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Molecular Basis for Deficient Acetaminophen Glucuronidation in Cats

AN INTERSPECIES COMPARISON OF ENZYME KINETICS IN LIVER MICROSOMES

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ABSTRACT. Cats are highly susceptible to acetaminophen toxicity because of deficient glucuronidation of this drug *in vivo*. The enzyme kinetic basis for this defect is unknown. Therefore, the kinetic properties of acetaminophen UDP-glucuronosyltransferase (acetaminophen-UGT) were investigated, using hepatic microsomes from cats (N = 4) compared with those of species that are less sensitive to acetaminophen intoxication including dogs (N = 4), humans (N = 4), and six other mammalian species (one liver from each). Gunn rats were also studied, since they express defective UGT family 1 isoenzymes and are also prone to acetaminophen toxicity. Acetaminophen kinetics were biphasic in all instances with distinct high and low affinity components. K_m values for the high affinity activity in cat microsomes (0.31 ± 0.1 mM; mean ± SEM) were intermediate between those of dogs (0.11 ± 0.02 mM) and humans (0.60 ± 0.06 mM) and other species (0.22 to 6.7 mM; range). On the other hand, high affinity V_{max} values were over 10-fold less in cat microsomes (0.025 ± 0.006 nmol/min/mg) than in dogs (0.92 ± 0.09 nmol/min/mg) and humans (0.27 ± 0.09 nmol/min/mg); and over 5-fold less compared with microsomes from other species (range 0.13 to 7.63 nmol/min/mg). Gunn rat microsomes showed a similar 10-fold difference in high affinity V_{max} values between the homozygous mutant (0.67 nmol/min/mg) and homozygous normal (6.75 nmol/min/mg) animals. These results demonstrate that, relative to a number of other species, cats have remarkably low hepatic levels of a high affinity acetaminophen-UGT. This difference is sufficient enough to explain poor glucuronidation of acetaminophen *in vivo* and susceptibility to acetaminophen intoxication. BIOCHEM PHARMACOL 53;7:1041–1047, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. UDP-glucuronosyltransferase; acetaminophen; enzyme kinetics; microsomes; Gunn rats; cats

Over 40 years ago, it was recognized that the domestic cat differed significantly from other mammals in the ability to form glucuronide conjugates of certain xenobiotics, particularly low molecular weight phenolic derivatives [1–3]. Specifically, it was observed that when cats were administered these compounds, they were either metabolized slowly resulting in a prolonged half-life, metabolized by alternative pathways, or eliminated by different mechanisms [4, 5]. A consequence of this difference is that cats are exquisitely sensitive to the adverse effects of many drugs and toxins that are normally glucuronidated before elimination [6].†

The most striking example of this idiosyncrasy of cats is susceptibility to the toxic effects of the phenolic analgesic drug acetaminophen. Minimally toxic weight-normalized doses of acetaminophen are 3–5 times lower in cats compared with humans or dogs [7, 8]. The basis for this difference appears to be a lack of significant drug glucuronidation in the cat accompanied by rapid saturation of the predominant sulfation pathway with increasing acetaminophen dosage. This results in prolonged half-life of the parent drug, and significant formation of a highly reactive oxidative metabolite (*N*-acetyl-*p*-benzoquinone imine) [9]. Although the *in vitro* metabolism of acetaminophen has not yet been reported for the cat, a limited number of studies using other simple planar phenols as substrates have shown substantially reduced hepatic microsomal UGT‡ activity in cats compared with a number of other species [10–12]. The enzyme kinetic basis for this difference is unknown.

In the present study, we investigated whether cats differed from other species in the affinity or apparent amount of enzyme responsible for glucuronidating acetaminophen in the liver. Specifically, we characterized the kinetic properties of acetaminophen-UGT in hepatic microsomes derived from cats in comparison with species that are less sensitive to acetaminophen toxicity, including humans, dogs, and six other mammalian species. We also determined

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† Jernigan AD, Isiosyncrasies of feline drug metabolism. In: *Proceedings of the 12th Annual Kal Kan Symposium*, pp. 65–70, 1988.

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‡ *Abbreviations:* UGT, UDP-glucuronosyltransferase; and UDPGA, UDP-glucuronic acid.

acetaminophen-UGT kinetics in liver microsomes from Gunn rats, a mutant strain of rats, which are sensitive to acetaminophen toxicity as a result of a genetic defect of all UGT family 1 isoenzymes [13].

MATERIALS AND METHODS

Chemicals and Reagents

Brij 58 (polyoxyethylene 20-cetyl ether), UDPGA (sodium salt), and acetaminophen were purchased from the Sigma Chemical Co. (St. Louis, MO). 2-Acetamidophenol was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Acetaminophen glucuronide standard was provided by the McNeil Consumer Products Co. (Fort Washington, PA). All reagents were of the highest grade commercially available.

Liver Microsomes

Table 1 gives relevant details of liver tissue used in these studies. Animals were either acquired specifically for these studies, or were untreated control animals from studies in which tissue other than liver was being collected. Collection of tissue was approved by the Tufts University Animal Research Committee. Immediately after animals were killed, liver tissue was collected and placed in plastic freezer bags on dry ice and transported for final storage at −80°. Donors of human liver tissue had no history of liver disease but had failed a tissue match to possible recipients.

Liver microsomes were prepared by differential centrifugation as previously described [14]. The resultant pellet was reconstituted in 20% glycerol/phosphate buffer, divided into aliquots, and stored at −80°. Microsomes prepared in this fashion in this laboratory have been shown to be stable for at least 12 months. Frozen microsomes were thawed immediately prior to use. Microsomal protein concentrations were determined using the bicinchoninic acid assay (Pierce, Rockford, IL).

TABLE 1. Relevant details of liver tissue

Species	N	Breed/Strain/Race	Age	Sex
Cat*	4	Domestic short hair	1.5 years	M
Dog†	4	Beagle/mixed	1–3 years	M (2); F (2)
Human‡	4	Caucasian	39–66 years	F (3); M (1)
Cow§	1	Holstein	6 months	F
Horse§	1	Quarter horse	13 years	M
Monkey‖	1	Crab-eating macaque	Unknown	Unknown
Mouse¶	4	CD-1	12 weeks	M
Pig§	1	Yorkshire	1 year	F
Rabbit**	1	New Zealand White	9 months	F
Rat*	3	Gunn	14 weeks	M

* Harlan Sprague Dawley Inc. (Indianapolis, IN). Gunn rats included one each of j/j (homozygous mutant); j/+ (heterozygous mutant); and +/+ (homozygous normal).

† Marshall Farms (North Rose, NY).

‡ International Institute for the Advancement of Science (Exton, PA).

§ Tufts University School of Veterinary Medicine (North Grafton, MA).

‖ Unknown origin.

¶ Charles River Laboratories Inc. (Wilmington, MA). Microsomes were prepared from pooled livers of 4 individual animals.

** Hazelton Research Laboratories (Denver, PA).

Acetaminophen Glucuronidation Assay

The method used was similar to that previously described with some modifications [14]. Disposable glass culture tubes were prepared on ice containing phosphate buffer (50 mM; pH 7.5), microsomes (0.8 mg protein/mL), Brij 58, 5 mM $MgCl_2$, and acetaminophen to a total volume of 150 μL. These solutions were then equilibrated in an agitated water bath at 37° for 3 min. Reactions were initiated by the addition of 100 μL of UDPGA and allowed to proceed for 30 min (180 min for cat liver microsomes), at which time the reaction was stopped by the addition of a mixture of 25 μL of 1 N HCl and 10 μg of 2-acetamidophenol (the internal standard) in 25 μL of methanol, vortexed, and then immediately cooled on ice. The resultant mixture was transferred to 1.5-mL microfuge tubes and centrifuged at 14,000 rpm on a benchtop Brinkmann microcentrifuge for 5 min. The supernatant was then transferred to vials for determination of product concentration by HPLC. The chromatography apparatus included a C_{18} column (μBondapack, 300 × 3.9 mm i.d., Waters, Milford, MA) with a mobile phase consisting of 100 mM KH_2PO_4 in water:glacial acetic acid:acetonitrile (96:1:3, by vol.) at a flow rate of 1.4 mL/min. Eluants were monitored by ultraviolet absorption at a wavelength of 254 nm (Lambda-Max model 481, Waters). Product identity was verified by demonstrating coelution with a known standard and by disappearance of product after treatment with β-glucuronidase. For each run, a calibration curve was prepared using a series of concentrations of pure acetaminophen glucuronide. Metabolite concentrations were calculated by linear regression of calibration curve data using measured metabolite/internal standard peak height ratios. Intra- and inter-assay coefficients of variation were found to be less than 8 and 12%, respectively. Recovery of metabolite from microsomes using this method was consistently greater than 90% for acetaminophen glucuronide concentrations up to 80 μg/mL.

Enzyme activity was expressed as a reaction velocity calculated by dividing the amount of product formed by the incubation time and microsomal protein content (nmol/min/mg). Linear dependencies of enzyme activity on incubation time (up to 240 min) and microsomal protein concentration (up to 1 mg/mL) were established in initial experiments both with and without detergents added. In addition, these were substantiated in each experiment for each species by demonstrating proportionally greater product formation when reaction mixtures contained 50% more microsomal protein or were incubated for 50% longer than usual at the lowest substrate concentration.

All kinetic experiments in this study were performed using optimal activating concentrations of Brij 58 deter-

mined individually for each liver. Details of these initial experiments are given elsewhere [15]. In summary, optimal Brij 58 concentrations were found to be 0.012 ± 0.000% (w/v; mean ± SEM) for cat microsomes, 0.014 ± 0.001% for dog microsomes, 0.012 ± 0.002% for human microsomes, and 0.012 to 0.024% (range) for microsomes from other species. Associated increases in activity compared with baseline (no detergent) were 174 ± 16% for cat microsomes, 292 ± 31% for dog microsomes, 66 ± 40% for human microsomes, and 251–563% for microsomes from other species.

Enzyme Kinetic Analysis

UDPGA kinetics were determined initially for all livers using a fixed acetaminophen concentration (50 mM; the highest soluble concentration) and a range of UDPGA concentrations (0.25 to 50 mM). Based on these data, a fixed UDPGA concentration of 20 mM (over 10 times UDPGA K_m) and a range of acetaminophen concentrations between 0.125 and 30 mM were used in subsequent studies to determine the kinetics of acetaminophen association. The lowest acetaminophen concentration investigated was 0.125 mM since activities at lesser concentrations could not be quantitated reliably.

A nonlinear least-squares regression analysis program (Sigmaplot, Jandel Scientific, San Rafael, CA) was used to derive the Michaelis–Menten kinetic parameters V_{max} and K_m according to the following relationship:

$$V = \frac{V_{max} \cdot S}{K_m + S} \quad (1)$$

where S is the concentration of substrate that was varied and V is enzyme activity. In instances where Eadie–Hofstee plots (V vs V/S) were biphasic, data were additionally analyzed by nonlinear regression using the following equation:

$$V = \frac{V_{max1} \cdot S}{K_{m1} + S} + \frac{V_{max2} \cdot S}{K_{m2} + S} \quad (2)$$

where subscripts 1 and 2 represent V_{max} and K_m values for high and low affinity enzyme activities, respectively. In some instances, data were not described adequately by either equation 1 or 2, and there was an obvious convexity of high velocity data on Eadie–Hofstee plots. Therefore, data were also analyzed by incorporation of the sigmoid V_{max} model (equivalent to the Hill equation):

$$V = \frac{V_{max1} \cdot S}{K_{m1} + S} + \frac{V_{max2} \cdot S^b}{K_{m2}^b + S^b} \quad (3)$$

Initial estimates of parameters for curve fitting were obtained from Hanes–Wolf plots (S vs S/V) for the one-enzyme model, or by the iterative procedure described by Spears *et al.* [16] for two-enzyme models. These initial estimates and fitted parameters obtained by nonlinear regression were found to differ by less than 30%. The least complex equation that best fit the data (Equation 1, 2, or 3) was ascertained by visual comparison of the curves of best fit, plots of residuals, and by use of Akaike Information Criterion (AIC) analysis [17], a statistical comparison method that accounts for model complexity (i.e. number of parameters). The validity of individual fitted parameter estimates was assessed by use of standard error values calculated by the regression program. Standard errors of accepted parameter fits were less than 50% of the estimate.

Data Analysis

Data from cat, dog, and human hepatic microsomes are presented as means ± SEM. For other species in which individual livers (per species) were used, experiments were conducted in triplicate and results given as mean values. For purposes of comparison, data from cow, horse, monkey, mouse, pig, rabbit, and rat (homozygous normal Gunn rat) hepatic microsomes are also summarized as means ± SEM (denoted as "other species" group). These data were analyzed subsequently by ANOVA using the rank transformation approach with Student-Newman-Keuls post hoc multiple comparisons testing [18]. A difference of $P < 0.05$ was considered significant. By use of the heterogenous "other species" group, we were able to determine which of the homogenous groups (cat, dog, or human) most likely represented the species "outlier" and which were close to the "norm" when differences between these homogenous groups were detected.

RESULTS

UDPGA Kinetics

UDPGA kinetics were described adequately by the linear Michaelis–Menten model in all species examined (Fig. 1). Table 2 shows UDPGA kinetic parameters derived by nonlinear regression analysis. Although K_m values varied minimally between species groups ($P = 0.448$, ANOVA), V_{max} values were 3- to 4-fold lower in cats and humans than in dogs and the "other species" group ($P = 0.0002$, ANOVA).

Acetaminophen Kinetics

In all instances, Eadie–Hofstee plots of acetaminophen glucuronidation at a fixed UDPGA concentration showed biphasic kinetics (Figs. 2 and 3). Confirming this observation, nonlinear regression analysis showed that data were described more appropriately by the two-enzyme models (Equation 2 or 3) rather than the single-enzyme model (Equation 1). In species other than dog, mouse, and cow, data either could not be fitted to Equation 1 or 2, or resultant fits were very poor (standard errors of estimates >100%). In addition, Eadie–Hofstee plots of these data showed convexity of points associated with the low affinity component (see plots of cat and human data in Fig. 3). In these instances, data were best described by the sigmoid

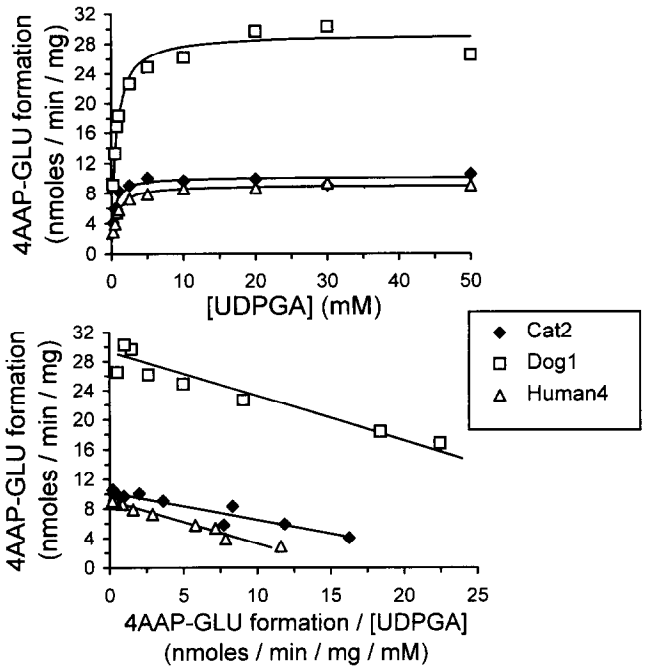

FIG. 1. Effect of UDPGA concentration on acetaminophen-glucuronide (4AAP-GLU) formation (top panel) in representative cat, dog, and human microsomes. Acetaminophen concentration was 50 mM. Corresponding Eadie–Hofstee plots of the data are shown in the bottom panel. Both panels show measured data points and associated curves representing the enzyme kinetic function of best fit (Equation 1) determined by nonlinear regression analysis. R^2 values for the fits were 0.923 (Cat2), 0.970 (Dog1), and 0.990 (Human4).

V_{max} modification of the low affinity component of the biphasic model (Equation 3).

Derived acetaminophen kinetic parameters are given in Table 3. V_{max} values for the high affinity component of acetaminophen-UGT activity in cat microsomes were more than 10-fold less than equivalent values for microsomes from dogs, humans, and the "other species" group ($P < 0.0001$, ANOVA). Likewise, the homozygous mutant Gunn rat had a high affinity V_{max} value 7 and 10 times smaller, (respectively) than V_{max} values determined for heterozygous mutant Gunn rat and the homozygous normal Gunn rat (measured using one individual of each type of rat). K_m values for the high affinity component were less than 1 mM for microsomes from all species except for rat microsomes (Wistar and Gunn mutants), which had values between 5 and 7 mM. Dog microsomes had significantly lower K_m values for the high affinity activity compared with microsomes from cats, humans, and the "other species" group ($P = 0.0014$, ANOVA).

DISCUSSION

The results of this *in vitro* study confirm previous *in vivo* observations of deficient glucuronidation of acetaminophen in cats compared with a number of other species. Furthermore, we have demonstrated by enzyme kinetic analysis

TABLE 2. Acetaminophen-UGT kinetics determined with variable UDPGA concentrations (0.25 to 50 mM) and constant acetaminophen concentration (50 mM)

Livers	K_m (mM)	V_{max} (nmol/min/mg)	N*
Cat	0.66 ± 0.12	7.8 ± 2.1† (d,o)‡	4
Dog	0.63 ± 0.04	21.6 ± 4.2 (c,h)	4
Human	0.58 ± 0.08	6.6 ± 1.6 (d,o)	4
"Other species"§	0.90 ± 0.15	33.7 ± 8.3 (c,h)	7
Cow	1.04	28.1	1
Horse	0.21	19.9	1
Monkey	0.51	13.5	1
Mouse	1.20	39.9	1
Pig	1.16	42.0	1
Rabbit	0.82	76.9	1
Gunn rat (+/+)	1.33	15.9	1
Gunn rat (j/+)	1.47	13.1	1
Gunn rat (j/j)	1.80	9.7	1

* N: number of individual livers. When N = 1, measured data represent means of three determinations.

† Data are given as means ± SEM.

‡ c, d, h, or o: Significantly different ($P < 0.05$) vs cat (c), dog (d), human (h), or "other species" group (o).

§"Orher species" group includes data from cow, horse, monkey, mouse, pig, rabbit, and homozygous normal Gunn rat (+/+).

that this difference may be attributable to markedly lower levels of a high affinity acetaminophen-UGT.

Glucuronidation reactions are catalyzed by the UGT enzymes, a group of more than 26 isozymes with distinct but overlapping patterns of substrate specificity and regulation [19]. Two distinct families of UGT enzymes have been identified by cDNA cloning and sequencing. Many of these enzymes have been characterized by substrate preference analysis of expressed cDNA. All UGT family 1 isozymes appear to originate from a single gene by alternative splicing to a common 3′ end. Preferred substrates include bili-

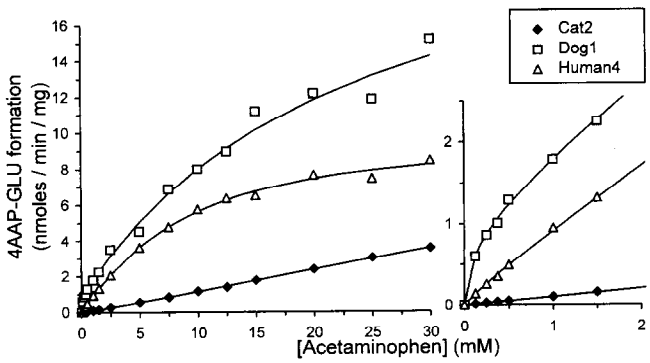

FIG. 2. Effect of acetaminophen (4AAP) concentration on acetaminophen-glucuronide (4AAP-GLU) formation in representative dog, cat, and human microsomes. The UDPGA concentration was 20 mM. Shown are measured data points and associated curves representing the enzyme kinetic function of best fit (Equation 2 for dog data; Equation 3 for cat and human data) determined by nonlinear regression. Insert at lower right shows magnification of plot at lower substrate concentrations. R^2 values for the fits were 0.999 (Cat2), 0.988 (Dog1), and 0.997 (Human4).

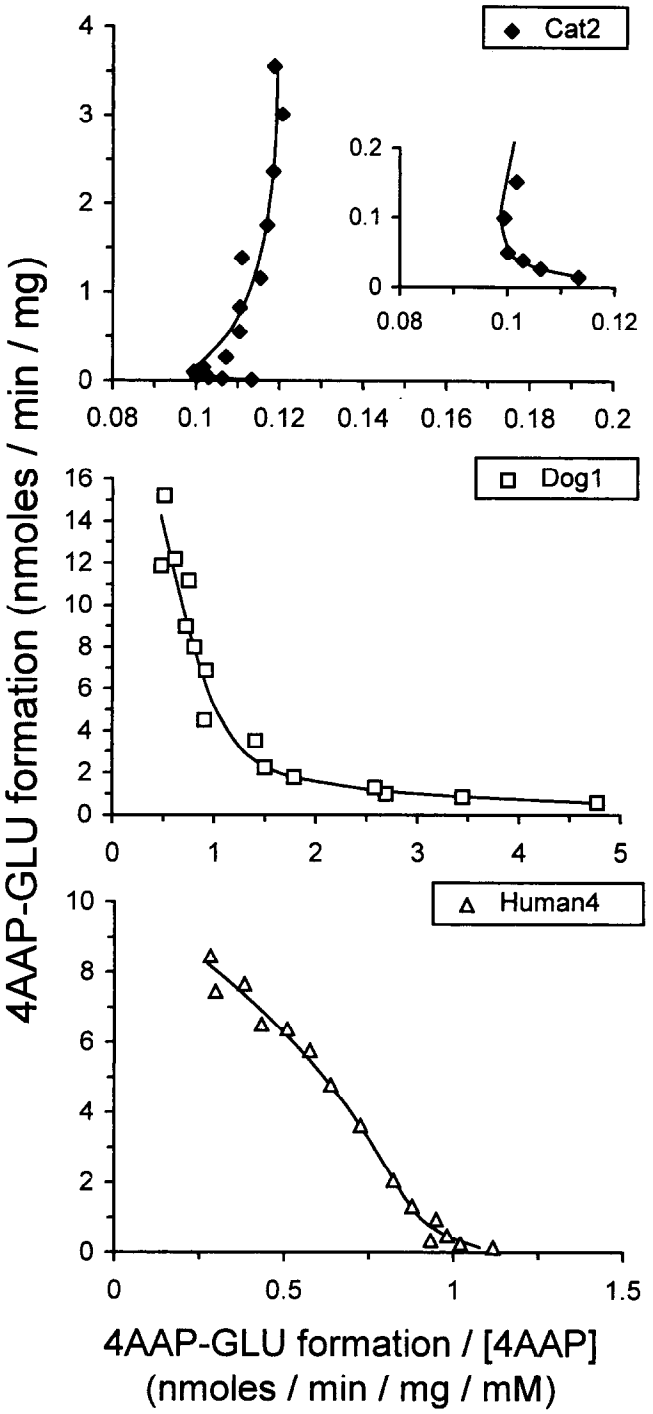

FIG. 3. Eadie–Hofstee plot of acetaminophen (4AAP) kinetics in representative dog, cat, and human microsomes (untransformed data is shown in Fig. 2). The UDPGA concentration was 20 mM. Shown are measured activity data points and associated curves representing the enzyme kinetic function of best fit (Equation 2 for dog data; Equation 3 for cat and human data) determined by nonlinear regression analysis. R^2 values for the fits were 0.999 (Cat2), 0.988 (Dog1), and 0.997 (Human4).

rubin (UGT1.1) [20, 21], tertiary amines (UGT1.4) [22], simple planar phenols (UGT1.6) [23, 24], and complex bulky phenols (UGT1.07) [23, 24]. Family 2 includes UGT isozymes specifically located in nasal epithelia (UGT2A), and hepatic enzymes (UGT2B) that conjugate carboxylic acids, hydroxylated compounds, and steroids [25].

Recent kinetic studies of acetaminophen metabolism using human and rat hepatic microsomes and expressed UGT enzymes have shown that multiple UGT isoforms are involved, and that UGT1.6 contributes significantly to the observed high affinity microsomal activity (K_m < 4 mM) [26]. Other UGT isoforms glucuronidate acetaminophen although with relatively low affinity (K_m > 12 mM). Based on studies in cats, dogs, mice, and humans, acetaminophen concentrations in blood and liver following therapeutic doses are generally less than 30 μg/mL (approximately 0.5 mM) and rarely exceed 300 μg/mL (approximately 5 mM) with toxic doses [8, 27, 28]. Consequently it is unlikely that the low affinity UGT isoforms contribute significantly to acetaminophen glucuronidation *in vivo* except following acetaminophen overdose. Furthermore, UGT1.6 would appear to be the relevant acetaminophen-UGT *in vivo*, although other, as yet unidentified, high affinity isoforms may also contribute. The 10-fold lower V_{max} of the high affinity acetaminophen-UGT that we observed in cat liver microsomes relative to other species is therefore sufficient enough to explain poor glucuronidation of acetaminophen *in vivo*. In addition, these results suggest that cats may either completely lack, or express very low levels of, a functional species homologue of UGT1.6.

The Gunn rat, a mutant strain of the Wistar rat, has been used for many years as an animal model of unconjugated hyperbilirubinemia [13]. The cause of this anomaly is a single point mutation that results in a premature stop codon in one of the common exons of the UGT family 1 gene locus and consequent dysfunction of all expressed UGT1 enzymes including UGT1.6 [29]. Like the cat, Gunn rats glucuronidate planar phenolic compounds poorly and are more susceptible to acetaminophen toxicity than normal rats [30]. In this study, we additionally showed that the high affinity V_{max} value was 10-fold less in microsomes from a homozygous mutant Gunn rat compared with a homozygous normal animal (it should be noted though that determinations were made using livers from individual rats). This difference in high affinity V_{max} is similar to that which we found when comparing cat microsomes with microsomes from other species. Unlike Gunn rats, which also have a defective bilirubin glucuronosyltransferase (UGT1.1), cats appear to have an adequate capacity to glucuronidate bilirubin [10]. Consequently, the defect of acetaminophen glucuronidation in cats is unlikely to involve all UGT family 1 enzymes.

As in previous studies of acetaminophen glucuronidation kinetics [26, 31], incubations in this study were performed in the presence of optimally activating concentrations of the detergent. Although this could potentially limit the direct *in vivo* applicability of the data, we did this in order to identify species differences in enzyme protein level (as indicated by V_{max} values) independent of differences in UGT protein–lipid interactions [32]. We have shown recently that significant species differences in the activation of acetaminophen-UGT by detergents exist, but are un-

TABLE 3. Comparative acetaminophen-UGT kinetics determined with variable acetaminophen concentrations (0.125 to 30 mM) and constant UDPGA concentration (20 mM)

Livers	K_{m1} (mM)	V_{max1} (nmol/min/mg)	K_{m2} (mM)	V_{max2} (nmol/min/mg)	b*	N†
Cat	0.31 ± 0.10‡ (d,h)§	0.025 ± 0.006 (d,h,o)	150 ± 65 (h,o)	22.1 ± 8.0 (h)	1.3 ± 0.1 (d)	4
Dog	0.11 ± 0.02 (c,h,o)	0.92 ± 0.09 (c)	46.2 ± 14.6 (h)	25.9 ± 3.7 (h)	1.0 ± 0.0 (c,h)	4
Human	0.60 ± 0.06 (d,c)	0.27 ± 0.09 (c,o)	9.3 ± 0.5 (c,d)	5.4 ± 1.7 (c,d,o)	1.4 ± 0.1 (d)	4
"Other species"‖	1.19 ± 0.74 (d)	3.15 ± 1.12 (c,h)	23.8 ± 8.1 (c)	22.9 ± 4.8 (h)	1.2 ± 0.1 (d)	7
Cow	0.61	1.44	33.1	28.5	1.0	1
Horse	0.96	1.00	7.3	12.1	1.4	1
Monkey	0.34	1.53	3.9	8.2	1.3	1
Mouse	0.26	7.63	62.8	38.9	1.0	1
Pig	0.36	0.13	27.7	34.9	1.3	1
Rabbit	0.22	3.56	2.9	28.1	1.4	1
Gunn rat (+/+)	5.58	6.75	28.6	9.9	1.2	1
Gunn rat (j/+)	5.36	4.65	35.0	9.2	1.2	1
Gunn rat (j/j)	6.70	0.67	29.7	10.2	1.1	1

* Fitted estimate of exponent in Equation 3.

† N: Number of individual livers. When N = 1, measured data represent means of three determinations.

‡ Data are given as means ± SEM.

§ c, d, h, or o: Significantly different ($P < 0.05$) vs cat (c), dog (d), human (h), or "other species" group (o).

‖ "Other species" group includes data from cow, horse, monkey, mouse, pig, rabbit, and homozygous normal Gunn rat (+/+).

likely to explain deficient acetaminophen glucuronidation in cats [15].

Consistent with previous studies using human microsomes [26], kinetic analyses of acetaminophen glucuronidation revealed the presence of distinct high and low affinity activities in all species examined. In dog, cow, and mouse microsomes, data were described satisfactorily by a simple biphasic kinetic model. However, in other species we were either unable to fit the data to this model, or resultant fits were very poor. Inspection of Eadie–Hofstee plots showed convexity of high velocity data points resulting in a distinctive sigmoidal shape to the plot (compare plots of cat and human data with the plot of dog data in Fig. 3). To obtain estimates of kinetic parameters for the high affinity acetaminophen-UGT, we used a modified biphasic model consisting of a high affinity Michaelis–Menten equation together with a low affinity sigmoidal V_{max} equation (equivalent to the Hill equation). Appropriateness of this model was confirmed by statistical analysis using the Akaike Information Criterion, which accounts for model complexity based on the total number of parameters that are used to fit the data. An identical approach was used recently to describe the complex enzyme kinetics of amitriptyline N-demethylation in human liver microsomes [33]. In that study, cytochrome P450 3A4 was shown to be primarily responsible for the sigmoidal low affinity component. Based on their findings and the results of a number of other studies, the nonlinear sigmoidal kinetics demonstrated by cytochrome P450 3A4 were suggested to result from cooperative substrate binding [34–38]. It is also possible that the sigmoidal kinetics observed for the low affinity acetaminophen-UGT in this study result from cooperative substrate binding; however, to confirm this would require further studies with purified enzyme.

In conclusion, we have demonstrated that cats have remarkably low hepatic levels of a high affinity acetaminophen-UGT relative to other species. This difference appears to be sufficient enough to explain poor *in vivo* acetaminophen glucuronidation and associated susceptibility to acetaminophen intoxication. Based on previous studies of substrate specificity of expressed UGT isoenzymes and by analogy to the effects of the genetic mutation in Gunn rats on acetaminophen glucuronidation, we hypothesize that cats may either lack or poorly express a functional species homologue of UGT1.6. This possibility warrants further investigation.

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