Toxicology Letters 235 (2015) 107–115

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Expression of hepatic cytochrome P450s and UDP-glucuronosyltransferases in PXR and CAR double humanized mice treated with rifampicin

Sang Yoon Lee [a], Ji-Yoon Lee [a], Young-Mi Kim [b], Sang Kyum Kim [a,*], Soo Jin Oh [c,**]

[a] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea
[b] College of Pharmacy, Hanyang University, Ansan, Gyeonggido 426-791 Republic of Korea
[c] Bio-Evaluation Center, KRIBB, 685-1 Yangcheong-ri, Ochang-eup, Cheongwon-gun, Chungbuk 363-883, Republic of Korea

HIGHLIGHTS

- Rifampicin increased microsomal protein and total CYP in the CAR/PXR humanized mice.
- Cyp2b10, Cyp2c, and Cyp3a11 were increased in the rifampicin-treated humanized mice.
- Rifampicin increased activities of Ugt1a6 and Ugt1a9 only in the humanized mice.
- Increase in Ugt1a1 activity by rifampicin was observed in both mice strains.
- The CAR/PXR humanized mice may be suitable for predicting drug–drug interactions.

ARTICLE INFO

Article history:
Received 9 February 2015
Received in revised form 25 March 2015
Accepted 27 March 2015
Available online 30 March 2015

Keywords:
Nuclear receptor
Humanized mice
Rifampicin
Cytochrome P450
UDP-glucuronosyltransferase

ABSTRACT

Nuclear receptor humanized mice models have been developed to predict regulation of drug metabolizing enzyme by xenobiotics. However, limited information is available concerning xenobiotic-induced regulation of drug metabolizing enzymes in multiple nuclear receptor humanized mice. The present study investigated the hepatic regulation of cytochrome P450s (CYPs) and UDP-glucuronosyltransferases (UGTs) in the pregnane X receptor (PXR) and the constitutive androstane receptor double humanized mice treated with rifampicin (RIF; 10 mg/kg) for 4 days. RIF increased hepatic microsomal protein and total CYP contents, and CYP reductase activity in the humanized mice, but not in normal mice. Moreover, hepatic induction of Cyp2b10, Cyp2c, and Cyp3a11 were observed only in the RIF-treated humanized mice, suggesting that the humanized mice are sensitive to RIF with respect to the regulation of the hepatic CYP system. Hepatic UGT activities using estradiol, serotonin, and mefenamic acid, but not chenodeoxycholic acid as substrates, increased in the RIF-treated humanized mice, and the glucuronidation activities of estradiol and chenodeoxycholic acid increased in RIF-treated normal mice. These results raise the possibility that a PXR-independent mechanism may be involved in hepatic regulation of UGTs by RIF.

1. Introduction

Hepatic drug metabolism plays a major role in the disposition of xenobiotics, including therapeutic drugs or environmental pollutants, and endogenous compounds (Kim and Novak, 2007). Cytochrome P450 (CYP) enzymes are responsible for the oxidative metabolism of xenobiotics with a variety of structures. Glucuronidation by UDP-glucuronosyltransferases (UGTs) is a conjugative clearance system with a large capacity. Hepatic expression of drug metabolizing enzymes may be altered in response to xenobiotics or pathophysiological conditions such as diabetes, long-term alcohol consumption, and inflammation (Kim and Novak, 2007). The change in CYP and UGT activity by xenobiotics is one of the most common causes of drug–drug interactions (DDI) that can result in drug toxicity and failure (Donato et al., 2010).

* Corresponding author at: College of Pharmacy and RCTCP, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Republic of Korea. Tel.: +82 42 821 5930; fax: +82 42 823 6566.
** Corresponding author at: Bio-Evaluation Center, KRIBB, Ochang, Chungbuk, Republic of Korea. Tel.: +82 43 240 6539; fax: +82 43 240 6529.
E-mail addresses: sangkim@cnu.ac.kr (S.K. Kim), diatree@kribb.re.kr (S.J. Oh).

http://dx.doi.org/10.1016/j.toxlet.2015.03.015
0378-4274/

Xenobiotic metabolism mostly occurs in the liver, but substantial biotransformation activity is observed in a variety of tissues. Hepatic regulation of drug metabolizing enzymes is mainly mediated at the transcriptional level through receptors, such as the aryl hydrocarbon receptor, and nuclear receptors including the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR). The nuclear receptors have a DNA binding domain and a ligand binding domain. Species differences in nuclear receptor ligand specificity have been observed (Oswal et al., 2013). In particular, mouse PXR is not activated by rifampicin (RIF), a strong ligand of human PXR. Conversely, pregnenolone-16alpha-carbonitrile (PCN) is a strong activator of mouse PXR, but not human PXR.

Pooled human liver microsomes (HLMs), human hepatocytes, and human liver-derived cell lines have been used as *in vitro* models to evaluate the DDI potential of xenobiotics. Pooled HLMs are not suitable to evaluate transcriptional regulation of drug metabolizing enzymes. Primary cultured human hepatocytes are widely used in drug metabolism studies, but huge variations in the magnitude of induction following treatment with prototypical inducers have been reported between human hepatocyte populations (Madan et al., 2003). Moreover, *in vitro* models cannot evaluate systemic factors involved in pharmacokinetic regulation and, consequently, pharmacological and toxicological events.

Transgenic humanized mice models were recently developed to overcome inter-species differences in ligand recognition of nuclear receptors. PXR and CAR play a critical role in the transcriptional regulation of CYPs, UGTs, glutathione *S*-transferases, and transporters. Studies have shown that PXR humanized mice developed using different transgenic strategies demonstrated a marked induction of Cyp3a11 by RIF but not PCN (Xie et al., 2000; Ma et al., 2007; Scheer et al., 2008). This suggests that humanized mice models may overcome species differences, and improve the study of drug metabolism and disposition. However, Cyp2b10 was not induced by RIF in PXR humanized mice (Scheer et al., 2008), although PXR has a role in controlling CYP2B through a cross-talk mechanism (Faucette et al., 2006; Nannelli et al., 2008).

Nuclear receptors play a key role in the regulation of target genes through interaction with other nuclear receptors, but limited information is available concerning xenobiotic-induced regulation of drug metabolizing enzymes in multiple nuclear receptor humanized mice. Moreover, most studies using nuclear receptor humanized mice have focused on a single or limited target CYPs. CAR and PXR are major xenobiotic receptors involved in the regulation of xenobiotic metabolizing enzymes. CAR, like PXR, exhibits species differences in ligand specificity (Chai et al., 2013) and there is a significant cross-talk between CAR and PXR in the regulation of drug metabolizing enzymes. The present study investigated the hepatic regulation of CYPs and UGTs in CAR and PXR double humanized mice treated with RIF.

2. Materials and methods

2.1. Chemicals and reagents

RIF, glycerol, sodium dithionite (sodium hydrosulfite), NADH, reduced NADPH, cytochrome c, resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, tolbutamide, 4-hydroxytolbutamide, chlorzoxazone, 6-hydroxychlorzoxazone, carbamazepine, saccharo-1,4-lactone, alamethicin, magnesium chloride, β-estradiol, chenodeoxycholic acid, serotonin, mefenamic acid, and uridine 5′-diphosphoglucuronic acid (UDPGA) were purchased from Sigma–Aldrich (St. Louis, MO). Chenodeoxycholic acid 24-acyl-β-D-glucuronide, serotonin β-D-glucuronide, and mefenamic acid acyl-β-D-glucuronide were purchased from Toronto Research Chemical (Toronto, Canada). β-Estradiol-3-glucuronide, midazolam or 1′-hydroxymidazolam was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), Bukwang Pharma Co. (Seoul, Republic of Korea) or BD Gentest Co. (Woburn, MA), respectively. Antibodies to Cyp1a2, Cyp2c and Cyp2e1 were purchased from Detroit R&D (Detroit, MI), the anti-Cyp2b10 and Cyp3a11 antibodies were purchased from Millipore (Temecula, CA), and anti-Gβ antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents and chemicals were of analytical or HPLC grade.

2.2. Animals

C57BL/6 male wild-type and humanized CARh-PXRh mice aged 7 weeks (C57BL/6-Nr1i2$^{tm1(NR1I2)\ Arte}$Nr1i3$^{tm1(NR1I3)\ Arte}$; Scheer et al., 2008) were obtained from Taconic Labs (Germantown, NY). All animals were maintained at Korea Research Institute of Bioscience and Biotechnology (Ochang, Republic of Korea). The mice were housed under temperature (22 ± 2 °C)- and humidity (55 ± 5%)-controlled conditions with a 12/12 h light/dark cycle for at least 1 week before the experiments. Laboratory chow and tap water were provided *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee and performed in accordance with the institutional guidelines.

2.3. Animal treatments and preparation of hepatic microsomes

The mice were intraperitoneally injected with either the vehicle or RIF (10 mg/kg/d) for 4 days and sacrificed 24 h after the last dose. The liver tissues were homogenized in 3 volumes of ice-cold buffer, consisting of 0.154 M KCl, 50 mM Tris–HCl, and 1 mM EDTA (pH 7.4), respectively. The homogenate was centrifuged (10,000 × *g*, 20 min) and the supernatant fraction was further centrifuged (104,000 × *g*, 65 min). The microsomal pellet was suspended and recentrifuged (104,000 × *g*, 65 min). The microsomes were diluted to an equivalent of 1.0 g of tissue/mL of buffer.

Table 1
Weights of the body and liver, and liver to body ratio in normal and humanized mice treated with RIF.

	Treatment	Body weight (g)	Liver weight (g)	Liver weight/body weight
Normal mice	Vehicle	26.2 ± 0.8	1.2 ± 0.1	0.045 ± 0.002a
	RIF	26.4 ± 1.1	1.2 ± 0.1	0.047 ± 0.002a
Humanized mice	Vehicle	24.7 ± 1.6	1.4 ± 0.1	0.056 ± 0.001b
	RIF	23.3 ± 1.2	1.5 ± 0.3	0.064 ± 0.013b

Mice were intraperitoneally treated with 10 mg/kg RIF once a day for 4 days and sacrificed 24 h after the last dose. Each value represents the mean ± SD for five mice. Significant differences among groups were determined using an analysis of variance (ANOVA) followed by the Newman–Keuls multiple comparison test. Values with different letters are significantly different from each other, $P < 0.05$. There was no significant difference between corresponding vehicle-treated group and refampicin-treated group (two-tailed Student's *t*-test).