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Glucuronidation in the chimpanzee (*Pan troglodytes*): Studies with acetaminophen, oestradiol and morphine

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Abstract

The chimpanzee has recently been characterized as a surrogate for oxidative drug metabolism in humans and as a pharmacokinetic model for the selection of drug candidates. In the current study, the glucuronidation of acetaminophen, morphine and oestradiol was evaluated in the chimpanzee to extend the characterization of this important animal model. Following oral administration of acetaminophen (600 mg) to chimpanzees ($n = 2$), pharmacokinetics were comparable with previously reported human values, namely mean oral clearance 0.91 vs. $0.62 \pm 0.05\,l\,h^{-1}\,kg^{-1}$, apparent volume of distribution 2.29 vs. $1.65 \pm 0.25\,l\,kg^{-1}$, and half-life 1.86 vs. 1.89 ± 0.27 h, for chimpanzee vs. human, respectively. Urinary excretions (percentage of dose) of acetaminophen, acetaminophen glucuronide and acetaminophen sulfate were also similar between chimpanzees and humans, namely 2.3 vs. 5.0, 63.1 vs. 54.7, and 25.0 vs. 32.3%, respectively. Acetaminophen, oestradiol and morphine glucuronide formation kinetics were investigated using chimpanzee ($n = 2$) and pooled human liver microsomes ($n = 10$). V_{max}^{app} and K_m^{app} (or S_{50}^{app}) for acetaminophen glucuronide, morphine 3- and 6-glucuronide, and oestradiol 3- and 17-glucuronide formation were comparable in both species. Eadie–Hofstee plots of oestradiol 3-glucuronide formation in chimpanzee microsomes were characteristic of autoactivation kinetics. Western immunoblot analysis of chimpanzee liver microsomes revealed a single immunoreactive band when probed with anti-human UGT1A1, anti-human UGT1A6, and anti-human UGT2B7. Taken collectively, these data demonstrate similar glucuronidation characteristics in chimpanzees and humans.

Keywords: *Glucuronidation, chimpanzees, human, pharmacokinetics, enzyme kinetics, sulphate conjugation*

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Introduction

Chimpanzees (*Pan troglodytes*) are the closest living relatives to man (Wildman, 2002), and the remarkable similarity in their genetics, anatomy, physiology, and endocrinology, has led to the use of chimpanzees in the discovery and development of medicines for humans. Previously, we evaluated the chimpanzee as a pharmacokinetic surrogate for humans in the drug candidate selection process (Wong et al. 2004), focusing on oxidative metabolism mediated by cytochromes P450. As part of the evaluation, we documented species similarities and differences with respect to *in vitro* P450 metabolic activities and immunoreactivity using chimpanzee and human liver microsomes.

Phase II conjugative reactions involve the addition of a polar moiety to a xenobiotic or a phase I metabolite facilitating the elimination of the resulting phase II metabolite in the urine and/or bile. Glucuronidation, the most prominent phase II reaction involved in the biotransformation and elimination of xenobiotics, is catalysed by UDP-glucuronosyltransferases (UGT) (Radominska-Pandya et al. 1999; Fisher et al. 2001). UGT isozymes are located within the lumen of the endoplasmic reticulum and are divided into two main families with overlapping substrate specificities (Radominska-Pandya et al. 1999; Fisher et al. 2001). To date there is very limited literature information on the glucuronidation of xenobiotics in the chimpanzee. Following the *in vivo* administration of cyproheptadine or diethylstilbestrol, urinary recoveries of both compounds as their respective glucuronide conjugates were similar in chimpanzees and humans (Metzler, 1976; Metzler et al. 1977; Fischer et al. 1980). More recent studies examining the biotransformation of nevirapine in both chimpanzees and human subjects indicate species similarities in the percent of dose recovered in urine as 2-hydroxy and 3-hydroxynevirapine glucuronide (Riska et al. 1999a, 1999b). Aside from these few studies, little is known about glucuronidation by chimpanzees *in vivo*, and much less is known about the microsomal conjugation of drug substrates *in vitro*.

To further our understanding of drug disposition in the chimpanzee, glucuronidation of the model substrates acetaminophen (APAP), oestradiol and morphine was determined (Figure 1). The *in vivo* disposition of acetaminophen was investigated following oral dosing to chimpanzees. The *in vitro* enzyme kinetics of APAP, oestradiol and morphine glucuronidation was examined using chimpanzee liver microsomes. Based on the limited literature information available from studies described above, our working hypothesis is that chimpanzees will show similarities to humans in their ability to glucuronidate xenobiotics. A better understanding of species similarities and differences in glucuronidation will allow for more appropriate use of chimpanzees as a model of human drug disposition.

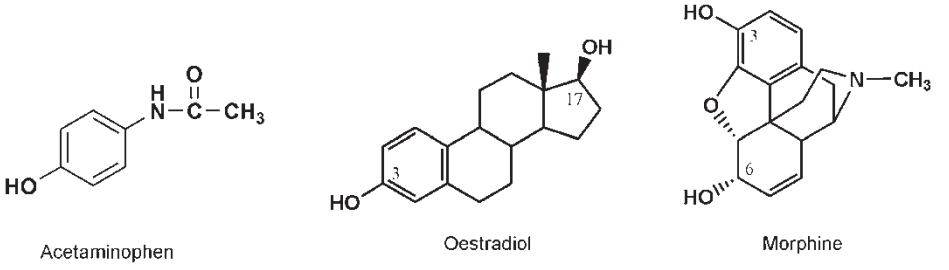

Figure 1. Chemical structure of acetaminophen, oestradiol and morphine.

Materials and methods

Chimpanzee in vivo *pharmacokinetic studies with acetaminophen*

The *in vivo* chimpanzee studies described in this paper were conducted according to protocols approved by the ACUC at the New Iberia Research Center (New Iberia, LA, USA). Two chimpanzees (chimpanzee 1, a 60.1-kg male; and chimpanzee 2, a 51.1-kg female; New Iberia) were administered a 600-mg oral dose of APAP (Tylenol extra strength liquid; McNeil Consumer Healthcare, Fort Washington, PA, USA) diluted in 100% Tang® orange drink (Kraft Foods, Northfield, IL, USA) to provide a total dosing volume of 1 ml kg^{-1}. Blood (approximately 5 ml) was collected at predose, 4, 6, 8, 10, 12, and 24 h post-dose. Blood was drawn from either the cephalic, femoral, or saphenous veins and collected in tubes containing ethylenediamine tetra-acetic acid (EDTA). Following collection, blood samples were centrifuged and the plasma supernatant was separated and stored frozen at −20°C. Cumulative urine samples were collected for 24 h via a collection pan placed under the chimpanzee's cage that drained into a vessel maintained at 4°C by wet ice. Urine samples were stored frozen at −20°C. Plasma and urine samples collected from the chimpanzees were analysed for APAP, acetaminophen glucuronide (APAPG), and acetaminophen sulphate (APAPS) using high-performance liquid chromatography-ultraviolet light (HPLC-UV) as described by Wang et al. (1986).

Pharmacokinetic analyses and comparisons with human data

All pharmacokinetic parameters were calculated by non-compartmental methods as described by Gibaldi and Perrier (1982) using WinNonlin® version 3.2 (Pharsight Corporation, Mountain View, CA, USA). Concentrations of APAP were not detected in the 12-h plasma sample from chimpanzee 1 and the 24-h plasma samples from both chimpanzees 1 and 2. Therefore, these samples were treated as zero in the pharmacokinetic analysis. Parameters are presented as a mean of the two chimpanzees followed by individual animal data in parentheses. Due to the limited sample sizes, no attempts have been made to compare the species statistically.

Chimpanzee liver microsomes preparation

Liver was collected by surgical resection under general anaesthesia from two different healthy adult male chimpanzees, 9 and 10 years old (chimpanzees 3 and 4). Both animals recovered fully from the surgery. The collected tissue was cut into small cubes and portions were flash frozen in liquid nitrogen. The tissue was processed into microsomes using standard ultracentrifugation methods as described by Lake (1987), and total protein concentrations were determined by the Lowry method (Lowry et al. 1951). Following preparation, the microsomes were stored in aliquots in 0.5-ml cryo vials at −80°C until use.

Acetaminophen glucuronidation assay

The glucuronidation of acetaminophen was examined in pooled human liver microsomes (a pool of ten males) (BD Gentest, Worburn, MA, USA) and in chimpanzee liver microsomes. Microsomes were pre-incubated on ice with alamethacin (50 μg mg^{-1} protein) (Sigma-Aldrich Co., St Louis, MO, USA) for 15 min. Alamethacin, a pore-forming peptide, was used to activate microsomes as previously described (Fisher et al. 2000). Following the pre-incubation period, the enzyme kinetics of acetaminophen glucuronidation was performed under the following incubation conditions: APAP (0.3–32 mM) (Sigma-Aldrich),

0.5 mg ml^{-1} microsomal protein, 2.5 mM $MgCl_2$, and 5 mM UDP-glucuronic acid (UDPGA) (Sigma-Aldrich) in 100 mM phosphate buffer (pH 7.1). Total incubation volume was 100 µl. The reactions were terminated at 60 min by the addition of 50 µl of ice-cold 25% formic acid containing internal standard (2-acetamidophenol at 2 mM final concentration; Sigma-Aldrich) followed by mixing. The resulting samples were centrifuged at 2000*g* for 20 min at 4°C and 40 µl of the supernatant were injected into the HPLC-UV for quantitation of APAPG. All reactions were performed in duplicate. In preliminary experiments, reactions performed under the described conditions were linear in chimpanzee and human liver microsomes up to 90 min (data not shown). Saccharolactone, a β-glucuronidase inhibitor, was not added to the incubations as it has been shown to reduce the rate of the acetaminophen glucuronidation reaction (Alkharfy and Frye, 2001).

APAPG (Sigma-Aldrich) was quantitated using a modification of a HPLC-UV method previously described by Court and Greenblatt (1997). Briefly, APAP, APAPG, and 2-acetamidophenol (the internal standard) were chromatographed on an Agilent 1100 Series HPLC (Agilent Technologies, Palo Alto, CA, USA) using a C_{18} column (Zorbax, 250 mm × 4.6 mm i.d., 5 µm; Agilent Technologies) with a mobile phase of 0.1% glacial acetic acid with 3.5% acetonitrile at a flow rate of 1.1 ml min^{-1}. The analytes were monitored using a UV detector (Agilent Technologies) set at wavelength of 254 nm. APAPG, APAP, and 2-acetamidophenol eluted at 7.5, 16.1, and 34.2 min, respectively. Compound identity of all analytes was verified using known standards. Concentrations of APAPG were quantitated using a calibration curve prepared with an authentic standard. Calibration curves for all assays performed were linear with $r^2 > 0.99$ and coefficients of variation (CVs) < 15%.

Oestradiol glucuronidation assay

Oestradiol glucuronidation was examined in both pooled human liver microsomes and chimpanzee liver microsomes using a similar method previously described by Williams et al. (2002). Briefly, microsomes were pre-incubated on ice with alamethacin (50 µg mg^{-1} protein) for 15 min. Following the pre-incubation period, the enzyme kinetics of oestradiol 3- and 17-glucuronidation were examined under the following incubation conditions: oestradiol (1.6–150 µM) (Sigma-Aldrich), 0.5 mg ml^{-1} microsomal protein, 1 mM $MgCl_2$, 5 mM UDPGA and 5 mM saccharolactone (Sigma-Aldrich) in 100 mM phosphate buffer (pH 7.1). Total incubation volume was 100 µl. Reactions were terminated at 20 min by the addition of 100 µl of ice-cold acetonitrile containing internal standard (4-nitrophenyl-β-D-glucuronide at 500 nM final concentration; Sigma-Aldrich) followed by mixing. The resulting samples were centrifuged at 2000*g* for 20 min at 4°C and the supernatant was removed, diluted twofold with water and injected (5 µl) into a liquid chromatograph coupled with a tandem mass spectrometer (LC/MS/MS) for quantitation of oestradiol 3-glucuronide (E3G) and oestradiol 17-glucuronide (E17G) (described below). All reactions were performed in duplicate. Formation of E3G and E17G were linear up to 30 min for both chimpanzee and human liver microsomes in preliminary experiments performed under the described conditions (data not shown).

LC/MS/MS quantitation of E3G (Sigma-Aldrich), E17G (Sigma-Aldrich), and 4-nitrophenyl-β-D-glucuronide (the internal standard) was performed using a Shimadzu VP Series HPLC (SCL-10A VP controller/LC-10AD VP pump; Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) and a Sciex API 4000 LC/MS/MS (Applied Biosystems/MDS Sciex, Foster City, CA, USA). Briefly, E3G, E17G and 4-nitrophenyl-β-D-glucuronide were chromatographed on a Phenomenex Synergi Fusion-RP column

(50 mm × 2 mm i.d., 4 μm; Phenomenex, Torrance, CA, USA) using a mobile phase gradient of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) at a flow rate of 300 μl min^{-1}. The following gradient was used: $t = 0$ min, 3% B; $t = 3.0$ min, 45% B; $t = 3.1$ min, 95% B; $t = 4.0$ min, 95% B; $t = 4.1$ min, 3% B; $t = 5.5$ min, 3% B; total run time 5.50 min. Quantitation was performed using negative electrospray ionization selective reaction-monitoring mode with the ionspray voltage set at −4000 V. The mass transition *m/z* 447.23 to 112.93 was monitored for both E3G and E17G and *m/z* 314.14 to 137.90 was monitored for 4-nitrophenyl-β-D-glucuronide. E3G, E17G, and 4-nitrophenyl-β-D-glucuronide eluted at 2.85, 3.05, and 2.11 min, respectively. The compound identity of all analytes was verified using known standards. Concentrations of E3G and E17G were quantitated using a calibration curve prepared with metabolite standards. Calibration curves for all assays performed were linear with $r^2 > 0.99$ and CVs < 15%.

Morphine glucuronidation assay

Morphine glucuronidation was determined in pooled human liver microsomes and in chimpanzee liver microsomes. Microsomes were pre-incubated on ice with alamethacin (50 μg mg^{-1} protein) for 15 min. Following the pre-incubation period, the enzyme kinetics of morphine 3- and 6-glucuronidation was performed under the following incubation conditions: morphine (0.05–5 mM) (Sigma-Aldrich), 0.5 mg ml^{-1} microsomal protein, 1 mM $MgCl_2$, and 5 mM UDPGA in 100 mM phosphate buffer (pH 7.1). Total incubation volume was 100 μl. The reactions were terminated at 45 min by the addition of 100 μl of ice-cold acetonitrile containing internal standard (dextromethorphan at 500 nM final concentration; Sigma-Aldrich) followed by mixing. The resulting samples were centrifuged at 2000*g* for 20 min at 4°C, diluted tenfold with solvent A (see below), and 4 μl of the diluted supernatant were injected into the LC/MS/MS for quantitation of morphine 3-glucuronide (M3G) and morphine 6-glucuronide (M6G) (described below). All reactions were performed in duplicate. In preliminary experiments, reactions performed under the described conditions were linear in chimpanzee and human liver microsomes up to 45 min for both the formation of M3G and M6G (data not shown). Saccharolactone was not added to the incubations as it had no effect on the rate of morphine glucuronidation in initial experiments (data not shown).

LC/MS/MS quantitation of M3G (Sigma-Aldrich), M6G (Sigma-Aldrich), and dextromethorphan (the internal standard) was performed using a Shimadzu VP Series HPLC (SCL-10A VP controller/LC-10AD VP pump; Shimadzu Scientific Instruments) and a Sciex API 4000 LC/MS/MS (Applied Biosystems/MDS Sciex). Briefly, M3G, M6G and dextromethorphan were chromatographed on a Waters YMC Basic column (50 mm × 2 mm i.d., 5 μm; Waters Corporation, Milford, MA, USA) using a mobile phase gradient of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) at a flow rate of 300 μl min^{-1}. The following gradient was used: $t = 0$ min, 0% B; $t = 4.10$ min, 10% B; $t = 4.15$ min, 10% B; $t = 4.50$ min, 70% B; $t = 4.80$ min, 100% B; $t = 6.00$ min, 100% B; $t = 6.10$ min, 0%B; $t = 6.50$ min, 0%B; total run time 6.50 min. Quantitation was performed using positive electrospray ionization selective reaction-monitoring mode with the ionspray voltage set at 4300 V. The mass transition *m/z* 462.27 to 286.26 was monitored for both M3G and M6G and *m/z* 272.10 to 147.16 was monitored for dextromethorphan. M3G, M6G, and dextromethorphan eluted at 2.9, 3.3, and 4.9 min, respectively. The compound identity of all analytes was verified using known standards. Concentrations of M3G and M6G were quantitated using a calibration curve prepared

with metabolite standards. Calibration curves for all assays performed were linear with $r^2 > 0.99$ and CVs < 15%.

Enzyme kinetics analysis

Enzyme kinetics experiments were fitted to either a classical Michaelis–Menten equation:

$$\left[V = \frac{S \times V_{\max}}{K_m + S}\right]$$

or the Hill equation:

$$\left[V = \frac{S^n \times V_{\max}}{S_{50}^n + S^n}\right]$$

using GraphPad Prism v.3.02 (GraphPad Software, San Diego, CA, USA). The fit to either equation was assessed by visual inspection of Eadie–Hofstee plots, and by minimization of the sum of squares of residuals and the standard error of parameter estimates.

Western blot analysis

Pooled human liver microsomes (a pool of ten males) (BD Gentest, Worburn, MA, USA) or chimpanzee liver microsomes ($n = 2$; male) were treated as described by Ausubel et al. (1994). Briefly, microsomal proteins (20 μg) were separated by sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis (PAGE), transferred to a nitrocellulose membrane, and reacted with antibodies recognizing human UGT1A1, UGT1A6 and UGT2B7 (BD Gentest). Proteins were visualized using a WesternBreeze chromogenic Western blot immunodetection kit (Invitrogen, Carlsbad, CA, USA).

Results

Oral pharmacokinetics of acetaminophen in chimpanzees

Figure 2 is a semilog plot of acetaminophen plasma concentration vs. time for the two chimpanzees given a single oral dose of 600 mg of APAP, and the oral pharmacokinetic parameters estimated for the two chimpanzees are presented in Table I. Human data from Iqbal et al. (1995) were used for comparison because the route of administration (i.e. oral) and dose (i.e. 500 mg) were similar enough to those employed in our chimpanzee studies such that a direct comparison of pharmacokinetic parameters could be made without having to perform dose normalizations. In general, the pharmacokinetic parameters in chimpanzees following oral dosing of acetaminophen agreed quite well with the human data. All chimpanzee pharmacokinetic estimates were less than 50% different than their corresponding human values (Table I).

Table II presents the urinary recoveries of acetaminophen and its glucuronide and sulphate conjugates. As with the oral pharmacokinetic parameters, the urinary recovery of acetaminophen and its main metabolites was similar in both species. Essentially, the entire dose administered to the chimpanzees was recovered as the parent compound and the two conjugates. This was similar to humans where 92% of the dose was accounted by these three entities.

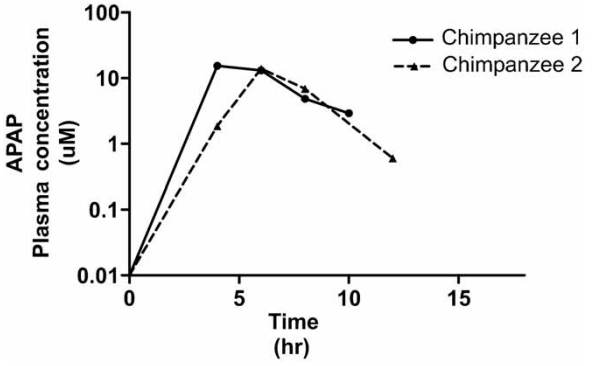

Figure 2. Plot of acetaminophen plasma concentration *vs*. time following administration of single oral doses (600 mg) of acetaminophen to two chimpanzees.

Table I. Oral pharmacokinetics of acetaminophen in chimpanzees and humans.

Species (n)	Dose (mg)	Cl/F ($l\,h^{-1}\,kg^{-1}$)	V_d/F ($l\,kg^{-1}$)	AUC ($\mu M^{\star}h$)	C_{max} (μM)	$t_{1/2}$ (h)
Chimpanzee ($n=2$)[a]	600	0.91 (0.69, 1.12)	2.29 (2.32, 2.26)	82.16 (95.06, 69.26)	14.65 (15.48, 13.83)	1.86 (2.31, 1.40)
Human ($n=10$)[b]	500	0.62 ± 0.05	1.65 ± 0.25	76.67 ± 5.23	24.81 ± 2.51	1.89 ± 0.27

Cl/F, oral clearance; V_d/F, apparent volume of distribution after oral dosing; AUC, area under the plasma concentration-time profile from time 0 extrapolated to infinity; C_{max}, maximum observed concentration achieved following oral dosing; $t_{1/2}$, half-life.
[a]Pharmacokinetic data are presented as the mean of estimates from two chimps with individual data in parentheses.
[b]From Iqbal et al. (1995) (pharmacokinetic data are presented as the mean ± standard error).

Table II. Recovery of acetaminophen and its glucuronide and sulphate conjugates in chimpanzee and human urine following single oral doses of acetaminophen.

	Percentage of the dose recovered in urine		
Species (n)	APAP	APAPG	APAPS
Chimpanzee ($n=2$)[a]	2.3 (2.9, 1.6)	63.1 (61.8, 64.4)	25.0 (28.1, 21.8)
Human ($n=8$)[b]	5.0	54.7	32.3

[a]Pharmacokinetic data are presented as the mean of estimates from two chimps with individual data in parentheses.
[b]From Forrest et al. (1982). Healthy subjects received a 20 mg kg^{-1} dose of acetaminophen.

Enzyme kinetics of acetaminophen in chimpanzee and human liver microsomes

Figure 3 shows Eadie–Hofstee plots of APAP glucuronidation kinetics in human and chimpanzee liver microsomes. Linear Eadie–Hofstee plots were observed for APAPG formation in microsomes from humans and chimpanzee 4 that are consistent with classical Michaelis–Menten kinetics (Figure 3A and C). In contrast, the Eadie–Hofstee plot for APAPG formation in microsomes from chimpanzee 3 possessed a distinctive 'hooked' profile characteristic of autoactivation kinetics described by the Hill equation (Figure 3B). Accordingly, APAPG formation rate vs. APAP concentration for chimpanzee 3 was fitted

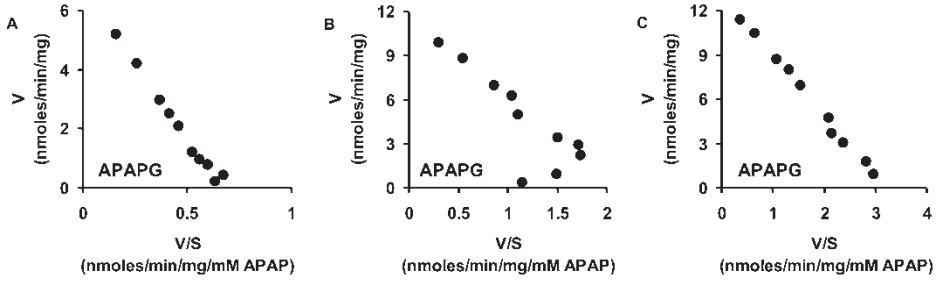

Figure 3. Eadie–Hofstee plots for acetaminophen (APAP) glucuronidation kinetics obtained using microsomes from humans (pooled of ten) (A), chimpanzee 3 (B) and chimpanzee 4 (C).

Table III. Summary of glucuronidation enzyme kinetics in human and chimpanzee liver microsomes for acetaminophen, oestradiol and morphine.

	Human liver microsomes (pool of ten)			Chimpanzee liver microsomes[b]		
Glucuronidation reaction[a]	V_{max}^{app}	K_m^{app} or S_{50}^{app}	n	V_{max}^{app}	K_m^{app} or S_{50}^{app}	n
APAPG (nmol min^{-1} mg^{-1}, mM)	6.9	10.6	1.0	12.1 (11.3, 13.0)	4.6 (5.1, 4.0)	(1.1, 1.0)
E3G (nmol min^{-1} mg^{-1}, μM)	0.36	36.3	1.5	0.23 (0.22, 0.24)	30.0 (29.8, 30.1)	(1.8, 1.7)
E17G (nmol min^{-1} mg^{-1}, μM)	0.16	13.6	1.0	0.34 (0.40, 0.27)	5.8 (6.1, 5.4)	1.0
M3G (nmol min^{-1} mg^{-1}, mM)	1.9	1.8	1.0	3.6 (3.8, 3.4)	0.77 (0.78, 0.76)	1.0
M6G (nmol min^{-1} mg^{-1}, mM)	0.24	1.2	1.0	0.40 (0.41, 0.39)	0.60 (0.62, 0.57)	1.0

V_{max}^{app}, apparent maximum velocity of an enzymatic reaction; K_m^{app}, apparent Michaelis-Menten constant; S_{50}^{app}, substrate concentration resulting in 50% of V_{max}^{app}; n, Hill coefficient.
[a]Units for V_{max}^{app} and K_m^{app} or S_{50}^{app} for each reaction are in parentheses as (V_{max}^{app} units, K_m^{app} or S_{50}^{app} units).
[b]Chimpanzee data are presented as the mean followed by individual estimates in parentheses as (chimpanzee 3 value, chimpanzee 4 value).

using the Hill equation. The estimated enzyme kinetic parameters are presented in Table III. V_{max}^{app} and K_m^{app}/S_{50}^{app} estimates from human and chimpanzee liver microsomes were comparable.

Enzyme kinetics of oestradiol 3- and 17-glucuronide in chimpanzee and human liver microsomes

Eadie–Hofstee plots for E3G and E17G formation in human and chimpanzee liver microsomes are presented in Figure 4. The kinetics of E3G formation in both human and chimpanzee liver microsomes had characteristic Eadie–Hofstee plots that were consistent with autoactivation kinetics described by the Hill equation (Figure 4A–C). In contrast, the Eadie–Hofstee plots for E17G formation were linear for both species. Fitting of the E3G data to the Hill equation resulted in comparable estimates of V_{max}^{app}, S_{50}^{app} and n in both species (Table III). Estimated V_{max}^{app} and K_m^{app} values obtained for E17G by fitting to the classical Michaelis–Menten equation were similar as well being approximately twofold of each other when comparing the human with the chimpanzee.

Enzyme kinetics of morphine 3- and 6-glucuronide in chimpanzee and human liver microsomes

Eadie–Hofstee plots for M3G and M6G formation in human and chimpanzee liver microsomes are presented in Figure 5, and the associated enzyme kinetic parameters

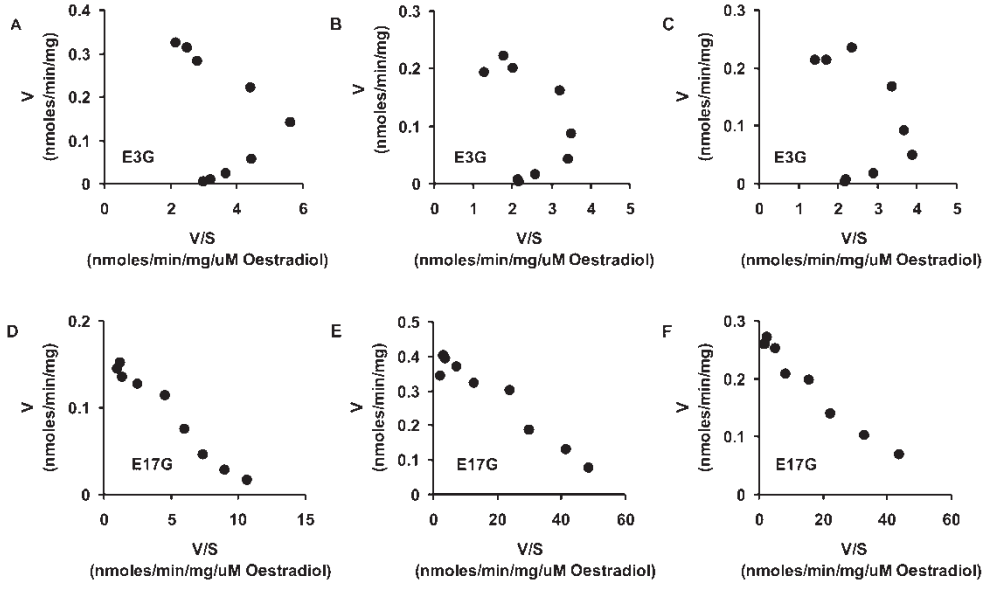

Figure 4. Eadie–Hofstee plots for E3G (A–C) and E17G (D–F) glucuronidation kinetics obtained using microsomes from humans (pooled of ten) (A, D), chimpanzee 3 (B, E), and chimpanzee 4 (C, F).

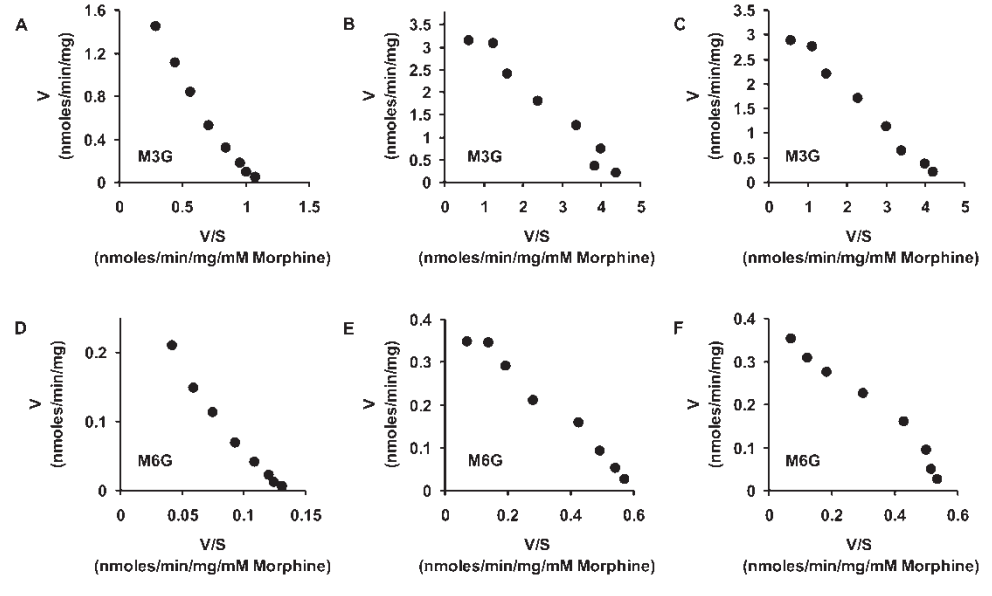

Figure 5. Eadie–Hofstee plots for M3G (A–C) and M6G (D–F) glucuronidation kinetics obtained using microsomes from humans (pooled of ten) (A, D), chimpanzee 3 (B, E), and chimpanzee 4 (C, F).

are presented in Table III. Eadie–Hofstee plots for M3G and M6G formation were linear for both species. Fitting to the Michaelis–Menten equation resulted in similar V_{max}^{app} and K_m^{app} estimates for human and chimpanzees that were approximately twofold of each other.

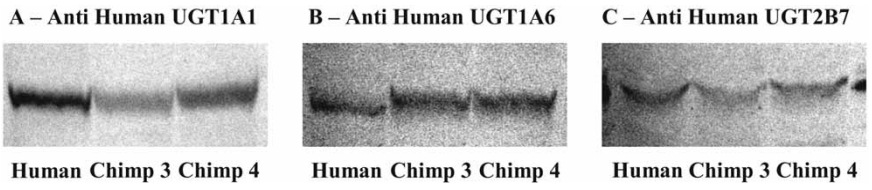

Figure 6. Western blot analysis of human (pooled) and male chimpanzee liver microsomes (20 μg of microsomal protein per lane) using antibodies reactive against human UGT1A1 (A), UGT1A6 (B) and UGT2B7 (C).

Western blots analysis of chimpanzee and human liver microsomes

A Western blot analysis of chimpanzee and human liver microsomes using antibodies reactive against human UGT 1A1, UGT 1A6, and UGT 2B7 is presented in Figure 6. The Western blot analysis is qualitative rather than quantitative in nature since the antibodies used in these studies were raised against human UGTs. Immunoreactive UGT proteins are present in chimpanzee liver microsomes analogous to each of the human UGTs investigated. For all three enzymes, the antibodies reacted with a single protein in chimpanzee liver microsomes migrating similarly to those found in human liver microsomes, suggesting similar molecular weights for the chimpanzee and human UGTs.

Discussion

Chimpanzees are a unique and limited resource for pharmacokinetic studies, yet this species may offer important advantages in the selection of promising drug candidates for human clinical trials. As with any non-clinical model, careful documentation of the similarities and differences in drug metabolism reactions with respect to humans must be provided, so that the model can be used intelligently. Whereas human tissue and recombinant enzymes are routinely available for biochemical studies, fresh tissue from healthy chimpanzees is rare and difficult to obtain. The studies reported here for the glucuronidation of important model drugs extends and complements earlier work characterizing drug disposition in this valuable animal model (Wong et al. 2004).

Acetaminophen or paracetamol (Figure 1) is a commonly used analgesic/antipyretic drug. In humans, APAP is primarily metabolized by the liver with only 2–5% excreted as unchanged drug in urine (Forrest et al. 1982). Its hepatic elimination is primarily via glucuronidation with sulfate conjugation being the second major pathway of elimination (Thomas et al. 1975; Forrest et al. 1982). The pharmacokinetic study with acetaminophen in chimpanzees provides the first reported comparison of the *in vivo* pharmacokinetics of a compound that is primarily eliminated by glucuronidation in chimpanzees and humans. The results suggest that the *in vivo* disposition of acetaminophen is similar in chimpanzees and humans. Both the *in vivo* pharmacokinetic parameters (Table I) and the urinary recoveries of acetaminophen and its glucuronide and sulphate conjugates (Table II) appear remarkably similar between the chimpanzees and humans when comparable oral doses are administered.

Acetaminophen glucuronidation has been previously assessed in human liver microsomes by others, and has shown a substantial degree of interindividual variability with reported activities varying from 7- to 15-fold (Fisher et al. 2000; Court et al. 2001). This large variability in activity may be a result of variability in the expression of UGT1A1, UGT1A6

or UGT1A9, which have been shown to glucuronidate acetaminophen at clinically relevant concentrations (Court et al. 2001). Of these UGT isozymes, UGT1A1, in particular, displays a dramatic range of protein expression and activities (Fisher et al. 2001). The V_{max}^{app} and K_m^{app} of APAPG formation generated in the current study using pooled human liver microsomes are comparable with literature values previously reported (Court et al. 2001). Enzyme kinetic parameters generated using chimpanzee liver microsomes were similar to estimates from pooled human liver microsomes, suggesting similar rates of APAPG formation. This is in contrast to a previous comparison using cynomolgus and rhesus monkey liver microsomes which showed APAPG formation rates that were five- to eightfold higher in macaque monkeys when compared with human liver microsomes (Sharer et al. 1995). Interestingly, Eadie–Hofstee plots of APAPG formation generated from chimpanzee 3 had the characteristic 'hooked' profile consistent with autoactivation kinetics, whereas the corresponding plot for chimpanzee 4 had a linear profile consistent with classical Michaelis–Menten kinetics. Both profiles have been observed previously in human liver microsomes (Court et al. 2001). Since APAP is glucuronidated by multiple UGTs in humans, the differences in the shapes of Eadie–Hofstee plots of APAPG formation in human liver microsomes may be related to both the kinetic characteristics and the relative expression or contribution of each UGT isoform involved in acetaminophen glucuronidation in individual human donors. An analogous situation in chimpanzees would explain the present observations, however, it is recognized that the limited number of individual chimpanzee liver microsomes used in the study may limit these conclusions.

The information from the Western blot analysis suggests that orthologues of human UGTs are present in the chimpanzee liver microsomes. In addition to acetaminophen, oestradiol and morphine were selected as probes of UGT catalytic activity in chimpanzee liver microsomes. The selection of these two substrates as probes was based upon their demonstrated selectivity for UGT1A1 and UGT2B7 in human liver microsomes (Senafi et al. 1994; Coffman et al. 1997; Court et al. 2003). In humans, UGT1A1 is primarily responsible for the glucuronidation of oestradiol at the 3-position (Senafi et al. 1994). Although the exact UGT isoforms responsible for E17G formation have yet to be identified, UGT 2B7 has shown to be involved (Gall et al. 1999). Eadie–Hofstee plots of both E3G and E17G formation in human liver microsomes are unique in that plots of E3G formation are 'hooked' shaped, which is consistent with autoactivation kinetics, whereas the corresponding plots for E17G are linear and consistent with Michaelis–Menten kinetics (Fisher et al. 2000; Alkharfy and Frye 2002). In the present experiments with chimpanzee liver microsomes, we observed similar characteristics in the Eadie–Hofstee plots of E3G and E17G formation. Furthermore, estimates of enzyme kinetic parameters observed for both metabolites were comparable with those obtained from human liver microsomes (Table III). Morphine 3- and 6-glucuronide formation is considered to be selective for UGT 2B7 activity in human liver microsomes (Coffman et al. 1997; Court et al. 2003). As noted, we observed species similarities in estimates of V_{max}^{app} and K_m^{app} for both M3G and M6G formation. In addition, Eadie–Hofstee plots of both morphine metabolites were similar being linear in both species. The regioselectivity of morphine glucuronidation at the 3- vs. the 6-position has been reported to be approximately 8:1 in human liver microsomes (King et al. 2000). In the rhesus monkey and dogs this ratio has been reported to be approximately 15:1 and approximately 50:1, respectively (King et al. 2000). Studies using marmoset monkey liver microsomes showed more similarity to the dog in that the regioselectivity of morphine glucuronidation was preferentially at the 3-position (Soars et al. 2001). The ratio of V_{max}^{app} for the formation of M3G vs. M6G in our experiments with pooled human liver microsomes is consistent with literature reports being

approximately 8:1 (Table III). This ratio is comparable in chimpanzee liver microsomes, being approximately 9:1 (Table III).

In summary, the current study documents the glucuronidation of three important substrates by chimpanzees. For the first time, the *in vivo* pharmacokinetics for compounds that are primarily metabolized by glucuronidation are compared directly for chimpanzees and humans, and *in vitro* studies of glucuronidation by chimpanzee liver are reported. While these data must be viewed cautiously because the number of animals used in the studies was low and little is known about substrate specifity in chimpanzees, these studies nonetheless add important, well-controlled data to the literature documenting animal–human similarities in drug disposition. Previous *in vitro* studies using liver microsomes from cynomolgus, rhesus, and marmoset monkeys have suggested higher rates of glucuronidation in non-primates when compared with humans (Sharer et al. 1995; Soars et al. 2001). The *in vivo* and *in vitro* information generated thus far collectively suggests that glucuronidation in chimpanzees is more similar to humans than previously observed for other non-human primates. Future studies with this species using compounds that are subject to glucuronidation, including those that undergo other types of glucuronidation such as *N*-glucuronidation and acyl-glucuronidation, will extend the characterization of glucuronidation in chimpanzees.

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