with inhibitors

Verapamil (P-glycoprotein inhibitor), nelfinavir (OATP1B1 inhibitor), quinidine (OCT1 inhibitor) were used to differentiate the inhibitory properties of these agents to the transporter gene expression levels in HepG2 and Huh-7 cells.

HepG2 and Huh-7 cells were exposed to verapamil (10, 25 and 50 μM) for P-glycoprotein inhibition, or nelfinavir (1, 2.5 and 5 μM) for OATP1B1 inhibition or quinidine (25, 63 and 125 μM) for OCT1 inhibition for 24 h. All the inhibitors were dissolved in double-distilled water then diluted with serum-free medium.

Data analysis

The data were presented in the form of means ± standard deviation. Graphs were created using GraphPad Prism software (GraphPad Software, Inc, USA).

RESULTS

Comparison of cell morphology and growth characteristics

HepG2 and Huh-7 cells morphology have closely related characteristics. The photographs of cell morphology are shown in Figure 1. HepG2 and Huh-7 cells display the typical epithelial-like morphology. Both cells show uniform shapes throughout the vessel. HepG2 cells are somewhat bigger and easier to observe under inverted microscope.

Growth characteristics

To observe growth characteristics of HepG2 and Huh-7 cells, suspension of cells containing 300,000 cells/well were applied to 6-well plates. Number of the cells was counted every day up to 6 days after plating. HepG2 cells grow faster and easier with calculated doubling time of 2.05 days and Huh-7 with 1.98 days [Figure 2].

Level of mRNA expressions of P-glycoprotein, OATP1B1 and OCT1

We calculated level of mRNA expressions of P-glycoprotein, OATP1B1 and OCT1 with the equation of 2^-d (Ct) (normalized to cyclophilin A as reference gene) [Figure 3]. We find that for all three transporters, HepG2 showed significantly lower level of mRNA expressions compared with those in Huh-7 cells.

Relative mRNA expression levels of P-glycoprotein, OATP1B1 and OCT1 in HepG2 cells after treatment with inhibitors

Figure 4 gives detail information on the inhibitory properties of verapamil, nelfinavir and quinidine on P-glycoprotein, OATP1B1 and OCT1 mRNA expression, respectively. Verapamil modifies P-glycoprotein mRNA expressions in a dose-dependent manner. Nelfinavir strongly modifies mRNA expressions of OATP1B1 also in a dose-dependent manner. While quinidine decreases the mRNA

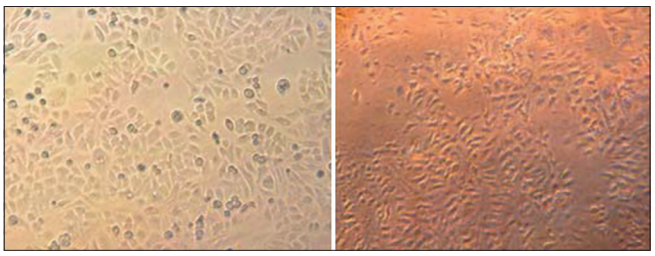

Figure 1: Morphology of cells used for analysis of transporter gene expression level. (a) HepG2 cells, (b) Huh-7 cells. Photographed under contrast phase microscope

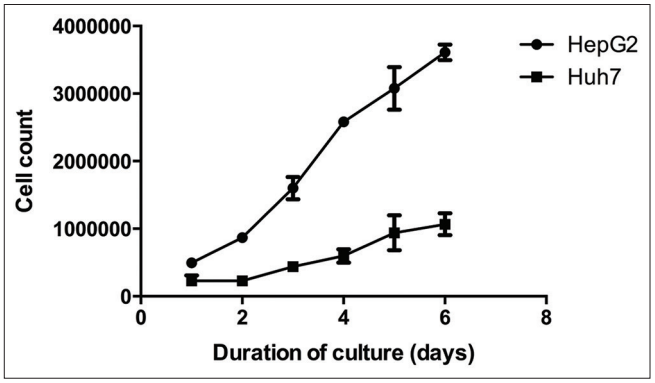

Figure 2: Growth of HepG2 and Huh-7 cells from day 1 to day 6. Calculated doubling time for HepG2 cells = 1.98 days, while for Huh-7 cells = 2.05 days

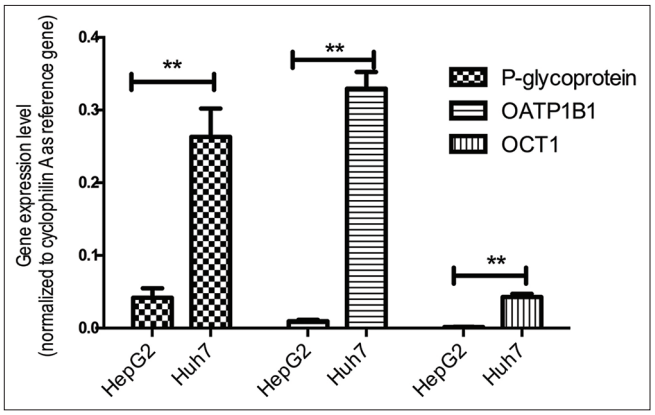

Figure 3: Messenger ribonucleic acid expression levels of P-glycoprotein, Organic Anionic Transporter Protein 1B1 and Organic Cationic Transporter-1 as calculated by 2^-d(Ct) (normalized to cyclophilin A as reference gene). ***P*<0.001

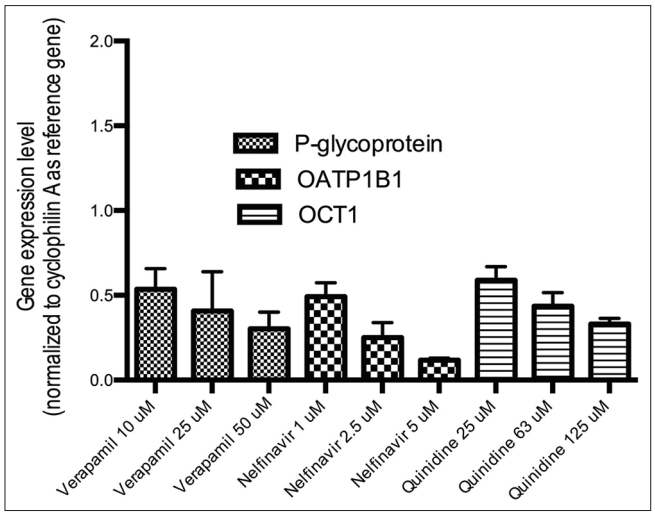

Figure 4: Gene expression levels of several drug transporter genes after treatment with inhibitors: Verapamil (P-glycoprotein inhibitor); nelfinavir (Organic Anionic Transporter Protein 1B1 inhibitor) and quinidine (Organic Cationic Transporter-1 inhibitor) in HepG2 cells

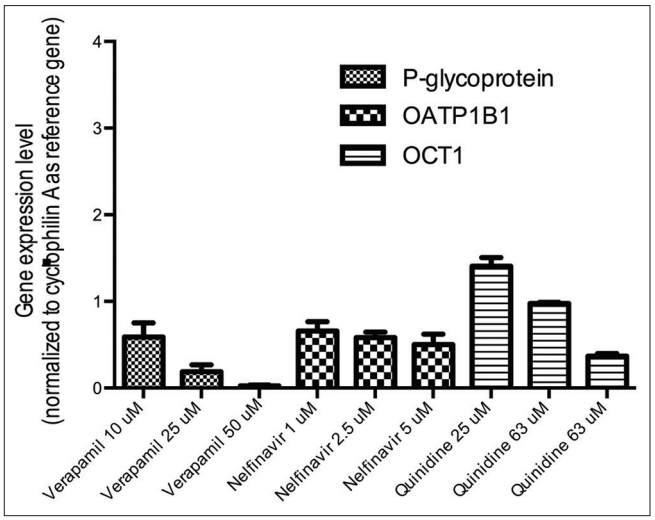

Figure 5: Relative expressions of several drug transporters gene after treatment with inhibitors: P-verapamil (P-glycoprotein inhibitor); nelfinavir (Organic Anionic Transporter Protein 1B1 inhibitor) and quinidine (Organic Cationic Transporter-1 inhibitor) in Huh-7 cells

expression level of OCT1 at concentrations of 25, 63 and 125 μM.

Relative mRNA expressions of P-glycoprotein, OATP1B1 and OCT1 in Huh-7 cells after treatment with inhibitors

Inhibitory effect of verapamil, nelfinavir and quinidine on P-glycoprotein, OATP1B1 and OCT1 were presented in Figure 5. Huh-7 exposure to verapamil resulted in the reduction of Pgp mRNA expression. Nelfinavir at concentration of 1, 2.5 and 5 μM reduces the expression of OATP1B1 mRNA expression in Huh-7 cells. Unlike the effect of quinidine in HepG2 cells, quinidine at concentrations of 25 and 63 μM did not result in the reduction of OCT1 expression. Quinidine can suppress the expression of OCT1 only at the highest concentration used (125 μM).

DISCUSSION

Cell lines are often used as *in vitro* models of human tissues.[18] However, gene expression levels were in general low in comparison with those in human tissues.[16] For the study of drug transport in the liver, primary human hepatocytes are still gold standard.[2,5] However, due to limited availability of human hepatocytes, cell culture can be used as an alternative. Therefore, characterization of drug transporters in hepatoma cell lines is important, that can provide valuable information for future studies.

Many of the studies on gene expressions for drug metabolism and transports for several cell lines have been reported previously.[6,19] Despite the wide use of HepG2 and Huh-7 as tools for pharmacological and toxicological studies,[6,7,10] recent findings by Guo *et al*.[19] in several hepatic cell lines showed that HepG2 and Huh-7 have a large difference in drug-metabolizing enzymes and transporters expression levels.

Our present study confirmed previous findings reported by Guo *et al*.[19] which showed that HepG2 cells expressed significantly lower levels of drug transporter genes. Our study were not only confirming existing knowledge on transporter gene expression levels, but also reported their response to inhibitors that have not been reported previously.

Transporters investigated in this study are three major transporters in the liver.[20,21] P-glycoprotein is the most recognized efflux transporter that mediates the transports of hydrophobic cations.[22] OATP1B1 and OCT1 are uptake transporters that extract its substrates from portal blood into the hepatocytes.[23] OATP1B1 has been shown to transport organic anionic compounds and OCT1 transports cationic organic substances.[24]

Our results showed that HepG2 expressed a low level of P-glycoprotein, OATP1B1 and OCT1. This result is in accordance with the Hilgendorf *et al*.[15] that showed that HepG2 express a low level of transporter genes. Huh-7 cells expressed a much higher level of P-glycoprotein, OATP1B1 and OCT1.

Our study showed that Huh-7 expressed moderate level of transporter genes. This results confirmed similar findings reported by Sivertsson *et al*.[10] and Guo *et al*.[19] However, choosing a specific cell lines as surrogate for human hepatocytes is rather difficult, given the markedly high variability across studies in

primary hepatocytes. The study by Nishimura and Naito[21] in normal hepatocytes showed that the overall expressions of drug transporters is low while other studies reported by Nishimura and Naito[25] reported that the expression of OCT1 is relatively high while for OATP1B1 and P-glycoprotein is very low.

After the determination of transporter's mRNA expression levels in HepG2 and Huh-7 cells, these cells were exposed to inhibitors of P-glycoprotein, OATP1B1 and OCT1. We followed EMA guideline for *in vitro* transport studies that suggests the use of substrate and inhibitors. We chose three concentrations of inhibitors that showed significant inhibitory effects in transporter gene expressions reported previously.[26-31]

Our results showed that HepG2 cells are more sensitive to drug inhibitions than Huh-7 cells. This might be due to the lower level of transporters expressed in HepG2 cells. Huh-7 showed significantly higher level of transporter's expressions that the cells also need significantly higher concentrations of inhibitors to suppress the level of expressions. The most plausible explanation for the difference might be due to the variability of transcription factors expressed in the two cells.[32] This different characteristic has to be taken to the account when one of the two cells must be chosen for drug transport studies. Our results are based on mRNA expression data, which may not always correlated with the expression of encoded proteins. However, for P-glycoprotein, in the literature we can find a good correlation between mRNA level, protein expressions and protein functionality.[33,34]

CONCLUSION

The present study showed a differential response of cells to the inhibitory effects of inhibitors used in our study (verapamil, nelfinavir and quinidine). We propose that both cells can be used as *in vitro* system for the study of transporters, but HepG2 cells are more sensitive to drug inhibitions than in Huh-7 cells.

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