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The effect of valproic acid on drug and steroid glucuronidation by expressed human UDP-glucuronosyltransferases

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Abstract

Valproic acid glucuronidation kinetics were carried out with three human UGT isoforms: UGT1A6, UGT1A9, and UGT2B7 as well as human liver and kidney microsomes. The glucuronidation of valproic acid was typified by high K_m values with microsomes and expressed UGTs (2.3–5.2 mM). The ability of valproic acid to interact with the glucuronidation of drugs, steroids and xenobiotics *in vitro* was investigated using the three UGT isoforms known to glucuronidate valproic acid. In addition to this the effect of valproic acid was investigated using two other UGT isoforms: UGT1A1 and UGT2B15 which do not glucuronidate valproic acid. Valproic acid inhibited UGT1A9 catalyzed propofol glucuronidation in an uncompetitive manner and UGT2B7 catalyzed AZT glucuronidation competitively ($K_i = 1.6 \pm 0.06$ mM). Valproate significantly inhibited UGT2B15 catalyzed steroid and xenobiotic glucuronidation although valproate was not a substrate for this UGT isoform. No significant inhibition of UGT1A1 or UGT1A6 by valproic acid was observed. These data indicate that valproic acid inhibition of glucuronidation reactions is not always due to simple competitive inhibition of substrates.

Keywords: Drug metabolism; Glucuronidation; Inhibition; Valproic acid; Interaction

1. Introduction

Valproic acid is a broad-spectrum antiepileptic drug that is also used in the treatment of bipolar disease and migraine headaches. Structurally, valproate is a short chain fatty acid, which is metabolized by the body in the same manner as endogenous fatty acids with greater than 97% eliminated by hepatic metabolism. Major pathways of valproate metabolism include glucuronidation by UGTs, mitochondrial β-oxidation and a minor cytochrome P450-dependent oxidation pathway and desaturation [1].

The UGTs are family of enzymes which catalyze the transfer of a glucuronic acid moiety from a donor co-substrate UDPGA to an aglycone [2]. The UGT family can be separated into two distinct families, the UGT1 family are all derived from a single gene by alternative splicing of four constant exons (exons 2–5) with a variable exon 1 which determines the substrate specificity of the isoform [3]. The UGT1 family members have demonstrated to be capable of glucuronidating a wide range of drugs, xenobiotics and endobiotics [4]. The UGT2 family of isoforms are encoded by single genes and are clustered on chromosome 4q [5]. This family have long been considered to be more involved in the glucuronidation of endobiotics including steroids and bile acids. UGT2 isoenzymes seem to favour these types of compound as substrates but can glucuronidate other chemical types.

Three UGT isoforms have been reported to be capable of glucuronidating valproic acid: UGT1A6 [6], UGT1A9 [7] and UGT2B7 [8]. Both UGT1A6 and UGT1A9 are capable of glucuronidating a wide range of drugs and xenobiotics [7]. UGT2B7 seems to be exceptional among the 2B family in its acceptance of drug molecules including morphine [9] and AZT [10,11] as well as glucuronidating a range of endogenous molecules including 4-hydroxyestrone and the bile acid hyodeoxycholic acid.

Valproate has been demonstrated to inhibit a wide variety of hepatic enzymes in humans and animal species

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

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both *in vivo* and *in vitro*. These include cytochrome P450, UGTs and epoxide hydrolase [12]. Overall, the largest inhibitory effect of valproate appears to be on drugs metabolized by the UGTs. The glucuronidation of valproic acid in humans is thought to affect the metabolism of other drugs which are glucuronidated. Lorazepam undergoes reduced clearance in the presence of valproic acid [13] as does lamotrigine with a corresponding increase in half life [14]. An interaction was observed in humans *in vivo* with concurrent dosing of valproic acid and zidovudine (AZT) which was used in an attempt to modulate the pharmacokinetics of AZT in HIV sufferers [15]. Co-administration of valproic acid significantly decreased the normally extensive first pass metabolism of AZT to its glucuronide conjugate and increased the ratio of urinary AZT glucuronide to unconjugated AZT by 50%. Valproic acid also inhibits the glucuronidation of parahydroxyphenobarbital, the major metabolite of phenorbarbital. This is thought to occur by valproate competing with parahydroxyphenobarbital at the UGT active site; this interaction has been observed in both *in vitro* and *in vivo* studies in rat [16,17]. Valproic acid has also been shown to inhibit the formation of 4-hydroxyandrostendione glucuronide, a synthetic steroid, in both *in vitro* and *in vivo* rat studies [18] an indication that valproic acid may also be able to interfere with the glucuronidation of steroids.

The aims of this study were to identify those UGT isoforms which could glucuronidate valproic acid and determine their kinetic constants to compare with those measured in human liver and kidney microsomal preparations. The effect of valproic acid on the glucuronidation of probe substrates, drugs and steroids known to be substrates for the human recombinant isoforms was to be investigated in order to assess the inhibitory potential of valproic acid on glucuronidation and whether metabolic interactions might be able to affect the glucuronidation of these compounds *in vivo*. To this end new analytical methods were developed to investigate the potential interactions between valproic acid and substrates for three UGT isoforms which glucuronidate valproic acid: UGT1A6, UGT1A9 and UGT2B7. In addition to this another two steroid glucuronidating UGT isoforms were included in the study. UGT1A1 has been reported to glucuronidate estriol and the synthetic steroid ethinylestradiol [19] and UGT2B15 which has been reported to glucuronidate a wide range of androgenic steroids [20].

2. Materials and methods

2.1. Chemicals

Substrates, UDP-glucuronic acid, AZT, AZT glucuronide and other reagents used in the assays were purchased from Sigma, Aldrich, BDH and were of the highest grade available. [^{14}C]UDPGA, [^{14}C] dihydrotestosterone and were purchased from NEN Dupont. Penicillin, streptomycin and geneticin were purchased from Gibco Life Technologies.

2.2. Tissue culture and human microsomes

The cloning of the UDP-glucuronosyltransferase isoforms used and their expression in V79 Chinese hamster lung fibroblast recombinant UGT cell lines have been published previously [21–23]. V79 cells expressing UGT1A1, UGT1A6, UGT1A9, UGT2B7 cells and Hek293 cells expressing UGT2B15 [20] (kindly donated by Dr. T. Tephly) were utilized in this series of studies. The V79 cell line heterologously expressing human UGTs were grown up in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 100 U/mL penicillin and 0.1 mg/mL streptomycin and optimized constant selection concentrations of geneticin (100 μg/mL). Hek293 cells expressing UGT2B15 were grown up in the media described above supplemented with 10 mM HEPES pH 7.4.

Human liver microsomes used were prepared from one liver, a female age 41 postmortem (subarrachnoid hemorrhage) and obtained through a liver bank. Human kidney tissue was a pathological specimen taken from a patient undergoing a nephrectomy. Microsomes were prepared from a single donor. Human tissues were collected after appropriate ethical approval.

2.3. Standardized cellular sonication

Cells were disrupted by a standardized sonication method. Pellets containing cells harvested from two 15 cm diameter tissue culture plates were thawed prior to assaying and resuspended in 200 μL of phosphate buffered saline pH 7.4. Each 200 μL suspension of cells was sonicated for 4–5 s bursts (MSE soniprep 150, Sanyo Gallenkamp) allowing at least 1 min on ice between bursts. Aliquots of cells prepared by this method were pooled prior to addition to the assays. Microsomes were diluted to the appropriate concentration were and were also activated by sonication. Protein concentrations were measured by the method of Lowry *et al.* [24].

2.4. UGT assays

The basic conditions for all the assays in this study were identical. Valproic acid or a probe substrate were incubated with either expressed human UGTs (250–350 μg protein per assay) or human kidney or liver microsomes (100–200 μg protein per assay) for 40 min at 37° in 100 mM Tris–Maleate buffer pH 7.4. Assays were terminated by addition of an equal volume of prechilled methanol and the precipitated protein removed by centrifugation (10,000 *g*, 10 min). A combination of radiochemical and LC–MS–MS analyses were applied to the supernatants depending on the substrate assayed.

2.5. Radiochemical HPLC analysis of glucuronidation

Standardized sonication and assays were performed according to the method of Ethell *et al.* [25]. Briefly the assay conditions were 100 mM Tris–Maleate pH 7.4, 5 mM $MgCl_2$, 2 mM UDPGA, 350–450 μg of sonicated cells (4–5 s bursts) or 200 μg of human liver/kidney microsomes. For kinetic determination of 4-hydroxyestrone with UGT2B7 the range of substrate concentrations for the probe substrates was 10–250 μM and the range used for the determination of valproate kinetics was 0.5–15 mM. Valproate stock solutions were made up from the sodium salt of the carboxylic acid which is readily soluble in water. There were no indications of substrate precipitation even at 15-mM assay concentrations. Assays were incubated for 40 min and terminated by an equal volume of methanol prechilled to −20°. The protein was removed by centrifugation and the supernatant injected onto HPLC. The HPLC conditions for these substrates comprised a binary gradient from 0 to 100% acetonitrile in ammonium acetate developed over 13 min on a Techsphere ODS2 column (HPLC technology). Detection of [^{14}C] labeled UDPGA and glucuronides was achieved using a Reeve 9701 radioactivity monitor (Reeve Analytical) fitted with a 200 μL heterogenous cerium activated lithium glass flowcell.

HPLC conditions varied depending on the probe substrate used in the interaction study. 17α-Ethinylestradiol, 2-hydroxyestradiol, β-estriol, 4-hydroxyestrone, 5α-androsterone, 3α,17β-diol and 8-hydroxyquinoline glucuronidation were also measured using the gradient method of Ethell *et al.* [25] as described above. Valproic acid glucuronide formation was linear with respect to time under these assay conditions.

The glucuronides of valproic acid, propofol and 4-hydroxyestrone which had become radiolabeled by incorporation of [^{14}C]UDPGA all co-eluted on the gradient radiochemical system and had to be resolved by isocratic separation. The isocratic separation used an acetonitrile/0.05 M ammonium acetate pH 5.7 mobile phase using the same column as used for the gradient method. For resolving propofol and valproic acid glucuronides the ratio was 76:24 and for 4-hydroxyestone and valproic acid it was 80:20 (Fig. 1).

Assays using dihydrotestosterone as substrate contained no [^{14}C]UDPGA, instead a radiolabeled substrate: [^{14}C] dihyrotesterone (0.2 μCi per assay) was used with all other the assay conditions remaining the same. Chromatograms illustrating the separation of the radiolabeled glucuronides are shown in Fig. 1. The gradient conditions were altered to allow the elution of unused [^{14}C] dihydrotestoterone using the same components of the binary gradient described for other substrates, also described previously [25]. The gradient used was 30–100% in 8.5 min, hold at 100% for 3 min with a 3 min re-equilibration step. The substrate was present at much lower concentrations than the co-substrate UDPGA (10–100 μM compared to 2 mM); therefore, levels of radiolabel incorporation into the dihydrotestosterone glucuronide were considerably higher and provided a more sensitive assay for this substrate.

2.6. LC–MS–MS analysis of AZT glucuronides

The rate of glucuronidation of AZT by UGT2B7 was relatively low compared to that of other probe substrates and the levels of activity measured by radiochemical HPLC methods did provide adequate sensitivity to study any potential competitive interaction between AZT and valproic acid glucuronidation.

A LC–MS–MS method was devised to provide the sensitivity necessary for this process. Authentic AZT glucuronide, 5 ng/μL, was infused at 10 μL/min (Pump 11, Harvard Apparatus) into the source of a Micromass Quattro LC mass spectrometer operating in negative ion mode. The formation of the parent ion of AZT glucuronide ($[M-1]^-$:442.2) was found to be optimal at cone and capillary voltages of 3.5 kV and 30 V, respectively. Other source conditions were: source block temperature 100°; desolvation temperature: 400°; nebuliser gas flow 100 L/hr

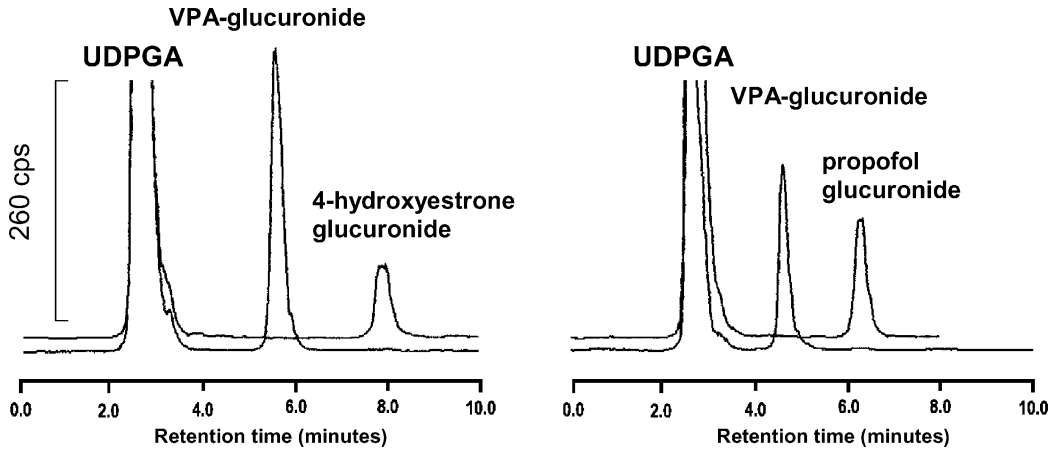

Fig. 1. HPLC chromatograms taken from test assays to illustrate the separation of valproic acid glucuronide (VPA-glucuronide) from UGT probe substrate glucuronides. The *Y*-axis is counts per second.

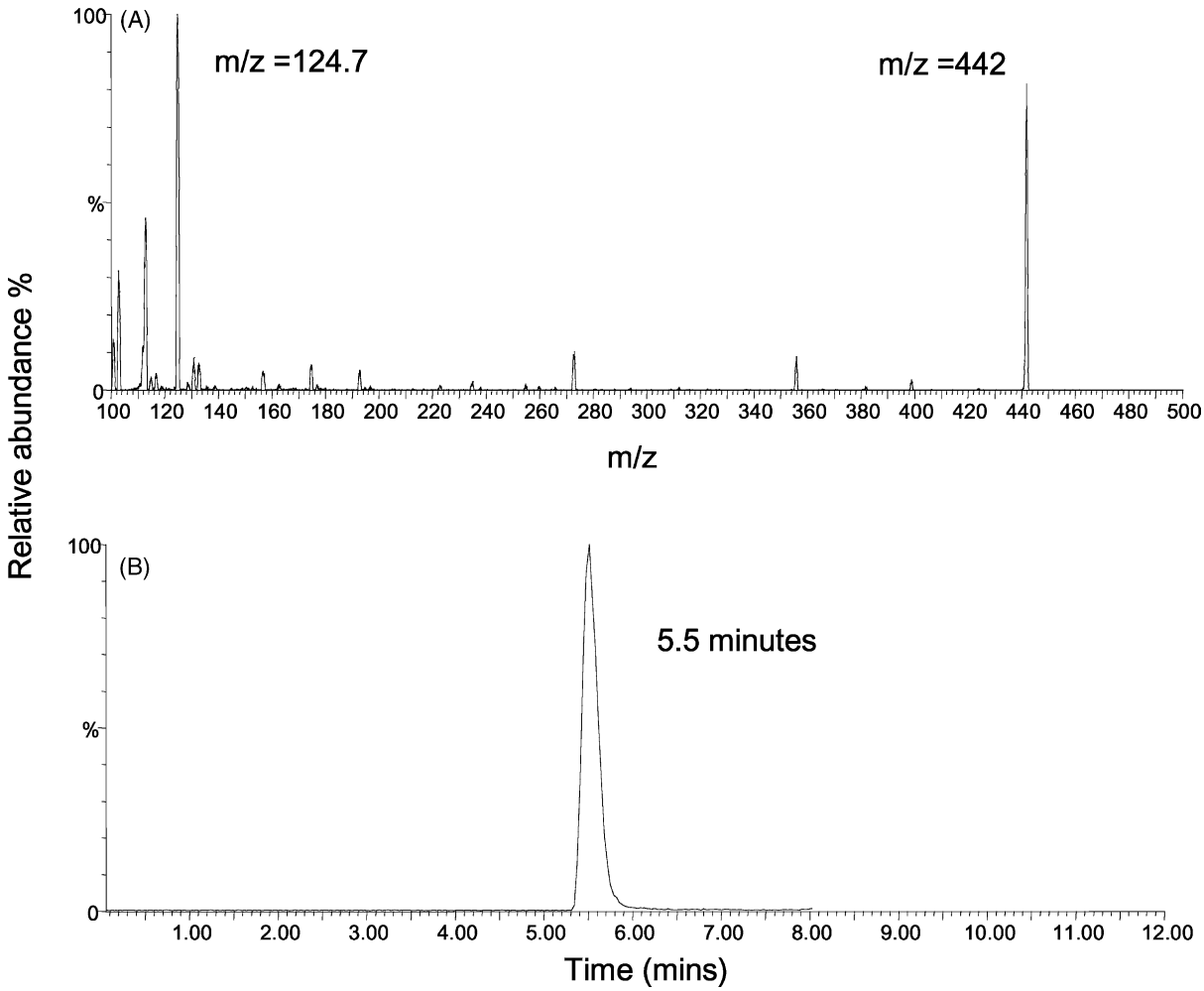

Fig. 2. (A) Daughter ion spectrum of AZT glucuronide at a collision energy of 20 eV. Remaining AZT glucuronide is present at $m/z = 442$, the largest fragment ion is illustrated at $m/z = 124.7$. (B) Sample chromatogram of AZT-glucuronide standard injected onto LC–MS–MS eluting at 5.5 min.

and desolvation gas flow: 300 L/hr. The parent ion was fragmented using argon as the collision gas and the daughter ion spectrum was recorded. The daughter ion of mass 124.7 was observed to be the most abundant ion (Fig. 2A) and was selected as the ion to be measured for a multiple ion monitoring (MRM) method. The cone voltage and collision energy were optimized for AZT glucuronide: cone voltage = 30 kV, collision energy = 25 eV. The LC conditions comprised a rapid gradient of 0–100% acetonitrile in 0.2% formic acid over 6 min with a 3 min re-equilibration step on a 15 cm × 2.1 mm i.d. Spherisorb ODS2 column (Waters) at a flow rate of 0.3 mL/min. AZT glucuronide eluted at 5.5 min (Fig. 2B) and assays incubated in the absence of UDPGA contained no peaks at all.

2.7. Interaction studies

The inhibitory effect of valproic acid on the glucuronidation of drug, xenobiotic and steroid substrates of glucurondation was evaluated with the following substrates for each UGT isoform: 17α-ethinylestradiol, 2-hydroxyestradiol and β-estriol glucuronidation by UGT1A1; 1-naphthol glucuronidation by UGT1A6; propofol glucuronidation by UGT1A9; 4-hydroxyestrone and AZT glucuronidation by UGT2B7 and dihydrotestosterone, 5α-androsterone, 3α,17β-diol and 8-hydroxyquinoline glucuronidation by UGT2B15. The substrate concentrations to be used to evaluate the inhibitory potential of valproic acid on expressed human UGTs were selected on the basis of their known kinetics, which have been published previously [7,10,25].

Initially each interaction experiment was performed with a minimum of three substrate concentrations which were selected to represent concentrations which were estimated from the known K_m of the glucuronidation reactions in question. The substrate concentrations employed in these studies were the equivalent of half the concentration of the K_m, the concentration at K_m and double the value of the K_m.

The inhibition of probe substrate glucuronidation was measured at each of these substrate concentrations with, 0, 0.5, 1 and 5 mM valproic acid. Three of the interaction experiments were repeated increasing the number of substrate concentration points in order to estimate more accurate K_i values. The interaction between valproic acid

and propofol glucuronidated by UGT1A9 was repeated at substrate concentrations of 0, 25, 100, 250, 500 and 750 μM propofol and 0, 0.5, 1 and 5 mM valproic acid. The interaction between valproic acid and 4-hydroxyestrone glucuronidation was repeated at substrate concentrations of 0, 25, 50, 100, 250 and 500 μM.

The conditions for the AZT interaction differed slightly from those of the other two substrates. The AZT LC–MS–MS assay was developed as an alternative to the radiochemical HPLC assays used previously. It was initially evaluated by measuring the effect of increasing valproic acid concentrations on a fixed concentration of AZT (1 mM) to determine an IC_{50} value (data not shown). From this result the concentrations of valproic acid were chosen for the interaction study. AZT was incubated with UGT2B7 at concentrations of 1, 2.5, 5, 7.5 and 10 mM in the presence of 0, 1 and 6 mM valproic acid.

The mode of valproic acid inhibition of each UGT isoform was determined by Eadie Hofstee plots and the inhibition constants were calculated from the experimental data by fitting the results to the Michaelis Menten equation for to the appropriate inhibition equation substituting the V_{max} and K_m values for each substrate in question into the equation (Prism 2, GraphPad Software).

3. Results

3.1. Glucuronidation of valproic acid by human UGTs

The kinetic parameters for valproic acid glucuronidation with expressed human UGTs and human tissue microsomes are listed in Table 1. No glucuronidation activity towards valproic acid could be measured for UGT1A1 or UGT2B15 but UGT1A6, UGT1A9 and UGT2B7 all clearly demonstrated significant activity towards this substrate. The K_m values for all the expressed UGTs are high typically the millimolar range (2.1–5.2 mM) and are consistent with the K_m values measured for valproic acid glucuronidation in both human liver and human kidney microsomes. The relative contribution of each isoform is difficult to assess and it is likely that all three play a role in the total microsomal glucuronidation of valproic acid.

Table 1
Enzyme kinetic parameters for glucuronidation of valproic acid using human liver and kidney microsomes and cloned and expressed human UGT isoforms

UGT isoforms	K_m (mM)	V_{max} (nmol/min mg protein)
UGT1A1	–	–
UGT1A6	3.2 ± 0.53	0.70 ± 0.04
UGT1A9	5.2 ± 0.84	0.98± 0.06
UGT2B7	2.1 ± 0.35	0.66 ± 0.04
UGT2B15	–	–
HKM	3.4 ± 0.63	0.48 ± 0.03
HLM	3.8 ± 0.30	3.2 ± 1.0

3.2. Valproic acid inhibition of glucuronidation catalyzed by human UGTs

The effect of the valproic acid on the glucuronidation on model substrates of the five human UGT isoforms used are shown in Table 2. The table also lists the K_m values of the substrates used which have been published elsewhere

Table 2
The effect of valproic acid on the glucuronidation of probe substrates for cloned and expressed human UGT isoforms

UGT isoform	Substrate	K_m (μM)	Inhibition (% activity of control) ± SD		
			[valproic acid] (mM)		
			0.5	1	5
UGT1A1	17α-Ethinylestradiol	55[a]	96 ± 4	93 ± 7	83 ± 8
	2-Hydroxyestradiol	35[a]	100 ± 0	93 ± 13	75 ± 5
	β-Estriol	76[a]	100 ± 0	98 ± 3	91 ± 10
UGT1A6	1-Naphthol	168[a]	98 ± 1	98 ± 1	96 ± 4
UGT1A9	Propofol	200[b]	90 ± 12	82 ± 5	60 ± 2
UGT2B7	4-Hydroxyestrone	24[c]	102 ± 8	101 ± 13	89 ± 4
	AZT	1600[d]	–	78 ± 12	46 ± 15[e]
UGT2B15	Dihydrotestosterone	42[a]	87 ± 4	77 ± 9	41 ± 11
	5α-Androsterone, 3α,17β-diol	33[a]	75 ± 23	95 ± 8	44 ± 4
	8-Hydroxyquinoline	96[a]	93 ± 12	100 ± 0	64 ± 10

Each inhibition point represents the mean inhibition of at least three substrate concentrations. The error is given as the standard error of the mean percentage inhibition.

[a] Originally published in [25].
[b] Originally published in [7,34].
[c] Compared to the previously published values of 12 and 16 μM [24].
[d] Originally published in [10].
[e] Assayed at 6 mM valproic acid not 5 mM (see Section 2).

[7,10,25]. The K_m for UGT2B7 4-hydroxyestrone was determined in this study ($K_m = 24 \pm 5$ μM, $V_{max} = 0.52 \pm 0.03$ nmol/min mg protein) and compares favorably to the values reported previously of 12 and 16 μM [26]. The results in Table 2 are expressed as percentage of control activity as measured in the absence of valproic acid.

UGT1A9 propofol glucuronidation, UGT2B7 AZT glucuronidation and UGT2B15 5α-androsterone, 3α,17β-diol glucuronidation were all inhibited by approximately 40–60%. Inhibition of UGT1A1 was only apparent at the highest valproic acid concentrations and UGT1A6 appeared not to be inhibited by valproic acid despite the fact that valproic acid is glucuronidated by this isoform.

The inhibition profiles for the valproic acid inhibition of propofol and AZT glucuronidation by UGT1A9 and UGT2B7 are shown in Fig. 3A and Fig. 4A. The mode of inhibition of these two isoforms is distinctly different. The inhibition of AZT glucuronidation by valproic acid is clearly competitive in nature (Fig. 3B) whereas the inhibition of propofol by the same substrate seems follows the form of uncompetitive inhibition (Fig. 4B) according to the Eadie Hofstee plot.

The data that was generated from the assays with UGT2B15 was more difficult to work with as the baseline activities for this isoform was much lower than the "ideal" substrates for the other UGT isoforms. Consequently, no linearization of the data was possible by Eadie Hofstee plots. Although the mode of inhibition could not be ascertained the results that were generated clearly demonstrate significant inhibition of UGT2B15 substrate glucuronidation.

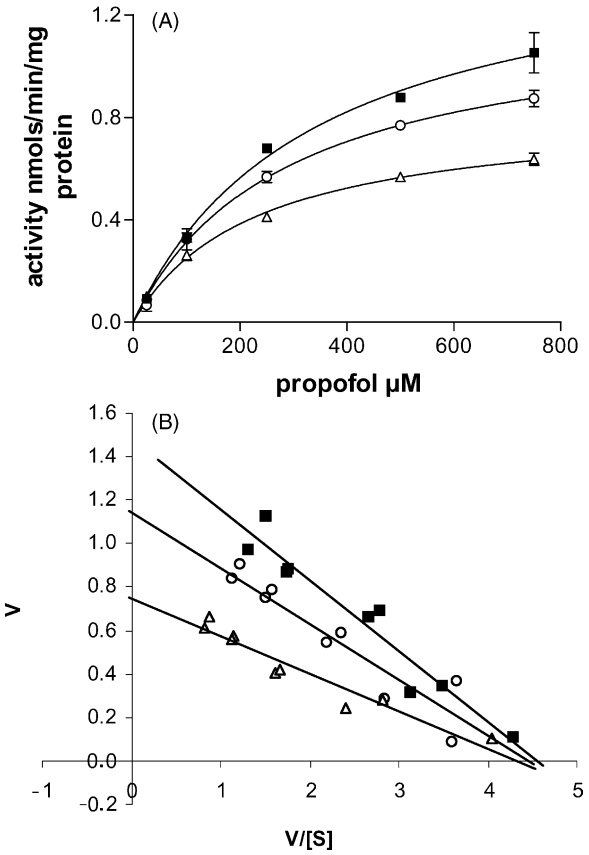

Fig. 3. Inhibition of UGT1A9 catalyzed propofol glucuronidation by valproic acid. (A) K_i determination for inhibition of AZT glucuronidation by valproic acid. (B) Eadie Hofstee plot of the data in (A) the form of the plot indicates uncompetitive inhibition. Closed squares: no valproic acid, open circles: 1 mM valproic acid, open triangles: 5 mM valproic acid.

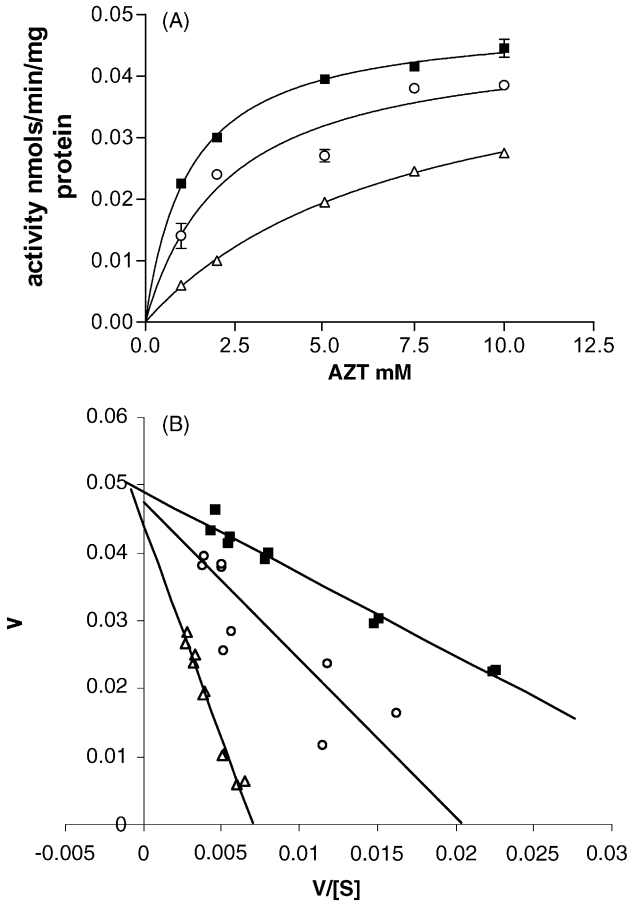

Fig. 4. Inhibition of UGT2B7 catalyzed AZT glucuronidation by valproic acid. (A) K_i determination for inhibition of AZT glucuronidation by valproic acid, $K_i = 1.6 \pm 0.06$ mM. (B) Eadie Hofstee plot of the data in (A), the form of the plot clearly indicates competitive inhibition. Closed squares: no valproic acid, closed circles: 1 mM valproic acid, open triangles: 6 mM valproic acid.

4. Discussion

The results of this study highlight the inherent difficulties in extrapolating from *in vitro* data to the *in vivo* situation. It is clear that a simple comparison of K_m values for two substrates metabolized by the same enzyme is insufficient when estimating potential for metabolic drug interactions.

Initially, simulations were run (Berkely Madonna software v. 8.0.1, Macey and Oster) making the assumption that $K_m = K_i$ using the equation for simple competitive inhibition substituting the kinetic constants measured for

each substrate for each UGT isoform into the equation. This was restricted to those UGT isoforms which did glucuronidate valproic acid. These simulations indicate that the levels of inhibition that would be expected for UGT1A6, UGT1A9 and UGT2B7 glucuronidation of 1-naphthol, propofol and 4-hydroxyestrone, respectively, would be virtually unmeasurable at 1 mM valproic acid and low at 5 mM valproic acid. The lack of activity towards valproic acid from both UGT1A1 and UGT2B15 suggested that there would be no inhibition of these isoforms in competition experiments. It was also predicted that a significant degree of inhibition would be measurable with AZT as a substrate for UGT2B7 due to the order of magnitude difference in the K_m values of AZT and 4-hydroxyestrone.

The predictions were borne out in the case of both UGT2B7 substrates, 1-naphthol glucuronidation by UGT1A6 and all UGT1A1 substrates showed no significant inhibition except a small effect at the very highest valproic acid concentration. The most noteworthy observations are the results which did not match the predictions that were made. UGT1A9 was inhibited to a much greater degree than was anticipated and the significant inhibition of all three UGT2B15 substrates was also unexpected. Application of Eadie Hofstee transformations of the kinetic data was applied to the UGT2B7 AZT and the UGT1A9 propofol inhibition data and is shown along with the untransformed data in Figs. 3 and 4. The plots of the linearized data clearly indicate that two different mechanisms of action are in effect with these two UGT isoforms. The UGT2B7 data is clearly identified as competitive in nature (and matches the simulated data almost perfectly, Fig. 5A) and the calculated K_i of 1.6 ± 0.04 mM is very similar to the measured K_m for valproic acid of 2.1 ± 0.35 mM. However, the inhibition of UGT1A9 propofol glucuronidation appears to be uncompetitive in nature. This type of inhibition is seen more often in enzymes which catalyze sequential reactions and has never been seen in the inhibition of UGT reactions prior to this report. Uncompetitive inhibition occurs when the inhibitor only binds enzyme substrate complex i.e. after binding of the first substrate has taken place. The interpretation of this mechanism is open to some speculation as no data is available on the order of binding in glucuronidation reactions. One possible scenario is that propofol binds to UGT1A9 enhancing the binding of valproic acid but either preventing access or preventing binding of UDPGA. The UGT1A9 active site is known to be capacious as demonstrated by the acceptance of an enormous variety of acceptor aglycones which differ greatly in size and shape [7] and as such the existence of multiple binding sites is quite possible. A great deal of further work would be necessary to elicit the precise mechanism of action and could give rise to a much greater understanding of the mechanism of glucuronidation reactions.

UGT2B15 inhibition was also not predicted although the mechanism of inhibition could not be deduced from the more limited data that was collected using this isoform. Competitive inhibition would indicate the binding of valproic acid in an orientation which is not catalytically active but does occur in the same region as the substrate but identification of uncompetitive inhibition in UGT1A9 means that this is a potential mode of inhibition in other UGT isoforms.

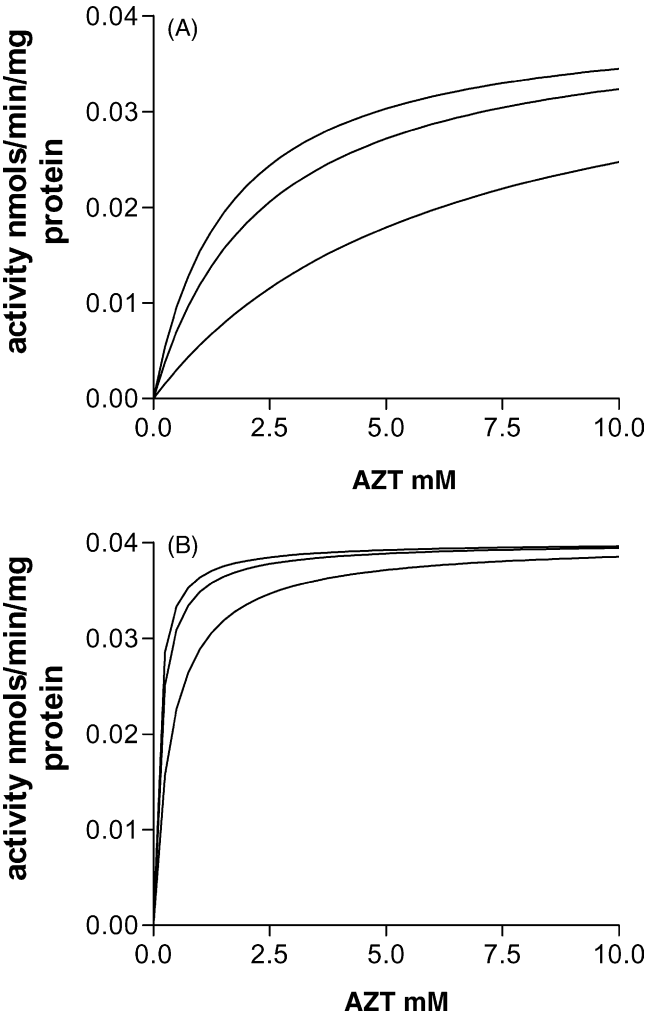

Fig. 5. Comparison of competitive inhibition simulations of AZT and valproic acid interactions with UGT2B7. (A) Simulated inhibition profiles of AZT glucuronidation with a K_m of 1.6 mM and (B) simulated inhibition profiles of AZT glucuronidation with a K_m of 100 μM. Valproic acid concentrations in the simulations were 0, 1 and 6 mM.

A discrepancy exists between the data reported herein and other published data describing AZT glucuronidation kinetics by UGT2B7. Two K_m values have been reported for this reaction and are very different, one is reported as 1600 μM and the other is substantially lower at approximately 100 μM [10,11]. UGT2B7 is the only UGT isoform reported to glucuronidate AZT and the K_m for human liver microsomal glucuronidation of AZT has been estimated at between 2.2 and 4.2 mM [27–32] and in one instance reported to be as high as 13 mM [33] although this value does seem extremely high. Competitive inhibition simulations were run using the high and low K_m values reported

and the results are shown in Fig. 5A and B. This clearly shows that if the K_m for UGT2B7 AZT glucuronidation was in the region of 100 μM, then virtually no inhibition of AZT glucuronidation by valproate would be observed. Whereas if the K_m was in millimolar range then the simulation matches the experimental observation very well indeed (Figs. 4A and 5A). This is consistent with the observations from human *in vitro* data which has been published, suggesting that the change in pharmacokinetics observed when both valproic acid and AZT are dosed to healthy volunteers is due to a metabolic interaction between these two compounds [15].

Acyl glucuronides are notoriously unstable and can undergo rearrangement to a reactive form which can than form protein adducts. Valproic acid glucuronide is relatively stable in comparison to the glucuornides of zomepirac and diflunisal. It has a half life of 60 hr which is considerably higher than the glucuronides of the aforementioned compounds which have half-lives of less than 1 hr [35]. It is therefore highly unlikely that the inhibition that has been observed in these incubations is due to any kind of adduct formation with the UGT protein as no significant rearrangement of the glucuronide will occur within the relatively short incubation time used.

The overall aim of this study was to investigate the potential interactions between valproic acid and other substrates of glucuronidation as catalyzed by recombinant enzymes. The results of this work have shown inhibition of glucuronidation and that there is a distinct possibility of *in vivo* metabolic interactions. It has also shown that UGT2B15 steroid glucuronidation can be inhibited to a significant degree *in vitro* and it would be of great interest to know the effects of valproic acid on other UGT2B isoforms other than the UGT2B15 and UGT2B7.

The information presented in this report has to be taken within the wider context of valproic acid effects *in vivo*. Other drug metabolizing enzymes are also inhibited by valproic acid [1] which may also affect the overall disposition of both VPA and drugs which are dosed concomitantly.

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