Faculty of Pharmaceutical Sciences[1], Okayama University, Okayama; Laboratory of Pharmaceutics[2], Laboratory of Clinical Pharmaceutics[3]; Laboratory of Biochemical Toxicology[4], Yokohama College of Pharmacy, Yokohama, Japan

In vitro glucuronidation of propofol in microsomal fractions from human liver, intestine and kidney: tissue distribution and physiological role of UGT1A9

M. Mukai[1], S. Tanaka[1], K. Yamamoto[1], M. Murata[2], K. Okada[3], T. Isobe[3], M. Shigeyama[3], H. Hichiya[4], N. Hanioka[4]

Received February 28, 2014, accepted March 28, 2014

Dr. Nobumitsu Hanioka, Professor, Laboratory of Biochemical Toxicology, Yokohama College of Pharmacy, 601 Matano-cho, Totsuka-ku, Yokohama 245-0066, Japan
nhanioka@hamayaku.ac.jp

Pharmazie 69: 829–832 (2014) *doi: 10.1691/ph.2014.4564*

Propofol (2,6-diisopropylphenol) is intravenously administered for anesthetic induction and maintenance, and is rapidly metabolized into its glucuronide, mainly by UDP-glucuronosyltransferase 1A9 (UGT1A9). In this study, propofol glucuronidation by liver microsomes (HLM), intestinal microsomes (HIM) and kidney microsomes (HKM) of humans were examined. The expression of UGT1A9 protein in HLM, HIM and HKM was analyzed by immunoblotting. The staining band intensities for UGT1A9 of HIM and HKM were 12% and 119% those of HLM, respectively. The kinetics of propofol glucuronidation by HLM and HKM exhibited substrate inhibition, whereas the kinetics by HIM followed the Michaelis-Menten model. The K_m, V_{max} and CL_{int} values of HLM were 41.8 μM, 5.21 nmol/min/mg protein and 126 μl/min/mg protein, respectively. The K_m value of HIM was significantly higher (6.7-fold) than that of HLM, and the V_{max} and CL_{int} values were significantly lower (56% and 8.3%, respectively) than those of HLM. The K_m value of HKM was comparable to that of HLM, and the V_{max} and CL_{int} values were significantly higher (2.1- and 3.7-fold, respectively) than those of HLM, respectively. These findings suggest that UGT1A9 expressed in the kidney as well as in the liver plays an important role in propofol glucuronidation. The information gained in this study should contribute to an appropriate use of drugs metabolized by UGT1A9.

1. Introduction

Propofol (2,6-diisopropylphenol) is intravenously administered for the induction or maintenance of anesthesia, because it has a smooth and rapid onset of action, which makes dose titration possible. It is rapidly eliminated from the body, and conjugation catalyzed by UDP-glucuronosyltransferase (UGT) in the liver is a major pathway of metabolism in humans (Fig. 1) (Langleyand Heel 1988; Vanlersberghe and Camu 2008). Among the isoforms of the human UGT family, UGT1A9 is predominant in the glucuronidation of propofol (Court 2005; Kiang et al. 2005). In addition, UGT1A8 expressed in extrahepatic tissues such as the small intestine and colon has been also reported to be capable of glucuronidating propofol (Cheng et al. 1999; Kiang et al. 2005). Oxidation (ω- and 4-hydroxylation) catalyzed by cytochrome P450 has also been observed (Murayama et al. 2007; Vanlersberghe and Camu 2008).

Human UGT1A9 is expressed not only in the liver but also in extrahepatic tissues such as the kidney and small intestine, and the expression levels of mRNA and protein in the kidney are about 3–5-fold higher than those in the liver (Ohno and Nakajin 2009; Harbourt et al. 2012; Fallon et al. 2013). Furthermore, large inter-individual variability in the expression levels of UGT1A9 mRNA and protein in the human liver have been suggested (Izukawa et al. 2009; Oda et al. 2012). Such variations can be expected to affect the plasma concentration, efficacy and side-effects of propofol. Recently, Gill et al. (2012) proposed the importance of renal metabolism to predict the physiological pharmacokinetics of drugs metabolized mainly by UGT1A9 based on static *in vivo-in vitro* methods. However, there is little information on the *in vitro* glucuronidation of propofol in extrahepatic tissues. This study was undertaken to analyze the kinetics of propofol glucuronidation by liver microsomes (HLM), intestinal microsomes (HIM) and kidney microsomes (HKM) of humans.

2. Investigations and results

2.1. Expression of UGT1A9 in HLM, HIM and HKM

The expression levels of UGT1A9 protein in HLM, HIM and HKM were assessed by immunoblotting using anti-human UGT1A9 antibody (Fig. 2). All microsomes yielded immunodetectable UGT1A9 protein. The staining intensity band of HKM was stronger than that of HLM, whereas the intensity of HIM was weak compared with that of HLM. The relative levels were HLM (100), HIM (12) and HKM (119), where the HLM level was normalized to 100.

2.2. Propofol glucuronidation activities in HLM, HIM and HKM

Glucuronidation activities of propofol in HLM, HIM and HKM were determined at substrate concentrations of 10, 100 and 1000 μM (Fig. 3). Glucuronidation activities in HLM at 10, 100

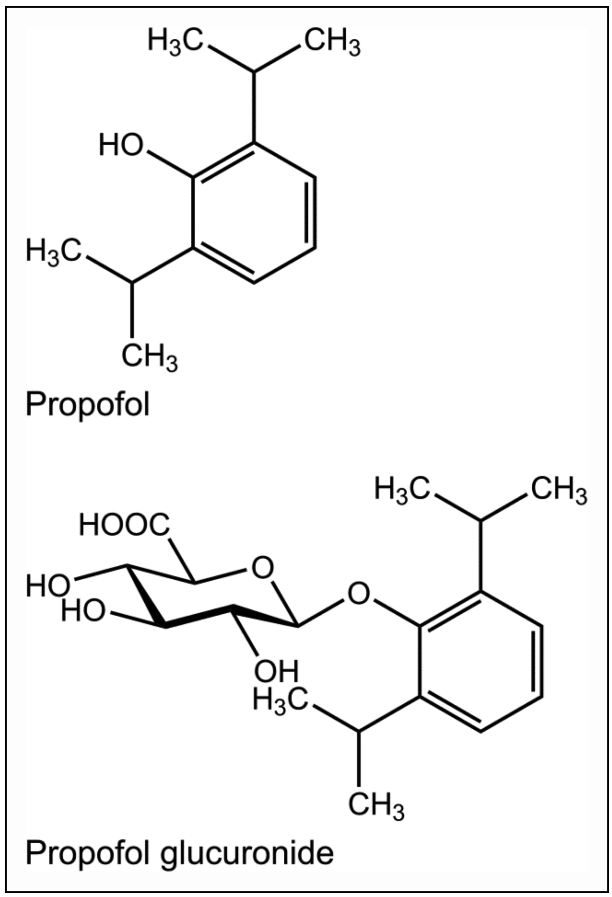

Fig. 1: Chemical structures of propofol and its glucuronide.

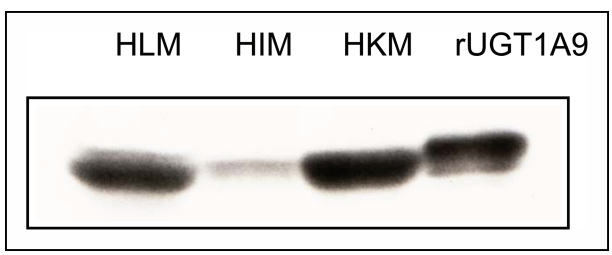

Fig. 2: Immunoblotting of HLM, HIM, HKM and recombinant human UGT1A9. Protein levels applied were HLM, HIM and HKM (each 50 μg protein/lane) and recombinant UGT1A9 (rUGT1A9, 20 μg protein/lane).

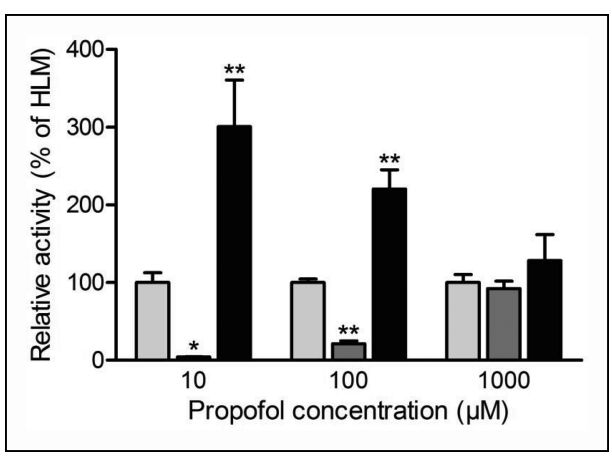

Fig. 3: Propofol glucuronidation activities in HLM, HIM and HKM. The results are expressed as a percentage of the activity of HLM. The activities of HLM at 10, 100 and 1000 μM substrate concentrations were 1.05 ± 0.13, 3.46 ± 0.16 and 2.42 ± 0.25 nmol/min/mg protein, respectively. Each column represents the mean ± SD of three separate experiments. □, HLM; ▒, HIM; ■, HKM. Significantly different from HLM (*$P < 0.05$, **$P < 0.01$).

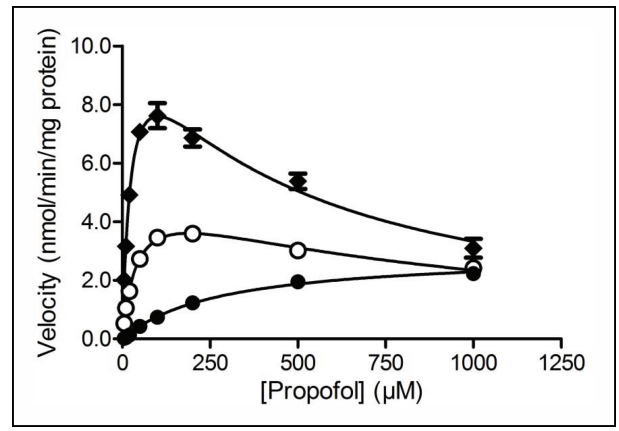

Fig. 4: Kinetics of propofol glucuronidation by HLM, HIM and HKM. Substrate concentrations were 5–1000 μM. Each point represents the mean ± SD of three separate experiments. ○, HLM; ●, HIM; ◆, HKM.

and 1000 μM propofol were 1.05, 3.46 and 2.42 nmol/min/mg protein respectively. Glucuronidation activities in HIM at 10 and 100 μM propofol were significantly lower (4.2% and 21%, respectively) than those in HLM. Glucuronidation activities in HKM at 10 and 100 μM propofol were significantly higher (3.0- and 2.2-fold, respectively) than those in HLM. However, glucuronidation activities in HIM and HKM at 10 μM propofol were comparable to that in HLM.

Kinetic analysis of propofol glucuronidation by HLM, HIM and HKM was performed for further detailed information. The V-[S] plots and parameters of kinetics are shown in Fig. 4 and Table. The kinetics for propofol glucuronidation by HLM exhibited substrate inhibition with a K_{si} value of 852 μM, and the K_m, V_{max} and CL_{int} values were 41.8 μM, 5.21 nmol/min/mg protein and 126 μl/min/mg protein, respectively. The kinetics for propofol glucuronidation by HIM followed the Michaelis-Menten model. The K_m value of HIM was significantly higher (6.7-fold) than that of HLM, and the V_{max} and CL_{int} values were lower (56% and 8.3%, respectively) than those of HLM. The kinetics for propofol glucuronidation by HKM exhibited substrate inhibition. The K_{si} value was significantly (52%) lower than that of HLM. The K_m value of HKM was 58% that of HLM, although no significant difference was observed between HLM and HKM. The V_{max} and CL_{int} values were significantly higher (2.1- and 3.7-fold, respectively) than those of HLM, respectively.

3. Discussion

Propofol is used clinically as an intravenous anesthetic which is chemically unrelated to other anesthetics. It is rapidly metabolized, and the majority of the dose is excreted into urine as propofol glucuronide in humans (Langleyand Heel 1988; Vanlersberghe and Camu 2008). The glucuronidation of propofol in humans is catalyzed mainly by UGT1A9 (Court 2005; Kiang et al. 2005). Since human UGT1A9 is extensively expressed not only in the liver but also in extrahepatic tissues such as the kidney and small intestine (Ohno and Nakajin 2009; Harbourt et al. 2012; Fallon et al. 2013), it is valuable to perform an *in vitro* study of propofol glucuronidation using enzyme sources from hepatic and extrahepatic tissues. In this study, propofol glucuronidation by HLM, HIM and HKM was investigated using enzymatic kinetics.

UGT1A9 protein was confirmed to be expressed in HLM, HIM and HKM by immunoblotting. The rank order of staining band intensity was HKM > HLM ≫ HIM. This tissue distribution of UGT1A9 expression generally correlated to previous reports (Harbourt et al. 2012; Fallon et al. 2013).

Table: Kinetic parameters for propofol glucuronidation by HLM, HIM and HKM

	K_m (μM)	V_{max} (nmol/min/mg protein)	CL_{int} (μl/min/mg protein)	K_{si} (μM)	Model
HLM	41.8 ± 4.2	5.21 ± 0.11	126 ± 14.2	852 ± 108	Substrate inhibition
HIM	$282 \pm 14^{**}$	$2.93 \pm 0.30^{*}$	$10.4 \pm 0.6^{**}$		Michaelis-Menten
HKM	24.4 ± 2.8	$11.2 \pm 1.3^{**}$	$463 \pm 46^{**}$	$447 \pm 129^{*}$	Substrate inhibition

Each value represents the mean ± SD of three separate experiments. Significantly different from HLM ($^{*}P<0.05$, $^{**}P<0.01$).

The kinetics for propofol glucuronidation by HLM and HKM exhibited substrate inhibition, whereas the kinetics by HIM fitted the Michaelis-Menten model. In addition, the values of kinetic parameters for propofol glucuronidation varied among HLM, HIM and HKM: K_m value, HIM $\gg$ HLM $\geq$ HKM; V_{max} value, HKM > HLM > HIM; CL_{int} value, HKM > HLM $\gg$ HIM. The results of V_{max} and CL_{int} values seem to reflect the expression levels of UGT1A9 in HLM, HIM and HKM. Ohno and Nakajin (2009) reported that the relative mRNA expressions of UGT1A9 of total UGTs in the human liver, small intestine and kidney were 1.8%, 0.5% and 51%, respectively. Furthermore, human UGT1A8 has been reported to contribute to the glucuronidation of propofol, although its *in vitro* activity is lower than that of UGT1A9 (Cheng et al. 1999; Kiang et al. 2005). The mRNA and protein of UGT1A8 have found to be expressed in the small intestine, colon and kidney, but not in the liver (Ohno and Nakajin 2009; Harbourt et al. 2012). The various kinetic models and parameter values for propofol glucuronidation among HLM, HIM and HKM are thought to be due to the difference in the relative and absolute expression levels of UGT1A9 and UGT1A8 in each tissue.

Several reports have proposed prediction methods of *in vivo* clearance to assess drug metabolism in each tissue from *in vitro* data (Obach et al. 1997; Soars et al. 2002; Yamanaka et al. 2007). According to previous reports, the *in vivo* clearance of hepatic, intestinal and renal glucuronidation of propofol in this study was estimated to be 112, 0.94 and 90.9 ml/min/kg body weight, respectively. The rank order of tissue clearance was liver $\geq$ kidney $\gg$ intestine, and it was suggested that UGT1A9 is expressed in the kidney as well as in the liver, but also that the kidney plays an important role in propofol glucuronidation. Therefore, the tissue distribution of UGT1A9 expression as well as the functional change of UGT1A9 *via* drug-drug interactions and genetic polymorphism (Kiang et al. 2005; Villeneuve et al. 2003; Jinno et al. 2003; Girard et al. 2004) is considered to be a factor in the inter-individual differences in the clinical response to propofol.

In conclusion, propofol glucuronidation by HLM, HIM and HKM was studied by kinetic analysis. The expression level of UGT1A9 protein in microsomes determined by immunoblotting was HKM > HLM $\gg$ HIM. The kinetics for propofol glucuronidation by HLM and HKM exhibited substrate inhibition, and the kinetics by HIM followed the Michaelis-Menten model. The K_m value of HIM was significantly higher than that of HLM, and the K_m value of HKM was comparable to that of HLM. The V_{max} and CL_{int} values of HIM and HKM were significantly low and high compared with those of HLM, respectively. These findings suggest that UGT1A9 expressed in the kidney as well as in the liver plays an important role in propofol glucuronidation. The information gained in this study should contribute to the appropriate use of drugs metabolized mainly by UGT1A9.

4. Experimental

4.1. Materials

Propofol and propofol glucuronide were from Santa Cruz Biotechnology (Santa Cruz, CA, USA); HLM, HIM and HKM were from XenoTech (Lenexa, KS, USA); recombinant human UGT1A9-expressing insect cells were from Corning (Corning, NY, USA); rabbit anti-human UGT1A9 was from Abnova (Taipei City, Taiwan); and peroxidase-conjugated goat anti-rabbit immunoglobulin was from Invitrogen (Carlsbad, CA, USA). All other chemicals and reagents used were of the highest quality commercially available.

4.2. Immunoblotting

HLM, HIM and HKM (each 50 μg protein/lane) and recombinant human UGT1A9 (20 μg protein/lane) as a positive control were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and electrotransferred to a polyvinylidene fluoride sheet. The sheet was incubated with rabbit anti-human UGT1A9 antibody (diluted at 1:2000) as the primary antibody and then with peroxidase-conjugated goat anti-rabbit immunoglobulin (diluted at 1:5000) as the secondary antibody. Immunoreactive proteins were visualized with chemifluorescence, and band densities were relatively determined with ImageJ v1.47 (National Institute of Health Sciences, Bethesda, MD, USA).

4.3. Assay for propofol glucuronidation activity

Propofol glucuronidation activities in HLM, HIM and HKM were determined according to a previous report with some modifications (Shimizu et al. 2003). The incubation mixture contained propofol (5–1000 μM), microsomes (HLM and HIM, 50 μg protein/ml; HKM, 20 μg protein/ml), alamethicin (20 μg/ml), 10 mM $MgCl_2$ and 2 mM UDP-glucuronic acid in a final volume of 200 μl of 50 mM Tris-HCl buffer (pH 7.4). After preincubation for 2 min at 37 °C, the reaction was initiated by adding UDP-glucuronic acid. Incubation was performed for 10 min (HLM and HKM) or 40 min (HIM) at 37 °C and terminated by adding 50 μl of 10% phosphoric acid and vortexing. The samples were centrifuged at 12000 *g* for 10 min at 4 °C. The supernatant was filtered with a polytetrafluoroethylene membrane filter (0.45 μm), and 50 μl of the filtrate was subjected to high-performance liquid chromatography with an Inertsil ODS-SP column (4.6 mm i.d. × 150 mm; GL Sciences, Tokyo, Japan). The column was maintained at 40 °C. Propofol glucuronide was isocratically eluted with 0.1% acetic acid/acetonitrile (60:40, v/v) at a flow rate of 1.0 ml/min. UV detection was performed at 220 nm. Standard curve samples spiked with propofol glucuronide were prepared in the same manner as incubation samples. Under these conditions, the retention times of propofol glucuronide and propofol were 4.2 and 33.8 min, respectively.

4.4. Data analysis

Kinetic parameters (K_m, V_{max} and K_{si}) for propofol glucuronidation were calculated by constructing velocity versus substrate concentration (V-[S]) plots using SigmaPlot v8.02 software (Systat Software, San Jose, CA, USA). *In vitro* clearance values were CL_{int} (V_{max}/K_m). All values are expressed as the mean ± S.D. of three separate experiments. Statistical comparisons were made with one-way analysis of variance with Dunnett's post-hoc test, and differences were considered significant when $P<0.05$.

Acknowledgements: This work was supported in part by JSPS KAKENHI Grant (23590148).

References

Cheng Z, Radominska-Pandya A, Tephly TR (1999) Studies on the substrate specificity of human intestinal UDP-glucuronosyltransferases 1A8 and 1A10. Drug Metab Dispos 27: 1165–1170.

Court MH (2005) Isoform-selective probe substrates for *in vitro* studies of human UDP-glucuronosyltransferases. Methods Enzymol 400: 104–116.

Fallon JK, Neubert H, Goosen TC, Smith PC (2013) Targeted precise quantification of 12 human recombinant uridine-diphosphate glucuronosyl transferase 1A and 2B isoforms using nano-ultra-high-performance liquid chromatography/tandem mass spectrometry with selected reaction monitoring. Drug Metab Dispos 41: 2076–2080.

Gill KL, Houston JB, Galetin A (2012) Characterization of *in vitro* glucuronidation clearance of a range of drugs in human kidney microsomes: comparison with liver and intestinal glucuronidation and impact of albumin. Drug Metab Dispos 40: 825–835.

Girard H, Court MH, Bernard O, Fortier LC, Villeneuve L, Hao Q, Greenblatt DJ, von Moltke LL, Perussed L, Guillemette C (2004) Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. Pharmacogenetics 14: 501–515.

Harbourt DE, Fallon JK, Ito S, Baba T, Ritter JK, Glish GL, Smith PC (2012) Quantification of human uridine-diphosphate glucuronosyl transferase 1A isoforms in liver, intestine, and kidney using nanobore liquid chromatography-tandem mass spectrometry. Anal Chem 84: 98–105.

Izukawa T, Nakajima M, Fujiwara R, Yamanaka H, Fukami T, Takamiya M, Aoki Y, Ikushiro S, Sakaki T, Yokoi T (2009) Quantitative analysis of UDP-glucuronosyltransferase (UGT) 1A and UGT2B expression levels in human livers. Drug Metab Dispos 37: 1759–1768.

Jinno H, Saeki M, Saito Y, Tanaka-Kagawa T, Hanioka N, Sai K, Kaniwa N, Ando M, Shirao K, Minami H, Ohtsu A, Yoshida T, Saijo N, Ozawa S, Sawada J (2003) Functional characterization of human UDP-glucuronosyltransferase 1A9 variant, D256N, found in Japanese cancer patients. J Pharmacol Exp Ther 306: 688–693.

Kiang TK, Ensom MH, Chang TK (2005) UDP-glucuronosyltransferases and clinical drug-drug interactions. Pharmacol Ther 106: 97–132.

Langley MS, Heel RC (1988) Propofol. a review of its pharmacodynamic and pharmacokinetic properties and use as an intravenous anaesthetic. Drugs 35: 334–372.

Murayama N, Minoshima M, Shimizu M, Guengerich FP, Yamazaki H (2007) Involvement of human cytochrome P450 2B6 in the ω- and 4-hydroxylation of the anesthetic agent propofol. Xenobiotica 37: 717–724.

Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, Rance DJ, Wastall P (1997) The prediction of human pharmacokinetic parameters from preclinical and *in vitro* metabolism data. J Pharmacol Exp Ther 283: 46–58.

Oda S, Nakajima M, Hatakeyama M, Fukami T, Yokoi T (2012) Preparation of a specific monoclonal antibody against human UDP-glucuronosyltransferase (UGT) 1A9 and evaluation of UGT1A9 protein levels in human tissues. Drug Metab Dispos 40: 1620–1627.

Ohno S, Nakajin S (2009) Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. Drug Metab Dispos 37: 32–40.

Shimizu M, Matsumoto Y, Tatsuno M, Fukuoka M (2003) Glucuronidation of propofol and its analogs by human and rat liver microsomes. Biol Pharm Bull 26: 216–219.

Soars MG, Burchell B, Riley RJ (2002) *In vitro* analysis of human drug glucuronidation and prediction of *in vivo* metabolic clearance. J Pharmacol Exp Ther 301: 382–390.

Vanlersberghe C, Camu F (2008) Propofol. Handb Exp Pharmacol 182: 227–252.

Villeneuve L, Girard H, Fortier LC, Gagné JF, Guillemette C (2003) Novel functional polymorphisms in the UGT1A7 and UGT1A9 glucuronidating enzymes in Caucasian and African-American subjects and their impact on the metabolism of 7-ethyl-10-hydroxycamptothecin and flavopiridol anticancer drugs. J Pharmacol Exp Ther 307: 117–128.

Yamanaka H, Nakajima M, Katoh M, Yokoi T (2007) Glucuronidation of thyroxine in human liver, jejunum, and kidney microsomes. Drug Metab Dispos 35: 1642–1648.