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Studies on induction of lamotrigine metabolism in transgenic *UGT1* mice

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Abstract

1. A transgenic 'knock-in' mouse model expressing a human *UGT1* locus (Tg-*UGT1*) was recently developed and validated. Although these animals express mouse UGT1A proteins, UGT1A4 is a pseudo-gene in mice. Therefore, Tg-*UGT1* mice serve as a 'humanized' UGT1A4 animal model.
2. Lamotrigine (LTG) is primarily metabolized to its *N*-glucuronide (LTGG) by hUGT1A4. This investigation aimed at examining the impact of pregnane X receptor (PXR), constitutive androstane receptor (CAR) and peroxisome proliferator-activated receptor (PPAR) activators on LTG glucuronidation *in vivo* and *in vitro*. Tg-*UGT1* mice were administered the inducers phenobarbital (CAR), pregnenolone-16 α -carbonitrile (PXR), WY-14643 (PPAR- α), ciglitazone (PPAR- γ), or L-165041 (PPAR- β), once daily for 3 or 4 days. Thereafter, LTG was administered orally and blood samples were collected over 24 h. LTG was measured in blood and formation of LTGG was measured in pooled microsomes made from the livers of treated animals.
3. A three-fold increase in *in vivo* LTG clearance was seen after phenobarbital administration. In microsomes prepared from phenobarbital-treated Tg-*UGT1* animals, 13-fold higher CL_{int} ($V_{\max}/K_m$) value was observed as compared with the untreated transgenic mice. A trend toward induction of catalytic activity *in vitro* and *in vivo* was also observed following pregnenolone-16 α -carbonitrile and WY-14643 treatment. This study demonstrates the successful application of Tg-*UGT1* mice as a novel tool to study the impact of induction and regulation on metabolism of UGT1A4 substrates.

Keywords

Lamotrigine; transgenic mice; uridine glucuronosyl transferases (UGTs); induction; *N*-glucuronide

Introduction

UDP-glucuronosyltransferases (UGTs) carry out the addition of glucuronic acid (β -D-glucopyranosiduronic acid) from uridine diphosphoglucuronic acid (UDPGA) to various

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functional groups in xenobiotics and endogenous compounds, thus forming the corresponding β -D-glucopyranosides (glucuronides). Glucuronides are more polar, ionizable at physiological pH and higher in molecular weight as compared with their parent drugs. This facilitates their renal as well as biliary excretion (Dutton 1966; Remmel et al. 2008, 2009). The UGT superfamily of proteins consists of three families, UGT1A and UGT2B being the most important. To date 19 human UGTs have been characterized. Members of UGT1A family are differentially expressed in organs such as liver, kidney, intestines, etc. and catalyse the conjugation of several planar and bulky phenols, 1°, 2°, 3° and aromatic amines, carboxylic acids, and endogenous substrates such as bilirubin, bile acids, and oestrogens (Mackenzie et al. 2000; Tukey and Strassburg 2001). UGT2B family enzymes are responsible for the glucuronidation of several xenobiotics, bile acids, prostanoids and steroidal substrates, especially androgens (Burchell et al. 1998; Mackenzie et al. 2000; Tukey and Strassburg 2001). The UGT1A family is exceptional because the entire *UGT1A* gene locus encodes for nine functional human UGT1A proteins, and is located on chromosome 2 (Ritter et al. 1992).

Phenobarbital (PB) is an activator of the nuclear receptor constitutive androstane receptor (CAR) (Kawamoto et al. 1999; Sugatani et al. 2001). Several studies have postulated that PB enhances the nuclear translocation of CAR *in vivo* (Moore et al. 2002; Sueyoshi and Negishi 2001) via a phosphorylation mechanism, based on ablation of phenobarbital induction in hepatocytes by okadaic acid (Sidhu and Omiecinski 1997). Compared with pregnane X receptor (PXR), CAR is found in low levels in the liver, has a limited tissue distribution, and is activated by fewer xenobiotics as compared with PXR (Moore et al. 2002, 2003). PXR, on the other hand, is less indiscriminate in its tissue expression and is activated by a broad range of xenobiotics, pregnenolone-16 α -carbonitrile (PCN) being an activator of murine PXR and rifampin an agonist of human PXR (Kliwer et al. 2002; Kobayashi et al. 2004; Moore et al. 2002). PXR is evolutionarily related to CAR (40% homology) and is thought to play a role in regulation of metabolism, transport and excretion, perhaps as a protective mechanism in cholestasis (Moore et al. 2002). A PXR-response element was identified and localized to a 386 bp region at -3529 to -3143 in the *UGT1A1* 5'-regulatory region. CAR/RXR α heterodimers also exhibited binding to the same element via sharing of the same DR-3 binding region. A similar DR-3 nuclear receptor response element was also identified in the *UGT1A6* promoter. Nuclear receptors such as PXR and CAR have been shown to be involved in regulation of *UGT1A1* gene expression both *in vitro* and *in vivo* (Barbier et al. 2004; Sugatani et al. 2001, 2005a; Yueh et al. 2001).

Lamotrigine (LTG) is a triazine anti-epileptic drug and is metabolized to a quaternary ammonium glucuronide, lamotrigine-2-*N*-glucuronide (LTGG) (Remmel and Sinz 1991; Sinz and Remmel 1991b), primarily by UGT1A4 (Green et al. 1998; Green and Tephly 1998). LTG, an anti-epileptic drug, is commonly co-administered with inducing anti-epileptic agents such as carbamazepine, phenytoin, and PB. Thus, there is clinical relevance in understanding these interactions. Lamotrigine is primarily a substrate for UGT1A4 and a weak substrate for UGT1A3, with the intrinsic clearance being at least ten-fold higher for UGT1A4 as compared with UGT1A3 (Argikar and Remmel 2009b). It is also understood that *UGT1A4* mRNA is higher in liver than *UGT1A3*. Miners and co-workers recently suggested UGT2B7 may be a low-capacity, low- K_m isoform for LTG glucuronidation (Rowland et al. 2006). However, we could not validate this finding in our laboratory with a sensitive liquid chromatography-mass spectrometry (LC-MS) assay. Because LTG is exclusively cleared by *N*-glucuronidation in humans and *UGT1A4* is a pseudo-gene in mice, it is good marker of UGT1A4 activity *in vivo* in the Tg-*UGT1* mice.

Recently, the Tukey laboratory has successfully developed and validated a novel transgenic mouse model that expresses a bacterial artificial chromosome encoding the entire human

UGT1 locus (Chen et al. 2005; Senekeo-Effenberger et al. 2007). Tg-*UGT1* mice have been useful for investigating the effect of induction and regulation on human UGT1A proteins and are directly applicable to a range of studies that involve tissue-specific protein expression, pharmacokinetic analysis of probe substrates, and other such studies involving xenobiotics which cannot be performed directly in humans (Argikar et al. 2008). The present study demonstrates application of Tg-*UGT1* mouse model for studying the effect of PXR, CAR and peroxisome proliferator-activated receptor (PPAR)- α , - β , and - γ activators on conjugative metabolism of LTG. The objectives of the current investigation were: (1) to compare the exposure profiles of LTG in Tg-*UGT1* mice and wild type mice; (2) to compare pharmacokinetic parameters of LTG in Tg-*UGT1* mice receiving different inducers: PCN (PXR activator), PB (CAR activator), WY-14643 (PPAR- α activator), ciglitazone (CGZ) (PPAR- γ activator), and L-165041 (PPAR- β activator); and (3) to study the kinetics of LTGG formation in liver microsomes obtained from control Tg-*UGT1*, induced Tg-*UGT1* and wild-type mice.

Materials and methods

Chemicals

LTG standard was obtained from Toronto Research Chemicals (North York, Ontario, Canada). LTGG standard was kindly provided by Glaxo (Upper Merion, PA, USA). High-performance liquid chromatography (HPLC)-grade formic acid was obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). HPLC-grade ammonium acetate, magnesium chloride, ethyl acetate, methanol and methyl-*t*-butyl ether (MTBE) were purchased from Fischer Scientific Co. (Fair Lawn, NJ, USA), the internal standard for the GC-MS assay—2,4-diamino-6-(4-methoxyphenyl)-1,3,5-triazine—was obtained from Aldrich (St Louis, MO, USA), *N*-(*t*-butyl dimethylsilyl)-*N*-methyl trifluoroacetamide (MTBSTFA) (derivatization grade), glacial acetic acid, Trisma buffer, D-saccharolactone, uridine diphosphoglucuronic acid (UDPGA), PCN, WY-14643, L-165041, β -naphthoflavone (BNF), fenofibric acid (FFA), and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich Co. (St Louis, MO, USA). PB was donated by Dr. M. Negishi. High-purity solvents used for MS—acetonitrile, ethyl acetate—were obtained from Honeywell Burdick & Jackson (Muskegon, MI, USA). Alamethicin was purchased from Fluka GmbH (Buchs, Switzerland). Nylon membrane filters were obtained from Whatman Co. (Maidstone, UK). Fresh human hepatocytes in a collagen/matrigel overlay, serum-free supplementation pack and William's E media were obtained from CellzDirect (Austin, TX, USA).

Study design

Tg-*UGT1* mice generated as previously described (Chen et al. 2005) were used in compliance with the National Institutes of Health (NIH) guidelines regarding the use of laboratory animals, as described by Senekeo-Effenberger et al. (2007). The study design is described in Table 1. Briefly, Tg-*UGT1* mice were divided into separate groups of three animals each for treatment with each activator and were matched by age and sex. This was to maximize the utilization of the generated Tg-*UGT1* mice.

Dosing of transgenic animals

Animals (20–30 g) were provided with food and water *ad libitum* and were maintained under controlled temperature (23°C) and lighting (12 h light/12 h dark cycles). PCN (PXR activator), WY-14643 (PPAR- α activator), CGZ (PPAR- γ activator), and L-165041 (PPAR- β activator) were dissolved in DMSO. PB (CAR activator) was dissolved in water at a concentration of 1 g l⁻¹. Mice in each group were administered the inducer every 24 h for 3 or 4 days. PCN (100 mg kg⁻¹) was dosed intraperitoneally, PB (0.1% w/v) was administered

in the drinking water *ad libitum*, and PPAR agonists WY-14643 (40 mg kg⁻¹), CGZ (15 mg kg⁻¹) and L-165041 (30 mg kg⁻¹) were administered intraperitoneally (Table 1). Twenty-four hours after the last dose, the mice were given LTG (20 mg kg⁻¹, as a solution formulated as 25% hydroxypropyl- β -cyclodextrin in water) via oral gavage in the morning. This dose, when scaled per body weight, falls within the prescribed dose range for humans. Blood samples were serially drawn by retro-orbital bleeding into heparinized capillary tubes at 1, 2, 4, 8, and 24 h after the oral bolus of LTG as per the guidelines of animal care and use committee. Blood samples (30–50 μ l) were transferred to microcentrifuge tubes after recording the weight and were stored in a –80°C freezer until bioanalysis. After the last sample of blood was collected, the mice were sacrificed and livers were collected and stored at –80°C.

Preparation of microsomes from mouse liver tissues

Equal amounts of liver tissues from each treatment group were pooled and pulverized under liquid nitrogen in a mortar. The pulverized tissue was homogenized in chilled 1.15% KCl with a Potter–Elvehjem Teflon homogenizer. The homogenate was centrifuged at 2000g for 10 min at 4°C (Sorvall swinging bucket, H1000B rotor) and the supernatant was further centrifuged at 9000g for 10 min at 4°C. The resulting supernatant was then subjected to two centrifugations at 100 000g for 60 min at 4°C each (Beckman SW-40Ti Rotor). The pellet was resuspended in buffer consisting of 50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, and 1 mM phenyl methyl sulfonyl fluoride. Protein concentration was determined by the Bradford method.

In vitro glucuronidation assays

LTG substrate concentrations were selected based on linearity studies with time and protein and $V_{\max}$ and K_m estimations in pooled human liver microsomes (Argikar and Remmel 2009a, 2009b). Glucuronidation incubations for each LTG concentration were carried out in triplicate by modification of a procedure developed earlier in this laboratory (Chen et al. 2005; Senekeo-Effenberger et al. 2007; Sinz and Remmel 1991a). In brief, microsomes (0.5 mg protein content ml⁻¹) obtained from the liver of the transgenic mice were pre-incubated with alamethicin (approximately 100 μ g mg⁻¹ of protein) on ice. Stock solutions of LTG were prepared in DMSO and 0.1 M acetic acid with the help of sonication. The final concentration of all organic solvents in each incubation did not exceed 1% of the total volume. Tris buffer (0.1 M, pH 7.4 at 37°C) was then added, followed by the addition of 5 mM MgCl₂, 5 mM D-saccharolactone and varying concentrations of LTG. The final pH was 7.2 due to the addition of the substrate in acetic acid. However, control incubations at this pH did not reveal any change in the substrate kinetics. The above incubation mixture was pre-incubated at 37°C for 2 min. The reaction was started by addition of freshly prepared 3 mM UDPGA. The final incubation volume of 200 μ l was incubated for 30 min at 37°C, 30 rpm in a waterbath (Model BS-11, Lab Companion, Seoul, Korea). Control incubations lacking UDPGA did not result in metabolite formation. An equal volume of chilled acetonitrile was used to stop the reaction, followed by centrifugation at 4500g for 5 min. The supernatant was then centrifuged through nylon spin filters at 4500g for 5 min and carried forward for LC-MS analysis.

Fresh human hepatocytes experiments

The fresh human hepatocytes (10⁶ cells/well in a twelvewell plate) were overlaid with Matrigel/collagen and maintained in a two-dimensional sandwich configuration for 48 h before addition of the inducer. To the regular Williams' E media (WEM) obtained from CellzDirect (Austin, TX, USA), a proprietary serum-free supplementation pack from CellzDirect containing penicillin–streptomycin was added to make up enriched Williams' E media (EWEM). Solutions of EWEM containing each inducer—100 μ M (PPAR- α), 1 mM

PB (CAR), 50 μ M RIF (PXR), 100 μ M BNF (aromatic hydrocarbon receptor, AhR) and 0.5% DMSO (control)—were prepared. DMSO was used as the vehicle control. The EWEM containing each inducer was added to the wells and the cells were incubated at 37°C in a 5% CO₂-humidified incubator. The media were replenished each day during the 3-day induction period. At the beginning of day 4, the cells were incubated with EWEM containing 1.25 mM LTG. After 4 h, the media were collected and analysed for formation of LTGG by the LC-MS method described below.

GC-MS assay for LTG in mouse blood

To quantitate LTG concentration in mouse whole blood, a sensitive GC-MS assay was developed on an Agilent 6890 series II gas chromatograph coupled with an Agilent 5973 series mass selective detector and an Agilent 7673 autosampler. Each whole blood sample (volume determined by weight) was mixed with 1 ml of MTBE for 30 s on a vortex mixer after addition of the internal standard (IS), followed by centrifugation at 13000g for 10 min in MicroMax centrifuge (International Equipment Co., White Lake, MI, USA). The organic layer was transferred to a test tube and evaporated under nitrogen on a Zymark Turbovac evaporator (Hopkinton, MA, USA). The residue thus obtained was reconstituted in MTBSTFA and transferred to an auto-sampler vial, sealed, and heated in a multi-block heater (Labline, Melrose Park, IL, USA) at 60°C for 60 min. An aliquot was injected onto a SolGel fused silica capillary column (0.25 mm i.d. $\times$ 60 m, 0.25 μ m; SGE, Ringwood, VIC, Australia). The injection port temperature was set to 250°C and the detector to 310°C. Upon injection, the oven temperature was held for 2 min at 60°C. This was followed with an increase by 9°C min⁻¹ to 150°C and held for 5 min, followed with an increase in temperature by 5°C min⁻¹ to 250°C where it was held steady for 10 min. Finally, the temperature was ramped up by 20°C min⁻¹ to 300°C and held for 5 min to elute high boiling-point contaminants. Derivatized LTG (m/z 426) and IS (m/z 388) eluted at 8.9 and 7.8 min, respectively, and were detected by selected-ion monitoring after electron-impact chemical ionization. Data were collected with the help of ChemStation system (Dayton, OH, USA). The limit of quantitation for this assay was 100 ng ml⁻¹.

LC-MS assay for LTGG

An LC-MS method was developed to provide the required sensitivity for quantitation of the quaternary ammonium glucuronide, as reported (Argikar and Remmel 2009b). In short, LTGG ([M]⁺; m/z 432.2) was monitored at an optimal spray voltage of 1.9 kV and detector voltage of 1.5 kV. A capillary temperature of 250°C, an interface temperature of 250°C, a heater block temperature of 200°C, and a nebulizing gas flow rate of 0.18 l min⁻¹ were optimized for the assay. LTGG was eluted at 2.5 min. The separation was carried out on a Higgins Analytical Haisil C-18 column (100 mm $\times$ 2.1 mm, 5 μ) at room temperature with a gradient comprised of 20–50% acetonitrile in 20 mM ammonium acetate buffer, pH 6.67, over a period of 8 min. The percentage of organic was ramped up to 80% over the next 2 min and held for 1 min, followed by a 4-min re-equilibration step. The flow rate for the analysis was 0.3 ml min⁻¹. Data acquisition was achieved on LCMS Solution Software® from Shimadzu, Inc. (Columbia, MD, USA). The limit of quantitation for this assay was 250 ng ml⁻¹.

Data analysis

Pharmacokinetic analysis for LTG in mouse whole blood was performed on a WINNONLIN non-compartmental analysis program (Pharsight, Mountain View, CA, USA), with an extra-vascular administration model for whole blood data. Dose time was set to zero minutes and doses and exact sample times for each individual mouse (amount of LTG in mg) were entered. A linear trapezoidal method with interpolation was used for the calculation of individual pharmacokinetic parameters. The data were weighted uniformly. The data

obtained from *in vitro* incubations were fit to Michaelis–Menten kinetic model with SigmaPlot (Systat Software, Richmond, CA, USA).

Results

In vivo pharmacokinetic analysis

LTGG levels in circulation are low. The limited sample volume from serial sampling of the *Tg-UGT1* mice afforded low sample volumes for bioanalysis. Furthermore, rate and extent of LTGG formation is minimal in wild-type mice, and hence could not be measured in whole blood. Due to these reasons, only LTG levels were measured in the whole blood obtained from the *Tg-UGT1* mice. Whole blood pharmacokinetic analysis of LTG yielded results as shown in Figure 1. In the mouse group receiving PCN as an inducer, the observed oral clearance of LTG ($CL_{obs} = CL/F$) was 1.5-fold higher than the control group receiving DMSO. Induction with PB resulted in three-fold higher observed clearance of LTG as compared with the control group receiving water (Figure 2). Amongst the animals receiving PPAR agonists, WY-14643 administration resulted in an increase in clearance of LTG by 1.4-fold. Administration of CGZ and L-165041 resulted in decreased clearance of LTG. Mean C_{max} and T_{max} values are reported in Table 2.

In vitro kinetic analysis in mouse liver microsomes

In the studies performed with pooled human liver microsomes, glucuronidation of LTG was found to be linear over a range of protein concentration from 0.1 to 1.0 mg ml⁻¹, over a period of 10–80 min (Argikar and Remmel 2009b). The substrate concentrations were 1.25 mM and 0.156 mM, which fall in the linear concentration range of enzyme activity, and are approximately equal to or lower than reported K_m values in human liver microsomes, as described in previous literature reports (Magdalou et al. 1992) and studies from our laboratory (Argikar and Remmel 2009b). At the same substrate concentration and protein concentration of 0.5 mg ml⁻¹, the formation of LTGG was found to be linear up to 40 min of incubation, after which a moderate loss of activity was observed. Results from these preliminary studies determined that formation of the glucuronide was linear at 0.5 mg protein ml⁻¹ for 30 min. The *in vitro* data were best fit to a Michaelis–Menten model as determined by residuals and V_{max} and K_m values were obtained. Intrinsic clearance was calculated as:

$$CL_{int} = V_{max}/K_m (\mu l \text{ min}^{-1} \text{ mg}^{-1} \text{ protein})$$

Results are reported in Table 3. Induction of CL_{int} was highest with PB (13-fold) as compared with the control group (Figure 2). Induction by PCN was about 3.5-fold higher in the liver microsomes as compared with the DMSO control group. All animals receiving PPAR agonists showed increase in CL_{int} of LTG. WY-14643, CGZ, L-165041 all showed more than a two-fold increase in turnover of LTG in the respective microsomes. Similar studies carried out in the wild-type mice as a part of validation of the *Tg-UGT1* mouse model resulted in minimal induction and the intrinsic clearances were much lower as compared with the transgenic mice (data not shown). Comparative CL_{int} and CL_{obs} from *in vitro* and *in vivo* data, respectively, are represented in Tables 2 and 3.

Induction of UGT1A4 activity in fresh human hepatocytes

A study in fresh human hepatocytes from a single donor was conducted to observe the induction by nuclear receptor activators. Results from this experiment illustrated that activators of PXR, CAR, PPAR- α and AhR increased the formation of LTGG as a result of 72-h pretreatment. After 4 h of incubation with LTG, the formation of LTGG was increased

by approximately 1.5–2.3 times in cells treated with four inducers as compared with control cells exposed to vehicle alone. Results from this experiment are illustrated in Figure 3.

Discussion

The results of the induction studies in this ‘humanized UGT1A4 transgenic mouse model’ (*UGT1A4* being a pseudo-gene in mice) indicate that CAR, and probably PXR and PPAR- α , up-regulate UGT1A4 expression *in vivo*. PPARs are transcription factors belonging to the superfamily of nuclear hormone receptors, which are activated by endogenous fatty acid ligands. Three isoforms of PPARs—PPAR- α , PPAR- β (also termed as PPAR- δ) and PPAR- γ —have been reported so far (Escriva et al. 1997; Michalik and Wahli 1999). The three isoforms are selectively activated by a range of endogenous and exogenous compounds and have varying tissue distribution. Fibrates such as clofibrate, fenofibric acid, and priniixic acid (WY-14643) are potent activators of PPAR- α (Heimbürger and Palmblad 1998; Hertz and Bar-Tana 1998), which has a moderate to high expression in liver. Although PPARs have been implicated to play a role in oxidative as well as conjugative metabolism of xenobiotics through modulation of drug metabolising enzymes, little information is available on the role of PPARs in the induction and regulation of specific UGTs. PPAR- α and PPAR- γ have previously been identified to play a role in up-regulation of drug-metabolizing enzymes, especially UGTs. Barbier and co-workers also found PPAR response elements in UGT2B4 (Barbier et al. 2003a) and UGT1A9 (Barbier et al. 2003b). PPAR- α has been implicated in increasing the levels of UGT1A4 mRNA in human hepatocyte cultures (Richert et al. 2003) and UGT1A1 in tissue culture cell lines (Brierley et al. 1996). Our group has recently shown that PPAR- α agonists induce several UGT isoforms including UGT1A1, UGT1A3, and UGT1A4 (Senkeo-Effenberger, 2007). Results from the present study indicated that induction with the PPAR- α agonist moderately increased CL_{int} and CL_{obs} of LTG *in vitro* and *in vivo* in the Tg-*UGT1* mice. In comparison with Tg-*UGT1* receiving DMSO, the Tg-*UGT1* mice receiving PPAR- α agonist WY-14643 showed a two-fold increase in microsomal CL_{int} and a 1.5-fold increase in the *in vivo* CL_{obs} . Thus, PPAR- α also appears to be involved in the regulation of UGT1A4. Tg-*UGT1* mice dosed with PPAR- β and - γ agonists pointed toward a lower CL_{obs} of LTG *in vivo*, but approximately a moderate two-fold higher microsomal CL_{int} . The paradoxical trend toward decrease in CL_{obs} of LTG in animals receiving CGZ and L-165041 cannot be explained at this time. It is known that interspecies differences exist between expression of the PPAR in rodents and humans that lead to resultant differences in peroxisome proliferation (Gonzalez et al. 1998; Tugwood et al. 1998). The results of our *in vivo* study demonstrate that treatment of the transgenic mice with WY-14643 results in a moderate two-fold induction of lamotrigine clearance in female mice, whereas a moderate decrease in clearance was observed with prototypical PPAR- β and - γ ligands. PPAR- γ is activated by thiazolidinediones or ‘glitazones’ such as pioglitazone, rosiglitazone, and CGZ (Ott et al. 1998; Parks et al. 1998; Spiegelman 1998), and has low expression in liver, with higher expression in spleen, intestine, and adipose tissue. PPAR- β , although not activated by a specific class of ligands, is activated by chemicals such as L165041, GW0742, GW2433, etc. (Desvergne and Wahli 1999; Devchand et al. 1999; Matsusue et al. 2004).

Recent work on the *UGT1A4* regulatory region has shown that hepatic nuclear factor 1a (HNF1a) is critical for the basal expression of UGT1A4 (Gardner-Stephen and Mackenzie 2007) and there are two xenobiotic response element (XRE) binding sites in the first 500 bp upstream of the UGT1A4 start site (Erichsen et al. 2008). Human UGT enzymes have previously been reported to be regulated following activation of PXR (Gardner-Stephen et al. 2004; Xie et al. 2003), CAR (Sugatani et al. 2005b; Xie et al. 2003), and AhR (Bock et al. 1998; Yueh et al. 2003). Significant species and ligand-dependent variation exist for activation of PXR (Moore et al. 2002). Several studies have demonstrated that human PXR

(hPXR) and mouse PXR (mPXR) are activated by exclusive ligands (Kliwer et al. 2002; Kobayashi et al. 2004; Moore et al. 2002). RIF acts as a strong activator of human and rabbit PXR where as it is a weak inducer of mouse PXR. DEX and PCN are commonly used to activate mouse and rat PXR. PB is a selective agonist of CAR in rodents (Moore et al. 2002; Sueyoshi et al. 1999). In the present study, Tg-*UGT1* mice receiving PB, a CAR agonist, showed approximately a 13-fold increase in microsomal CL_{int} and a three-fold increase in the observed LTG clearance as compared with control mice ($389 \pm 51 \text{ ml h}^{-1} \text{ kg}^{-1}$). Tg-*UGT1* mice receiving PCN, a mPXR agonist, showed a 3.5-fold increase in microsomal CL_{int} and a modest 1.5-fold increase in the observed LTG clearance as compared with control mice receiving DMSO ($254 \pm 32 \text{ ml h}^{-1} \text{ kg}^{-1}$). Mice receiving PB had a 13-fold increase in microsomal CL_{int} and the mean velocity increased more than 130-fold with a tenfold increase in K_m compared with microsomes from vehicle control-treated mice. In comparison, in microsomes from animals receiving PCN, the mean velocity increased 38-fold and the mean K_m increased ten-fold as compared with the DMSO-receiving group. Thus, PB resulted in the stronger inductive response in both liver microsomes and *in vivo* in relation to PCN. These data suggest that both PXR and CAR may function as modulators of hUGT1A4 in the liver and that murine PXR and CAR bind to the human response elements in the regulatory region of the *hUGT1A4* gene.

Full kinetic experiments in the liver microsomes revealed that in untreated mouse microsomes obtained from Tg-*UGT1* mice, LTG is glucuronidated by a low-capacity, low- K_m enzyme. Upon induction, both V_{max} and K_m increased substantially, indicating a switch to high capacity and high- K_m enzyme. *In vitro* experiments carried out in our laboratory suggest that hUGT1A4 has similar higher K_m values (Argikar and Rummel 2009b). The identity of the murine enzyme responsible for the LTG glucuronidation is not known. Miners and his co-workers recently suggested based on inhibition experiments that hUGT2B7 may be a low-capacity and a low- K_m isoform for LTG glucuronidation (Rowland et al. 2006). However, we could not confirm LTG *N*-glucuronidation with cloned and expressed hUGT2B7 with a sensitive LC-MS assay. Even though rodent UGT1A enzymes are known to be induced after AhR (Bock et al. 1998), the induction observed by an activator of CAR in the mice may not have been due to a mouse UGT1A isoform. This is because when wild-type mice were administered PB orally, the microsomal CL_{int} and *in vivo* CL_{obs} were not substantially higher than the untreated wild-type mice, thus implying that a hUGT enzyme was induced (Tables 2 and 3). Furthermore, in the wild-type mice induced with PB, the enzyme kinetics demonstrated only a low-capacity, low- K_m enzyme. Our findings demonstrate that mouse CAR is activated by PB in liver and can translocate from the cytosol and bind to CAR response elements on hUGT1A4 in the Tg-UGT1 mice (Bock et al. 1998; Koike et al. 2005). PB has been reported to be one of the most selective CAR inducers, though it is speculated that it may activate PXR at higher concentrations (Moore et al. 2002; Sueyoshi and Negishi 2001). CAR has been previously reported to play a role in modulation of hUGT1A1, the enzyme responsible for the metabolism of bilirubin, through its effect on the *UGT1A1* gene (Sugatani et al. 2001, 2005a, 2005b). The increase in *in vivo* clearance for LTG in the PB-treated group and increase in *in vitro* CL_{int} values for all treatment groups were statistically significant ($p < 0.05$, Student's *t*-test). However, the current study design is limited by the number of animals per group for further, more powerful statistical analysis among various groups treated with inducers. The current results point toward a possible induction of LTG glucuronidation in the Tg-*UGT1* mice as a result of administration of nuclear receptor inducers.

Our studies with fresh human hepatocytes also illustrated an increase in turnover of LTG in cells treated with various nuclear receptor agonists (Figure 3). It must be noted that the induction experiment in fresh human hepatocytes was conducted in cells obtained from a single donor and a comprehensive study in cells from multiple donors may be conducted to

validate these results further. Such a study, however, was not within the scope of the current investigation. Overall, the *in vitro* results point to involvement of PPAR, PXR and CAR in up-regulation of UGT1A4 enzyme in the liver. PCN, a murine PXR agonist, modulated the observed blood clearance as well as the hepatic intrinsic clearance of LTG. PB was found to be the most potent inducer and increased the blood CL_{obs} of LTG by three-fold. It can be predicted that nuclear receptors as above could be involved in similar LTG clearance modulation in humans. For a drug with a high extraction ratio, the *in vivo* hepatic clearance is predominantly determined by the hepatic blood flow and may not be altered to a great extent by an alteration in CL_{int}. However, the effects of inducing agents may be significant on clearance of drugs with lower hepatic extraction ratio like LTG, where hepatic clearance is primarily a function of CL_{int}.

The transgenic mice used in this study are valuable for functional studies with UGT1A4 because it is a pseudo-gene in rodents. However, these mice still express a murine UGT1A locus and induction of hUGTs relies on the murine transcription factors. Further progress in eliminating the mouse UGT1A background has recently been accomplished in the Tukey laboratory (Tukey 2008). Future work with this novel *in vivo* model of human glucuronidation will involve expression of the human transcription factors to obtain a truly 'humanized' mouse model for xenobiotic metabolism and toxicity studies. It is clear that activators of these receptors play a critical role in modulation of hepatic and extra-hepatic metabolism through their effects on *UGT1A* gene locus. The identification of critical pathways involved in the induction and regulation of the UGT1A enzymes will lend an additional perspective to the understanding of conjugative metabolism of xenobiotics, and the mechanisms of drug–drug interactions involving UGTs.

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References

- Argikar UA, Iwuchukwu OF, Nagar S. Update on tools for evaluation of uridine diphosphoglucuronosyltransferase polymorphisms. *Expert Opin Drug Metab Toxicol.* 2008; 4:879–94. [PubMed: 18624677]
- Argikar UA, Remmel RP. Effect of aging on glucuronidation of valproic acid in human liver microsomes and the role of UDP-glucuronosyltransferase UGT1A4, UGT1A8, and UGT1A10. *Drug Metab Dispos.* 2009a; 37:229–36. [PubMed: 18838507]
- Argikar UA, Remmel RP. Variation in glucuronidation of lamotrigine in human liver microsomes. *Xenobiotica.* 2009b; 39:355–63. [PubMed: 19387891]
- Barbier O, Duran-Sandoval D, Pineda-Torra I, Kosykh V, Fruchart JC, Staels B. Peroxisome proliferator-activated receptor alpha induces hepatic expression of the human bile acid glucuronidating UDP-glucuronosyltransferase 2B4 enzyme. *J Biol Chem.* 2003a; 278:32852–60. [PubMed: 12810707]
- Barbier O, Fontaine C, Fruchart JC, Staels B. Genomic and non-genomic interactions of PPARalpha with xenobiotic-metabolizing enzymes. *Trends Endocrinol Metab.* 2004; 15:324–30. [PubMed: 15350604]
- Barbier O, Villeneuve L, Bocher V, Fontaine C, Torra IP, Duhem C, Kosykh V, Fruchart JC, Guillemette C, Staels B. The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor alpha and gamma target gene. *J Biol Chem.* 2003b; 278:13975–83. [PubMed: 12582161]

- Bock KW, Gschaidmeier H, Heel H, Lehmkoetter T, Munzel PA, Raschko F, Bock-Hennig B. AH receptor-controlled transcriptional regulation and function of rat and human UDP-glucuronosyltransferase isoforms. *Adv Enzyme Regul.* 1998; 38:207–22. [PubMed: 9762354]
- Brierley CH, Senafi SB, Clarke D, Hsu MH, Johnson EF, Burchell B. Regulation of the human bilirubin UDP-glucuronosyltransferase gene. *Adv Enzyme Regul.* 1996; 36:85–97. [PubMed: 8869742]
- Burchell B, Brierley CH, Monaghan G, Clarke DJ. The structure and function of the UDP-glucuronosyltransferase gene family. *Adv Pharmacol.* 1998; 42:335–8. [PubMed: 9327909]
- Chen S, Beaton D, Nguyen N, Senekeo-Effenberger K, Brace-Sinnokrak E, Argikar U, Remmel RP, Trotter J, Barbier O, Ritter JK, Tukey RH. Tissue-specific, inducible, and hormonal control of the human UDP-glucuronosyltransferase-1 (UGT1) locus. *J Biol Chem.* 2005; 280 (45):37547–57. [PubMed: 16155002]
- Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev.* 1999; 20:649–88. [PubMed: 10529898]
- Devchand PR, Ijpenberg A, Devesvergne B, Wahli W. PPARs: nuclear receptors for fatty acids, eicosanoids, and xenobiotics. *Adv Exp Med Biol.* 1999; 469:231–6. [PubMed: 10667335]
- Dutton, GJ., editor. Glucuronic acid—free and combined. New York, NY: Academic Press; 1966.
- Erichsen TJ, Ehmer U, Kalthoff S, Lankisch TO, Muller TM, Munzel PA, Manns MP, Strassburg CP. Genetic variability of aryl hydrocarbon receptor (AhR)-mediated regulation of the human UDP-glucuronosyltransferase (UGT) 1A4 gene. *Toxicol Appl Pharmacol.* 2008; 230:252–60. [PubMed: 18433817]
- Escriva H, Safi R, Hanni C, Langlois MC, Saumitou-Laprade P, Stehelin D, Capron A, Pierce R, Laudet V. Ligand binding was acquired during evolution of nuclear receptors. *Proc Natl Acad Sci USA.* 1997; 94:6803–8. [PubMed: 9192646]
- Gardner-Stephen D, Heydel JM, Goyal A, Lu Y, Xie W, Lindblom T, Mackenzie P, Radominska-Pandya A. Human PXR variants and their differential effects on the regulation of human UDP-glucuronosyltransferase gene expression. *Drug Metab Dispos.* 2004; 32:340–7. [PubMed: 14977869]
- Gardner-Stephen DA, Mackenzie PI. Hepatocyte nuclear factor1 transcription factors are essential for the UDP-glucuronosyltransferase 1A9 promoter response to hepatocyte nuclear factor 4alpha. *Pharmacogenet Genomics.* 2007; 17:25–36. [PubMed: 17264800]
- Gonzalez FJ, Peters JM, Cattley RC. Mechanism of action of the nongenotoxic peroxisome proliferators: role of the peroxisome proliferator-activator receptor alpha. *J Natl Cancer Inst.* 1998; 90:1702–9. [PubMed: 9827524]
- Green MD, King CD, Mojarrabi B, Mackenzie PI, Tephly TR. Glucuronidation of amines and other xenobiotics catalyzed by expressed human UDP-glucuronosyltransferase 1A3. *Drug Metab Dispos.* 1998; 26:507–12. [PubMed: 9616184]
- Green MD, Tephly TR. Glucuronidation of amine substrates by purified and expressed UDP-glucuronosyltransferase proteins. *Drug Metab Dispos.* 1998; 26:860–7. [PubMed: 9733664]
- Heimbürger M, Palmblad J. The peroxisome proliferator-activated receptor alpha (PPARalpha) ligand WY 14,643 does not interfere with leukotriene B4 induced adhesion of neutrophils to endothelial cells. *Biochem Biophys Res Commun.* 1998; 249:371–4. [PubMed: 9712703]
- Hertz R, Bar-Tana J. Peroxisome proliferator-activated receptor (PPAR) alpha activation and its consequences in humans. *Toxicol Lett.* 1998; 102–3:85–90.
- Kawamoto T, Sueyoshi T, Zelko I, Moore R, Washburn K, Negishi M. Phenobarbital-responsive nuclear translocation of the receptor CAR in induction of the CYP2B gene. *Mol Cell Biol.* 1999; 19:6318–22. [PubMed: 10454578]
- Kliwer SA, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev.* 2002; 23:687–702. [PubMed: 12372848]
- Kobayashi K, Yamagami S, Higuchi T, Hosokawa M, Chiba K. Key structural features of ligands for activation of human pregnane X receptor. *Drug Metab Dispos.* 2004; 32:468–72. [PubMed: 15039302]
- Koike C, Moore R, Negishi M. Localization of the nuclear receptor CAR at the cell membrane of mouse liver. *FEBS Lett.* 2005; 579:6733–6. [PubMed: 16310787]

- Mackenzie PI, Miners JO, McKinnon RA. Polymorphisms in UDP glucuronosyltransferase genes: functional consequences and clinical relevance. *Clin Chem Lab Med*. 2000; 38:889–92. [PubMed: 11097345]
- Magdalou J, Herber R, Bidault R, Siest G. In vitro N-glucuronidation of a novel antiepileptic drug, lamotrigine, by human liver microsomes. *J Pharmacol Exp Ther*. 1992; 192:1166–73. [PubMed: 1545383]
- Matsusue K, Peters JM, Gonzalez FJ. PPARbeta/delta potentiates PPARgamma-stimulated adipocyte differentiation. *FASEB J*. 2004; 18:1477–9. [PubMed: 15247146]
- Michalik L, Wahli W. Peroxisome proliferator-activated receptors: three isotypes for a multitude of functions. *Curr Opin Biotechnol*. 1999; 10:564–70. [PubMed: 10600688]
- Moore JT, Moore LB, Maglich JM, Kliewer SA. Functional and structural comparison of PXR and CAR. *Biochim Biophys Acta*. 2003; 1619:235–8. [PubMed: 12573482]
- Moore LB, Maglich JM, McKee DD, Wisely B, Willson TM, Kliewer SA, Lambert MH, Moore JT. Pregnane X receptor (PXR), constitutive androstane receptor (CAR), and benzoate X receptor (BXR) define three pharmacologically distinct classes of nuclear receptors. *Mol Endocrinol*. 2002; 16:977–86. [PubMed: 11981033]
- Ott P, Ranek L, Young MA. Pharmacokinetics of troglitazone, a PPAR-gamma agonist, in patients with hepatic insufficiency. *Eur J Clin Pharmacol*. 1998; 54:567–71. [PubMed: 9832300]
- Parks DJ, Tomkinson NC, Villeneuve MS, Blanchard SG, Willson TM. Differential activity of rosiglitazone enantiomers at PPAR gamma. *Bioorg Med Chem Lett*. 1998; 8:3657–8. [PubMed: 9934490]
- Rommel RP, Sinz MW. A quaternary ammonium glucuronide is the major metabolite of lamotrigine in guinea pigs. In vitro and in vivo studies. *Drug Metab Dispos*. 1991; 19:630–6. [PubMed: 1680630]
- Rommel RP.; Zhou, J.; Argikar, UA. UDP-glucuronosyl transferases. In: Rodrigues, DA., editor. *Drug–drug interactions*. 2. New York, NY: Informa Health Care; 2008. p. 744
- Rommel RP.; Zhou, J.; Argikar, UA. Uridine diphospho glucuronosyl transferases. In: Pearson, P.; Wienkers, L., editors. *Handbook of drug metabolism*. 2. New York, NY: Informa Health Care; 2009. p. 630
- Richert L, Lamboley C, Viollon-Abadie C, Grass P, Hartmann N, Laurent S, Heyd B, Manton G, Chibout SD, Staedtler F. Effects of clofibrate acid on mRNA expression profiles in primary cultures of rat, mouse and human hepatocytes. *Toxicol Appl Pharmacol*. 2003; 191:130–46. [PubMed: 12946649]
- Ritter JK, Chen F, Sheen YY, Tran HM, Kimura S, Yeatman MT, Owens IS. A novel complex locus UGT1 encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes with identical carboxyl termini. *J Biol Chem*. 1992; 267:3257–61. [PubMed: 1339448]
- Rowland A, Elliot DJ, Williams JA, Mackenzie PI, Dickinson RG, Miners JO. In vitro characterization of lamotrigine N2-glucuronidation and the lamotrigine-valproic acid interaction. *Drug Metab Dispos*. 2006; 34:1055–62. [PubMed: 16565174]
- Senekeo-Effenberger K, Chen S, Brace-Sinnokrak E, Bonzo JA, Yueh MF, Argikar U, Kaeding J, Trottier J, Rommel RP, Ritter JK, Barbier O, Tukey RH. Expression of the human UGT1 locus in transgenic mice by 4-chloro-6-(2,3-xylydino)-2-pyrimidinylthioacetic acid (WY-14643) and implications on drug metabolism through peroxisome proliferator-activated receptor alpha activation. *Drug Metab Dispos*. 2007; 35:419–27. [PubMed: 17151188]
- Sidhu JS, Omiecinski CJ. An okadaic acid-sensitive pathway involved in the phenobarbital-mediated induction of CYP2B gene expression in primary rat hepatocyte cultures. *J Pharmacol Exp Ther*. 1997; 282:1122–9. [PubMed: 9262383]
- Sinz MW, Rommel RP. Analysis of lamotrigine and lamotrigine 2-N-glucuronide in guinea pig blood and urine by reserved-phase ion-pairing liquid chromatography. *J Chromatogr*. 1991a; 571:217–30. [PubMed: 1810949]
- Sinz MW, Rommel RP. Isolation and characterization of a novel quaternary ammonium-linked glucuronide of lamotrigine. *Drug Metab Dispos*. 1991b; 19:149–53. [PubMed: 1673389]
- Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes*. 1998; 47:507–14. [PubMed: 9568680]

- Sueyoshi T, Kawamoto T, Zelko I, Honkakoski P, Negishi M. The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. *J Biol Chem*. 1999; 274:6043–6. [PubMed: 10037683]
- Sueyoshi T, Negishi M. Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu Rev Pharmacol Toxicol*. 2001; 41:123–43. [PubMed: 11264453]
- Sugatani J, Kojima H, Ueda A, Kakizaki S, Yoshinari K, Gong QH, Owens IS, Negishi M, Sueyoshi T. The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase UGT1A1 gene and regulation by the nuclear receptor CAR. *Hepatology*. 2001; 33:1232–8. [PubMed: 11343253]
- Sugatani J, Nishitani S, Yamakawa K, Yoshinari K, Sueyoshi T, Negishi M, Miwa M. Transcriptional regulation of human UGT1A1 gene expression: activated glucocorticoid receptor enhances constitutive androstane receptor/pregnane X receptor-mediated UDP-glucuronosyltransferase 1A1 regulation with glucocorticoid receptor-interacting protein 1. *Mol Pharmacol*. 2005a; 67:845–55. [PubMed: 15557560]
- Sugatani J, Sueyoshi T, Negishi M, Miwa M. Regulation of the human UGT1A1 gene by nuclear receptors constitutive active/androstane receptor, pregnane X receptor, and glucocorticoid receptor. *Methods Enzymol*. 2005b; 400:92–104. [PubMed: 16399345]
- Tugwood JD, Holden PR, James NH, Prince RA, Roberts RA. A peroxisome proliferator-activated receptor- α (PPAR α) cDNA cloned from guinea-pig liver encodes a protein with similar properties to the mouse PPAR α : implications for species differences in responses to peroxisome proliferators. *Arch Toxicol*. 1998; 72:169–77. [PubMed: 9520140]
- Tukey RH. Expression of the human UGT1 locus in transgenic and humanized mice. Abstract presented at the 12th International UGT Workshop; 2008. Abstract Number 34
- Tukey RH, Strassburg CP. Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract. *Mol Pharmacol*. 2001; 59:405–14. [PubMed: 11179432]
- Xie W, Yeuh MF, Radominska-Pandya A, Saini SP, Negishi Y, Bottroff BS, Cabrera GY, Tukey RH, Evans RM. Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. *Proc Natl Acad Sci USA*. 2003; 100:4150–5. [PubMed: 12644700]
- Yueh MF, Huang YH, Hiller A, Chen S, Nguyen N, Tukey RH. Involvement of the xenobiotic response element (XRE) in Ah receptor-mediated induction of human UDP-glucuronosyltransferase 1A1. *J Biol Chem*. 2003; 278:15001–6. [PubMed: 12566446]
- Yueh MF, Nguyen N, Famourzadeh M, Strassburg CP, Oda Y, Guengerich FP, Tukey RH. The contribution of UDP-glucuronosyltransferase 1A9 on CYP1A2-mediated genotoxicity by aromatic and heterocyclic amines. *Carcinogenesis*. 2001; 22:943–50. [PubMed: 11375903]

Figure 1.

Pharmacokinetic profiles of LTG in various treatment groups after oral gavage of LTG. LTG concentrations in whole blood of Tg-*UGT1* mice (three mice per group) receiving specific activators of nuclear receptors—PCN (PXR activator), PB (CAR activator), WY-14643 (PPAR- α activator), CGZ (PPAR- γ activator), and L-165041 (PPAR- β activator)—were monitored by GC-MS. Standard deviation from the mean was within 20% at each concentration.

Figure 2. Kinetic profiles of LTG in Tg-*UGT1* mouse whole blood. LTG clearance in Tg-*UGT1* mice (three mice per group) administered: 0.1% PB in drinking water for 4 days (◆) and water control (■).

Figure 3.

Induction of LTG glucuronidation in fresh human hepatocytes. LTG glucuronidation activity was assessed in human hepatocytes in primary culture (Matrigel/collagen sandwich) from a single donor. Activation by inducers of hPXR (RIF), AhR (BNF), PPAR- α (FFA) and CAR (PB) was compared with control (DMSO). Bars represent the means of recorded values from two wells containing 10^6 cells/well.

Table 1

Study design for the *in vivo* portion of this investigation. The study consisted of groups of three animals each, matched by age, gender and weight. Each inducer group was evaluated against a vehicle control. Administration of LTG was carried out orally, 24 h after the administration of the last dose of a given inducer.

Inducer	Receptor	Dose	Days	Number of animals per group	Gender of animals	Type of animals	Dose of LTG (oral, mg kg ⁻¹)
PCN	PXR	100 mg kg ⁻¹	3	3	Male	Tg-UGT1	20
DMSO	Control	–	3	3	Male	Tg-UGT1	20
PB	CAR	0.1% w/v in water	4	3	Male	Tg-UGT1	20
Water	Control	–	4	3	Male	Tg-UGT1	20
WY-14643	PPAR-α	40 mg kg ⁻¹	3	3	Female	Tg-UGT1	20
L-165041	PPAR-β	30 mg kg ⁻¹	3	3	Female	Tg-UGT1	20
CGZ	PPAR-γ	15 mg kg ⁻¹	3	3	Female	Tg-UGT1	20
DMSO	Control	–	3	3	Female	Tg-UGT1	20
None	–	–	–	3	Male	Tg-UGT1	20
None	–	–	–	3	Male	Wild-type	20
PB	CAR	0.1% w/v in water	4	3	Male	Wild-type	20

Table 2

LTG whole blood clearance in Tg-*UGT1* mice. DMSO was a vehicle control in Tg-*UGT1* mice groups treated with activators of PCN where drinking water was a control for groups treated with PB. CL/F is represented as mean value of three animals $\pm$ standard deviation.

Group	Gender	$C_{\max}$ ($\mu\text{g ml}^{-1}$)	$T_{\max}$ (h)	CL/F ($\text{ml h}^{-1} \text{kg}^{-1}$)
DMSO	Male	5.0 ± 0.2	2 ± 0.4	254 ± 32
PCN	Male	5.5 ± 0.2	2 ± 0.2	386 ± 33
Water	Male	4.0 ± 0.2	1 ± 0.5	389 ± 51
PB	Male	2.7 ± 0.1	1 ± 0.2	$1133 \pm 511^*$
DMSO	Female	6.3 ± 0.2	2 ± 0.2	347 ± 149
WY-14643	Female	3.0 ± 0.1	1 ± 0.2	486 ± 96
CGZ	Female	6.7 ± 0.2	1 ± 0.4	280 ± 129
L-165041	Female	6.5 ± 0.2	2 ± 0.2	206 ± 63
–	Male	8.9 ± 0.2	2 ± 0.1	240 ± 116
–	Male	9.1 ± 0.2	2 ± 0.1	261 ± 128

* Indicates statistical significance, $p < 0.05$ (Student's t-test).

Table 3

Michaelis–Menten kinetics for LTG glucuronidation in Tg-*UGT1* mice liver microsomes. DMSO was used as a control vehicle for Tg-*UGT1* mice groups treated with all nuclear receptor activators except PB. Drinking water was used as a control vehicle for Tg-*UGT1* mice treated with PB. Consideration was given to matching the genders of animals while choosing the activators and control groups. $V_{\max}$ and K_m are represented as means of triplicate measurements in pooled liver microsomes (from animals within each inducer group) $\pm$ standard deviation.

Group	Gender	$V_{\max}$ (nmoles $\text{min}^{-1} \text{mg}^{-1}$)	K_m (mM)	$CL_{\text{int}} = V_{\max}/K_m$ (ml $\text{min}^{-1} \text{mg}^{-1}$)
DMSO	Male	0.4 ± 0.02	0.2 ± 0.01	2.0
PCN	Male	18.9 ± 0.3	2.7 ± 0.2	7.0
Water	Male	0.5 ± 0.01	0.2 ± 0.01	2.5
PB	Male	68.3 ± 2.6	2.2 ± 0.5	31.1 *
DMSO	Female	0.6 ± 0.02	0.2 ± 0.02	3.0
WY-14643	Female	23.1 ± 0.6	3.6 ± 0.3	6.4
CGZ	Female	13.7 ± 0.5	2.0 ± 0.2	6.9
L-165041	Female	10.8 ± 0.7	1.5 ± 0.1	7.2
–	Male	0.3 ± 0.01	0.1 ± 0.01	3.0
–	Male	0.4 ± 0.02	0.1 ± 0.01	4.0

* Indicates statistical significance, $p < 0.05$ (Student's t-test).