IDENTIFICATION OF UDP-GLUCURONOSYLTRANSFERASES INVOLVED IN THE HUMAN HEPATIC METABOLISM OF GV150526, A NOVEL GLYCINE ANTAGONIST

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SUMMARY

The major metabolic pathway for elimination of GV150526 is by glucuronidation exerted by glucuronosyl transferases (UGTs). Potential exists for the modification of GV150526 pharmacokinetics by drugs capable of inhibiting the glucuronidation of GV150526. Using human liver microsomes, 44 compounds were screened for inhibition of GV150526 glucuronidation. These compounds were selected because they are extensively glucuronidated themselves or are used as concomitant medication in the treatment of acute stroke. For 11 compounds out of the 44, full inhibition kinetics were performed to determine their K_i-value and mechanism of inhibition. To predict possible *in vivo* drug-drug interactions, the theoretical percentage of inhibition (*i*) was determined, based on *in vitro* determined K_i-values, and the expected C_{max} plasma levels of GV150526 and the inhibitor. Of the 11 compounds examined, only propofol had an *i*-value of 6.6; for all other compounds *i*-values were lower than 2.1. These results indicate that although *in vitro* inhibition is observed, the likelihood of *in vivo* drug-drug metabolic interactions occurring is low. The inhibition results suggest that in addition to UGT1A1, also UGT1A3, UGT1A8/9, and UGT2B4 are involved in the glucuronidation of

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GV150526. The involvement of UGT1A1 and UGT1A8/9 was confirmed from studies using cDNA expressed human UGT cell lines.

KEY WORDS

UDP-glucuronosyltransferase, human liver metabolism, drug-drug interactions, GV150526, NMDA receptor, stroke

INTRODUCTION

The 2-carboxyindole derivative, GV150526 (3-[-2(phenylcarbamoyl) ethenyl]-4,6-dichloroindole-2-carboxylic acid) (Fig. 1) is a highly potent antagonist of N-methyl-D-aspartate (NMDA)-induced convulsions /1/. Its effect is reversed by D-serine (glycine agonist) confirming that GV150526's mechanism of action is through the glycine site associated with the NMDA receptor. GV150526 has a clear neuroprotective action both pre- and post-ischemic administration as tested in the permanent MCA occlusion model in rat /1,2/. Moreover, no side effects at doses significantly higher than the neuroprotective ones are observed in rat and mouse /1/. The latter suggests that antagonism of glycinergic activity during abnormal overactivation of the NMDA receptor following ischemic stroke might be an efficacious and safe approach to block the neurodegeneration resulting from stroke.

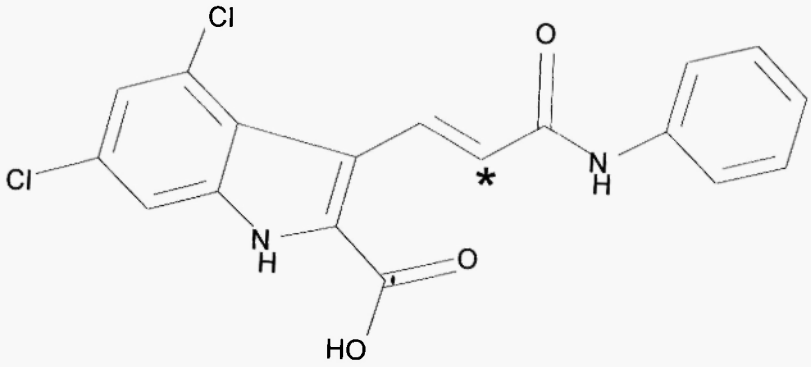

Fig. 1: Structure of GV150526 (GV150526A is the sodium salt). *Position of ^{14}C label.

In vitro studies utilizing human hepatic liver slices and subcellular fractions showed that GV150526 is predominantly converted by UDP-glucuronosyltransferase(s) (UGTs) /3/. After single intravenous administration of GV150526 to healthy volunteers, LC-MS/MS revealed that the major metabolites in plasma, urine and feces were acyl glucuronides /3/. Multiple isomers were present due to acyl migration /4/. In addition to the direct glucuronidation, a minor pathway of GV150526 biotransformation was identified involving P450 enzymes. While the P450 enzymes involved in the minor biotransformation pathway of GV150526 were identified /3/, this was not the case for the major metabolic pathway. Extensive clinical pharmacokinetic studies are difficult and expensive to undertake. In addition, pharmacokinetic interactions are difficult to predict, because they involve several distinct processes, including absorption, distribution, protein binding, metabolism and excretion. However, modifications of hepatic metabolism appear to constitute the major source of drug interactions /5/. Therefore, *in vitro* systems, such as human liver microsomes, offer the opportunity to screen many potential GV150526 drug interactions rapidly and cost effectively. We thus tested 44 different compounds to measure their capacity to inhibit the glucuronidation of GV150526 *in vitro* and predict its *in vivo* relevance and to identify the UGT isoforms capable of converting GV150526.

MATERIALS AND METHODS

Chemicals and materials

The 2-carboxyindole derivatives GV150526 (as the sodium salt, purity >98%) and ^{14}C-GV150526 (4.14 mBq/mg, 112 μCi/mg, purity >99%) were synthesized by the Department of Medicinal Chemistry and Chemical Development, GlaxoWellcome S.p.A. (Verona, Italy).

Acetamidophen, alamethicin, amoxicillin, 3′-azido-3′-deoxythymidine, bilirubin, bovine serum albumin (BSA), carbamazepine, chlorpromazine, cimetidine, cyclosporin A, desipramine, diazepam, diclofenac, diflunisal, digoxin, erythromycin, 17β-estradiol, 17α-ethinylestradiol, fenoprofen, furosemide, 4-hydroxy-biphenyl, hyodeoxycholic acid, ibuprofen, imipramine, ketoprofen, lithocholic acid, magnesium chloride, metoprolol, morphine, 1-naphthol, (S)-naproxen, nicardipine, nifedipine, omeprazole, *dl*-propranolol, phenobarbital,

quinidine, ranitidine, rifampicin, saccharo 1,4-lactone, salicylic acid, taurocholic acid, troleandromycin, UDP-glucuronic acid, verapamil and (±)-warfarin were from Sigma Chemical Co. (Milan, Italy). Chloramphenicol was from Aldrich Chemicals (Milan, Italy) and Cefoperazone from Pfizer (Florence, Italy). Propofol was from ICN Pharmaceuticals (Milan, Italy). Lacidipine (GR43365X) was from GlaxoWellcome (Verona, Italy). cDNA expressed UGT proteins were obtained from Gentest (Woburn, USA) (1A1, 1A4 and 1A6) or Prof. B. Burchell (University of Dundee, UK) (1A8/9, 2B7 and 2B15). All other materials were of analytical or HPLC grade and used without further purification.

Human liver preparations

Human liver tissue (5-100 g, histologically normal) was obtained from patients undergoing liver resection surgery at Borgo Roma Hospital (Verona, Italy), with the consent of the donors and the hospital. Human liver microsomes were prepared according to the method described by Gorrod *et al.* /6/. Briefly, 50% (w/v) homogenates were made with a Potter S homogeniser (Braun, Milan, Italy) fitted with a teflon pestle in ice-cold 0.1 M phosphate buffer (pH 7.4). Homogenates were centrifuged for 20 min at 9000 *g* (4°C), and the resulting supernatant for 60 min at 100,000 *g* (4°C). The pellet was resuspended in 1 ml of 0.1 M phosphate buffer (pH 7.4) per 1 g of wet liver tissue, and designated microsomes. Total cytochrome P450 and cytochrome b_5 levels were determined according to Omura and Sato /7/. Protein concentrations were determined with the Bio-Rad protein solution (Bio-Rad, Milan, Italy) based on Bradford /8/, using BSA (fraction V) as standard. Microsomes were aliquoted and stored at -80°C until use.

Pooled human liver microsomes (from six male and three female Caucasians) were prepared in order to minimize the effect of the known interindividual variability in human cytochrome P450 activities /9,10/. Total cytochrome P450, cytochrome b_5 levels and protein concentration were determined as described above. The organ donors were 54, 59 and 62 year-old women and six men aged 32, 57, 60, 65, 66 and 67 years.

Determination of inhibitory effect of various drugs on the glucuronidation of GV150526

Glucuronidation of GV150526 was performed as described previously /3/. Briefly, the assay mixtures in 0.1 M phosphate buffer (pH 7.4) contained 4 mM UDP glucuronic acid, 4 mM saccharo 1,4-lactone, 10 mM magnesium chloride and 0.4 mg/ml protein (pooled human liver microsomes). To this mixture the inhibitor solution (1, 10 or 100 μM) was added; the reaction was started by the addition of 10 μM ^{14}C-GV150526 (0.112 μCi) and the final volume of the assay mixture was 200 μl. Assay mixtures were incubated for 30 min at 37°C and terminated by addition of 200 μl acetonitrile to deproteinize the mixture. After centrifugation, 100 μl supernatant was analyzed by HPLC (see below). All inhibitors were dissolved in 50% acetonitrile/ water except for bilirubin which was dissolved in 0.2 M NaOH (10 mg/ml) and then diluted to 0.48 mg/ml with prewarmed 100 mM Tris-HCl (pH 7.4). The amount of acetonitrile in the assay was always less than 1%, and had no effect on the glucuronidation of GV150526.

Determination of enzyme kinetics

Enzyme kinetics for the glucuronidation of GV150526 was performed as previously described /3/. Briefly, the assay mixures in 0.1 M phosphate buffer (pH 7.4) contained: substrate ^{14}C-GV150526 (0.5-250 μM at varying specific activities), 4 mM UDPGA, 4 mM saccharo 1,4-lactone, 10 mM magnesium chloride, and 0.4 mg/ml human liver microsomes, in a total volume of 200 μl. The enzymatic reaction was initiated by addition of protein and incubated for 30 min at 37°C. Glucuronidation of GV150526 using cDNA expressed protein was conducted as follows: to 0.1 M Tris/HCl (pH 7.4) was added 3.0 mM UDPGA, 5 mM magnesium chloride, 0.5 mg/ml protein, substrate ^{14}C-GV150526 (0.5-250 μM at varying specific activities) and 20 μg alamethicin (only for Gentest cDNAs: 1A1, 1A4 and 1A6), in a total volume of 200 μl. Incubation was for 30 min at 37°C. All reactions were terminated by the addition of 200 μl acetonitrile and subsequently samples were centrifuged at 900 *g* for 10 min at 4°C. Of the resulting supernatant 100 μl was analyzed by HPLC (see below). Full kinetic studies were performed for 11 endogenous and xenobiotic compounds to determine their K_i values. Thus, a small volume of

buffer was replaced by inhibitor solution (1, 10, 100, 1000 or 2000 μM) and GV150526 enzyme kinetics was determined.

Data analysis

Initial estimates of apparent K_m and V_{max} and apparent K_i values were obtained by linear regression of Lineweaver-Burk plots. These values were then used as the first estimates for a Michaelis-Menten model (Microsoft Graphit, version 3.0, an extended least-squares modelling program).

The theoretical percentage of inhibition obtainable *in vitro* at physiological concentrations (C_{max} plasma levels were used) of inhibitor and GV150526 were calculated using the equation:

$$i=100 * [I]/\{Ki(1+[S]/Km)+[I]\},$$

assuming competitive inhibition /11/. *i* represents the percentage inhibition (= potential inhibition), and [I] and [S] are plasma concentrations of the inhibitor and GV150526, respectively.

HPLC quantification of metabolites

GV150526 and its glucuronide metabolites were separated on a Hypersil ODS column (250 x 4.6 mm, 5 μm) at 35°C pre-fitted with a RP8 (20 x 2 mm, 30-40 μm) guard column. The HPLC system comprised a Waters model 510 (Waters, Milan, Italy) delivery system equipped with a Jasco 975 UV-detector (λ = 343 nm). Elution was with buffer A (25 mM ammonium acetate, pH 8.0) and B (100% methanol) at a flow rate of 1.0 ml/min. The gradient employed was as follows: methanol concentration was increased linearly from 40% to 50% over 5 min, thereafter to 60% over 15 min, followed by elevation to 85% over 15 min. Radiochemical detection was used to quantify the amount of GV150526 glucuronide formed. Metabolites were detected in the HPLC effluent by using an on-line radioactive monitor (Flo-one-β, Camberra Packard, Milan, Italy). The scintillator flow was 4 ml/min and cell size 0.5 ml.

RESULTS

Inhibition of GV150526 glucuronidation by drugs, xenobiotics and endogenous compounds

The inhibition of GV150526 glucuronidation by 37 clinically used drugs and two xenobiotics is outlined in Table 1. At a low (1 or 10 μM) inhibitor concentration, four drugs (diazepam, propofol, nicardipine and 17α-ethinylestradiol) and the xenobiotic, 4-hydroxybiphenyl, inhibited the glucuronidation of GV150526 by more than 50%. It should, however, be noted that only one out of the 37 drugs tested (propofol) showed high glucuronidation inhibition of GV150526 at a low (1 μM) concentration. At a high (100 μM) inhibitor concentration, five other drugs (morphine, diclofenac, nifedipine, lacidipine, and digoxin) and 1-naphthol also inhibited GV150526 glucuronidation by more than 50%. In addition, inhibition of GV150526 glucuronidation by various endogenous compounds was determined and the results are shown in Table 2. Only at 100 μM was inhibition by more than 50% observed for 17β-estradiol, bilirubin and hyodeoxycholic acid.

Inhibition kinetics of GV150526 glucuronidation

For morphine, diazepam, nicardipine, phenobarbital, warfarin, 17α-ethinylestradiol, hyodeoxycholic acid, 1-naphthol, propofol, and 4-hydroxybiphenyl, full inhibition kinetics of GV150526 glucuronidation was determined. Most of these molecules were examined because they inhibited the glucuronidation of GV150526 readily at low concentrations *in vitro*. However, phenobarbital and (+/-)-warfarin, which did not inhibit the glucuronidation of GV150526 readily, were also included. Competitive inhibition was observed with 17α-ethinylestradiol, morphine, nicardipine, and 4-hydroxybiphenyl. Mixed and non-competitive inhibition was observed with bilirubin, hyodeoxycholic acid, 1-naphthol and propofol, and diazepam and phenobarbital, respectively. K_i values for molecules which themselves are known to be glucuronidated ranged from 0.5 to 43.5 μM (Table 3). Two molecules for which no glucuronidation pathway has been described, nicardipine and diazepam, also had low K_i values. Not surprisingly, the K_i values for warfarin and phenobarbital were high because both molecules are not directly glucuronidated (Table 3).

TABLE 1

Screening of different drugs as inhibitors of GV150526 glucuronidation in human liver microsomes[a]

Therapeutic class	Drugs	Percentage of control activity	
		Drug concentration	
		10 μM	100 μM
Analgesics	Acetamidophen	111.0	105.0
	Salicyclic acid	79.0	79.0
	Morphine	69.0	24.0
NSAIDs	Ketoprofen	100.0	96.0
	Ibuprofen	95.0	106.0
	Fenoprofen	75.0	70.0
	Diflusinal	92.0	50.0
	Diclofenac	65.0	35.0
	(S)-naproxen	97.0	91.2
Anti-convulsants	Phenobarbital	50.7	67.2
	Carbamazine	90.0	89.0
Anxiolytic	Diazepam	34.0	3.4
Anesthetic	Propofol	44.0[b] 31.6	ND[c]
H_2-Antagonists	Cimetidine	97.0	107.0
	Omeprazole	104.0	50.0
	Ranitidine	87.0	89.0
Ca^{2+}-channel blockers	Nifedipine	97.0	28.0
	Lacidipine	57.0	31.9
	Nicardipine	28.0	12.4
	Verapamil	82.0	66.6
Diuretic	Furosemide	90.0	89.0
Antibiotics	Rifampicin	88.0	70.0
	Chloramphenicol	99.0	106.0
	3′-Azido-3′-deoxythymidine	83.0	99.0
	Cefoperazone	98.0	102.0
	Amoxicillin	94.0	92.0
	Troleandomycin	84.0	83.5
TCA	Imipramine	100.0	102
	Desimpramine	92.0	101.0
Neuroleptic antidepressant	Chlorpromazine	77.0	97.0
Contraception	17α-Ethinylestradiol	36.5	20.0
Immunosuppressor	Cyclosporin A	68.0	105.3
β-Blockers	Propranalol	69.0	90.3
	Metaprolol	82.0	61.0
Cardiac glycoside	Digoxin	73.0	46.0
Anticoagulant	(+/-)-Warfarin	74.0	68.0
Antiarrhythmic	Quinidine	97.0	101.0
Xenobiotics	1-Naphthol	64.0	0
	4-Hydroxybiphenyl	39.0	4.6

NSAIDS = non-steroid anti-inflammatory drugs; TCA = tricyclic antidepressants

[a]GV150526 concentration was kept at 10 μM, glucuronidation velocity was 120 + 10 pmol/min/mg protein (n=8 determinations). Data are given as the means of two separate determinations. [b] Propofol concentration was 1 μM. [c] ND = not determined.

TABLE 2

Screening of different endogenous compounds as inhibitors of GV150526 glucuronidation in human liver microsomes [a]

		Percentage of control activity	
		Inhibitor concentration	
		10 μM	100 μM
Endogenous compounds	17β-Estradiol	96.0	27.4
	Bilirubin	97.0	23.9
Bile acids	Hyodeoxycholic acid	82.0	37.6
	Lithocholic acid	87.0	133.0
	Taurocholic acid	94.0	108.7

[a] GV150526 concentration was kept at 10 μM, glucuronidation velocity was 120 ± 10 pmol/min/mg protein (n=8 determinations). Data are given as the means of two separate determinations.

The competitive and mixed mechanisms of inhibition exerted by some molecules as well as their low K_i values suggest that GV150526 is glucuronidated by UGT1A1 (17α-ethinylestradiol), UGT1A3 (hyodeoxycholic acid), UGT1A6 (1-naphthol), UGT1A8/9 (propofol), UGT2B4 (hyodeoxycholic acid) and UGT2B7 (morphine). To determine whether these UGTs are indeed involved in the glucuronidation of GV150526, cDNA expressed human UGT cell line microsomes were used (see below).

To predict possible human metabolic drug-drug interactions *in vivo* we calculated the theoretical percentage of inhibition (*i*) or potential inhibition. This *i*-value is based on the *in vitro* determined K_i of GV150526 glucuronidation by a given inhibitor and the plasma C_{max} values of GV150526 and inhibitor. The average C_{max} value of GV150526 plasma concentration observed in phase 2 clinical studies is 267 μM (106 μg/ml) /12/. Using this C_{max} value and C_{max} values for the various inhibitors (Table 3), it was shown that the highest *i*-value (6.6) was obtained for propofol. Interestingly, *i*-values for the

TABLE 3

Apparent K_i values for the inhibition of GV150526 glucuronidation by some drugs in human liver microsomes

Compound	Ki (μM)	i[a]	Type of inhibition	Isoenzyme[b]	Plasma concentration at C_{max} (μM)[c]	Reference
Endogenous						
bilirubin	43.5	0.3-2.1	mixed	UGT1A1[1]	3.42-27.4	f
hyodeoxycholic acid	16.4	0-1.6	mixed	UGT1A3[2]/2B4[3]	0-8.1	f
Drugs						
diazepam	10.4	0.1-0.4	non-competitive	None[d]	0.26-1.11	/17/
17α-ethinylestradiol	17.5	0.0	competitive	UGT1A1[4]	0.0004	/18/
morphine	17.3	0.23 0.01	competitive	UGT2B7[5]	1.2 (i.v) 0.04 (p.o)	/19/ /20/
nicardipine	8.3	0.2	competitive	None	0.2	/21/
phenobarbital	5225	0.03-0.06	non-competitive	None	40-100	/22/
propofol	9.8	6.6	mixed	UGT1A8/9[1]	10	/26/
(+/-)-warfarin	320	0.02 0.03	mixed	None	1.6 (S-warfarin) 2.8 (R-warfarin)	/23/ /23/
Xenobiotics						
1-naphthol	0.5	N.A.[e]	mixed	UGT1A6[1]	N.A.	
4-hydroxybiphenyl	2.1	N.A.	competitive	UGT2B4[1]	N.A.	

[a] i-value was calculated as outlined in the experimental procedures. [b] References are 1, /15/; 2, /26/; 3, /23/; 4, /14/; and 5, /24/.
[c] C_{max} of GV150526 is 207 μM. [d] None means no direct glucuronidation possible, functionalization by cytochrome P450s occurs prior to glucuronidation. [e] N.A. = means not applicable. [f] Dal Negro (Glaxo Wellcome) unpublished results.

endogenous compounds bilirubin and hyodeoxycholic acid were 2.1 and 1.6, respectively. All other *i*-values were lower than 0.5 (Table 3).

Glucuronidation of GV150526 using human cDNA expressed UGTs

Human cDNA expressed UGT enzymes (1A1, 1A4, 1A6, 1A8/9, 2B7 and 2B15) were used to delineate which isoenzymes are involved in the glucuronidation of GV150526 (Table 4). Glucuronidation of GV150526 with these cDNA expressed cell line microsomes was performed at a low (1 μM) and high (50 μM) substrate concentration. As shown in Table 4, GV150526 is readily glucuronidated by UGT1A1 and 1A8/9 at both substrate concentrations. No glucuronidation of GV150526 was observed using UGT1A4, 1A6, 2B7 or 2B15.

TABLE 4

Glucuronidation of GV150526 by cDNA expressed human UGT enzymes

	Velocity (pmol glucuronide formed/min/mg protein)	
cDNA expressed UGT	**GV150526** (1 μM)	**GV150526** (50 μM)
1A1	55.0 ± 1.8 [a]	317.0 + 10.6
1A4	0	0
1A6	0	0
1A8/9	3.2 ± 0.5	55.2 + 3.6
2B7	0	0
2B15	0	0

[a] Data are given as the means of three determinations.

Glucuronidation kinetics of GV150526 using pooled microsomes and cDNA expressed UGT1A1 and UGT1A8/9

The conversion of GV150526 to its glucuronic acid conjugate by pooled microsomes and cDNA expressed proteins followed simple Michaelis-Menten kinetics. However, at GV150526 concentrations

exceeding V_{max}, substrate induced inhibition of GV150526 glucuronidation occurred (not shown).

The apparent K_m values for human liver microsomes, UGT1A1 and UGT1A8/9 were 9.0, 5.5 and 11 μM, respectively. The apparent V_{max} values for human liver microsomes, UGT1A1 and UGT1A8/9 were 260, 430 and 140 pmol/min/mg, respectively (Table 5).

TABLE 5

Glucuronidation kinetics of GV150526 using cDNA expressed protein and human liver microsomes

	^{app}Km (μM)	$^{app}Vmax$ (pmol/min/mg protein)
human liver microsomes	9.0 ± 2.7 [a]	260 ± 24
UGT1A1	5.5 ± 4.4	430 ± 120
UGT1A8/9	11.0 ± 5.6	140 ± 25

[a] Data are given as the means of three determinations.

DISCUSSION

GV150526 is a novel drug developed to reduce neuronal damage after ischemic stress. In rats and dogs the main metabolic pathway is by glucuronidation. The acyl glucuronic acid conjugates of GV150526 are transported to the bile and thereafter excreted from the body. It is suspected that a similar process occurs in man. Because acyl glucuronidation is likely to be the rate-limiting step in GV150526 elimination, a range of drugs and endogenous compounds which may be glucuronidated were screened for their ability to inhibit human liver microsomal GV150526 glucuronidation. These inhibition studies served a two-fold purpose: identification of the UGT isoenzymes involved in the glucuronidation of GV150526, and the *in vitro* determination of possible drug-drug interactions.

From a general point of view our results demonstrate that most drugs had no inhibitory effect on GV150526 glucuronidation. Only one drug (propofol) and two endogenous compounds (bilirubin and hyodeoxycholic acid) had a low K_i and "high" potential inhibition (*i*)

value, indicating a possible relevance for clinical drug interaction involving GV150526.

Most drugs tested did not inhibit GV150526 glucuronidation *in vitro*. Hence, if at 100 μM, less than 50% inhibition is observed, the corresponding K_i value is expected to be higher than 100 μM. Thus, when a compound is essentially non-inhibitory in the *in vitro* system, it is unlikely to inhibit *in vivo*. For example, based on our *in vitro* results, (±)-warfarin should not inhibit the glucuronidation of GV150526 and interfere with the clearance of GV150526. Indeed, the pharmacokinetics of GV150526 administered during daily dosing of warfarin was consistent with those observed previously in healthy volunteers and stroke patients (F. Hoke, unpublished results).

For those drugs, xenobiotics and endogenous compounds that inhibited the glucuronidation of GV150526, they did so because they are extensively glucuronidated themselves. However, no direct glucuronidation of diazepam and nicardipine has been demonstrated. There are two possibilities for the inhibition exerted by these compounds on the glucuronidation of GV150526. First, these drugs may undergo glucuronidation, but this pathway has never been proven. Second, they could interact with UGTs by an unknown mechanism (e.g. formation of inactive complex, steric hindrance, physical or chemical perturbation of the microsomal membrane). The inhibitory mechanism of diazepam could be explained because it has an affinity for UGT binding sites without being a substrate for these enzymes /13/.

From a clinical point of view, an important consideration for evaluating the potential clinical relevance of the *in vitro* findings is the magnitude of the K_i of the competitive inhibitor that is clinically achievable /14/. In other words, if the K_i of an inhibitor is much higher than plasma concentrations achieved in patients, such inhibition may occur *in vitro* but not *in vivo*. Accordingly, diazepam, 17α-ethinylestradiol, morphine, nicardipine, phenobarbital, and warfarin are not likely to be clinically important inhibitors of GV150526 glucuronidation, because their estimated K_i exceeds their clinically achievable corresponding plasma concentration. In contrast, the K_i values of propofol, bilirubin and hyodeoxycholic acid approximate their peak physiological concentrations and these compounds might thus interfere with the hepatic glucuronidation of GV150526. On the basis of the theoretical percentage of inhibition, propofol appears to be the most potent inhibitor of GV150526 glucuronidation. Inhibition of 3′-

azido-3′-deoxythymidine glucuronidation by certain co-administered drugs had *i*-values of 30 /11/, and the authors concluded that this value was of no clinical relevance. Therefore, it seems doubtful whether the *i*-value of 6.6 for propofol obtained in this study has any clinically significant relevance.

Data presented here also allow another important prediction concerning GV150526 elimination in humans. The apparent K_m of GV150526 glucuronidation in pooled human microsomes was 9 μM. Data *in vitro* and *ex vivo* indicate that GV150526 is highly bound to human plasma proteins (fraction unbound = 0.002%, /12/). It is thus inconceivable that unbound plasma concentrations *in vivo* will approach the estimated K_m value, even if a protein binding displacement was to occur. Thus, it is unlikely that the plasma clearance of GV150526 would alter with dose due to saturation of hepatic glucuronidation.

In vitro results have always to be used with caution when extrapolating to *in vivo* situations. Plasma concentrations may not be the best parameter to use when interpreting *in vitro* results. Drug concentrations in the hepatocyte may be much higher than the total or free plasma concentration due to active transport drug uptake and/or slow hepatic elimination. *In vitro* results must thus be extrapolated with caution.

Identification of the UGT isoform(s) responsible for GV150526 glucuronidation is of paramount importance since this allows prediction of the genetic, environmental and physiological factors likely to alter drug clearance *in vivo*. The high level of inhibition of GV150526 glucuronidation by 17α-ethinylestradiol, estradiol and bilirubin suggests the involvement of UGT1A1, since these compounds are mainly glucuronidated by this isoform /15/. Similarly, involvement of UGT1A8/9 is suspected since propofol inhibited the glucuronidation of GV150526 to a high degree. The capacity of both isoforms to glucuronidate GV150526 was confirmed using cDNA expressed proteins. Although the affinity of UGT1A1 for GV150526 appeared to be higher than UGT1A8/9, the apparent K_m for GV150526 glucuronidation by both cDNA expressed UGTs closely matched the K_m observed for human liver microsomal GV150526 glucuronidation. This suggests that both UGT isoforms may contribute equally to the glucuronidation and hence elimination of GV150526.

The involvement of UGT1A3 and UGT2B4 (hyodeoxycholic acid inhibition), UGT1A6 (1-naphthol inhibition) and UGT2B7 (morphine inhibition) was suspected. However, both UGT1A6 and UGT2B7 cDNA expressed proteins did not glucuronidate GV150526. These data suggest that 1-naphthol and morphine inhibit more than one UGT isoform. Indeed, 1-naphthol is glucuronidated by UGT1A6 by 50% only, and morphine is glucuronidated by UGTs other than UGT2B7 /16/. Moreover, acetaminophen (paracetamol) (UGT1A6 substrate) and most NSAIDs (UGT2B7 substrates) did not inhibit the glucuronidation of GV150526 to the same extent as did 1-naphthol and morphine. Taken together, it thus appears that UGT1A6 and UGT2B7 are not involved in the glucuronidation of GV150526. No cDNA expressed proteins of UGT1A3 and UGT2B4 were present to determine their involvement in the glucuronidation of GV150526. Overall, our data indicate that GV150526 is glucuronidated by at least two UGT isoforms and possibly four. Since more than one UGT isoform is involved in the elimination of GV150526, drug-drug interactions at the metabolic level are not likely to occur.

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