Minireview

DRUG-DRUG INTERACTIONS FOR UDP-GLUCURONOSYLTRANSFERASE SUBSTRATES: A PHARMACOKINETIC EXPLANATION FOR TYPICALLY OBSERVED LOW EXPOSURE (AUCi/AUC) RATIOS

J. Andrew Williams, Ruth Hyland, Barry C. Jones, Dennis A. Smith, Susan Hurst, Theunis C. Goosen, Vincent Peterkin, Jeffrey R. Koup, and Simon E. Ball

Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Ann Arbor, Michigan (J.A.W., S.H., T.C.G., V.P., J.R.K., S.E.B.), and Sandwich, Kent, United Kingdom (R.H., B.C.G., D.A.S.)

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ABSTRACT:

Glucuronidation is a listed clearance mechanism for 1 in 10 of the top 200 prescribed drugs. The objective of this article is to encourage those studying ligand interactions with UDP-glucuronosyltransferases (UGTs) to adequately consider the potential consequences of in vitro UGT inhibition in humans. Spurred on by interest in developing potent and selective inhibitors for improved confidence around UGT reaction phenotyping, and the increased availability of recombinant forms of human UGTs, several recent studies have reported in vitro inhibition of UGT enzymes. In some cases, the observed potency of UGT inhibitors in vitro has been interpreted as having potential relevance in humans via pharmacokinetic drug-drug interactions. Although there are reported examples of clinically relevant drug-drug interactions for UGT substrates, exposure increases of the aglycone are rarely greater than 100% in the presence of an inhibitor relative to its absence (i.e., AUCi/AUC ≤2). This small magnitude in change is in contrast to drugs primarily cleared by cytochrome P450 enzymes, where exposures have been reported to increase as much as 35-fold on coadministration with an inhibitor (e.g., ketoconazole inhibition of CYP3A4-catalyzed terfenadine metabolism). In this article the evidence for purported clinical relevance of potent in vitro inhibition of UGT enzymes will be assessed, taking the following into account: in vitro data on the enzymology of glucuronide formation from aglycone, pharmacokinetic principles based on empirical data for inhibition of metabolism, and clinical data on the pharmacokinetic drug-drug interactions of drugs primarily cleared by glucuronidation.

Clinical Relevance of Cytochrome P450 versus UDP-Glucuronosyltransferase Inhibition in Vitro?

In considering the clinical relevance of pharmacokinetic drug-drug interactions mediated by drug-metabolizing enzymes, efficacy linked to dosage requirements and/or toxicity can be considered as appropriate endpoints. The conduct of these studies in early drug discovery (Williams et al., 2003) and for definitive purposes (Bjornsson et al., 2003) has been previously described. For metabolized drugs primarily cleared by cytochrome P450 (P450) substrates, numerous examples exist of clinically relevant pharmacokinetic drug-drug interactions. Ketoconazole inhibition of CYP3A4-catalyzed terfenadine metabolism, leading to as much as a 35-fold increase in drug exposure [area under the curve (AUC)] is a well known example (Boxenbaum, 1999). The clinical consequences were cardiac arrhythmias, torsades de pointes, and even death in some cases (Monaghan et al., 1990). Also, exposure (AUCi/AUC) Ratios >2 for UGT Substrates: Absence of Evidence?

Figure 1 indicates that approximately three quarters of the top 200 prescribed drugs in the United States in 2002 are cleared by metabolism (www.rxlist.com). The classical view that cytochrome P450-catalyzed hydroxylations precede glucuronidation of the newly available nucleophilic oxygens holds true for many drugs. However, compounds such as zidovudine (Miners and McKenzie, 1991) are cleared primarily by direct glucuronidation without the requirement of P450 enzymes. Also, many drugs are cleared by multiple metabolism pathways of the parent compound. An example is that of olanzapine, which is metabolized by UGT1A4-catalyzed N-glucuronidation (Lin-
with compounds cleared by P450 enzymes, the number of drugs that appear to have glucuronidation as a clearance mechanism is small. However, they are sufficient in number for consideration of the effects (or lack thereof) of coadministration with potent in vitro inhibitors of UGT enzymes. Table 1 lists those drugs in the top 200 prescribed in 2002 that have glucuronidation as a clearance mechanism, alongside recombinant UGT enzymes known to contribute to their glucuronidation. UGT2B7 is the most commonly listed enzyme (Table 1, 7 structurally diverse compounds of 20; 35%), followed by UGT1A4 (N-glucuronidated compounds, 20%) and UGT1A1 (15%).

For pharmacokinetic interaction studies that have been conducted for drugs cleared by glucuronidation, changes in drug exposure measured by area under the plasma concentration versus time curve (AUC) are typically less than two-fold the value observed in the absence of inhibitor (Miners and Mackenzie, 1991; Liston et al., 2001; Boase and Miners, 2002; Lin and Wong, 2002). For example, valproate coadministration increases the plasma AUC of lorazepam by 20% (Samara et al., 1997). One study reporting a four-fold increase in exposure of the now withdrawn drug zomepirac after concomitant administration with probenecid is an exception (Smith et al., 1985), but probably also includes a probenecid-inhibition component of renal elimination in humans (Burckhardt and Burckhardt, 2003). Evidence of an active transport component to drug-drug interactions mediated by P450 enzymes is an area under scrutiny (Benet et al., 2003). For UGT substrates, our understanding of the interplay between glucuronidation and transport of glucuronides will improve with mechanistic studies (Cummings et al., 2004) to complement clinical study designs (Smith et al., 1985).

Evidence of toxicity as a result of inhibition of UGTs is rare. An exception is lamotrigine, for which coadministration with valproic acid appears to increase the risk of rash (Garrett, 2002). However, significant synergistic effects associated with increased efficacy also result from coadministration of valproic acid with lamotrigine (Garrett, 2002).

Exposure (AUC/AUC) Ratios >2 for UGT Substrates: Evidence of Absence

I. Pharmacokinetic Principles and UGT Enzymology. Table 2 lists characteristics of drugs, which, in the presence of an inhibitor of clearance (metabolism in this case), increase the risk of a large (>5) ratio of exposure in the presence of an inhibitor compared with its absence (AUC/AUC). When combined, these listed risk factors present the potential for the highest AUC/AUC ratio. Therefore, high extraction drugs metabolized by a single enzyme (like terfenadine; Boexenbaum, 1999), simultaneously presented to the liver along with an inhibitor in which the ratio of liver concentration ([I]) to potency of inhibition (K_inh) is high, are at the greatest risk of having a high (>5) AUC/AUC ratio (Table 1), as are compounds with a low in vivo K_m. Thus, the potent CYP3A inhibitor ketoconazole increases the AUC of terfenadine, which is primarily metabolized by CYP3A enzymes (Ling et al., 1995), and has a hepatic extraction ratio of 0.9 (90% of the dose is metabolized on first pass through the liver) by approximately 35-fold (Boxenbaum, 1999).

As indicated above, observed AUC/AUC ratios for glucuronidated drugs coadministered with UGT inhibitors are typically less than 2. This is because drugs primarily cleared by glucuronidation generally do not have the characteristics necessary to lead to high AUC/AUC ratios, as described below and in Table 2. The data in Table 1 indicate that UGT substrates are often metabolized by multiple UGTs and have high K_m values in vitro compared with substrates of P450 enzymes. In addition, in vitro data also suggest that inhibitor/K_m ratios would also be typically low for inhibitors of UGT2B7-catalyzed AZT (3’-azido-


**TABLE 1**

Dugs that have glucuronidation listed as a clearance mechanism, including those in the top 200 U.S. prescribed drugs in 2002

<table>
<thead>
<tr>
<th>Drug</th>
<th>Listed UGT</th>
<th>Rank in Top 200</th>
<th>In Vitro $K_{m}$</th>
<th>Reference</th>
<th>Total (Unbound) [Plasma]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>1A4</td>
<td>&gt;200</td>
<td>10</td>
<td>Dehal et al. (1992)</td>
<td>0.76</td>
<td>Franke et al. (2003)</td>
</tr>
<tr>
<td>Carvediol</td>
<td>1A1, 2B4, 2B7</td>
<td>174</td>
<td>27</td>
<td>Ohno et al. (2004)</td>
<td>0.6 (0.003)</td>
<td>Therapeutic Drugs (A, 1994)</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>2B7</td>
<td>&gt;200</td>
<td>N/A</td>
<td>Jin et al. (1993)</td>
<td>532710</td>
<td>Sallustio et al. (1991)</td>
</tr>
<tr>
<td>Cocodeine</td>
<td>&gt;200</td>
<td>2600</td>
<td>Courtil et al. (2003)</td>
<td>0.83 (0.66)</td>
<td>Therapeutic Drugs (B, 1994)</td>
<td></td>
</tr>
<tr>
<td>Cyclobenzaprine</td>
<td>1A4</td>
<td>61</td>
<td>N/A</td>
<td>Hawes (1998)</td>
<td>0.16</td>
<td>Hucker and Stauffer (1976)</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>2B7</td>
<td>197</td>
<td>18</td>
<td>Kumar et al. (2002)</td>
<td>0.94</td>
<td>Therapeutic Drugs (C, 1994)</td>
</tr>
<tr>
<td>DMXAA</td>
<td>N/A</td>
<td>&gt;200</td>
<td>100</td>
<td>Miners et al. (1997)</td>
<td>787 (32)</td>
<td>Zhou et al. (2002)</td>
</tr>
<tr>
<td>Etosipone</td>
<td>&gt;200</td>
<td>440</td>
<td>Watanabe et al., 2003</td>
<td>5.4 (0.27)</td>
<td>Therapeutic Drugs (C, 1994)</td>
<td></td>
</tr>
<tr>
<td>Fenofobrate</td>
<td>1A9</td>
<td>130</td>
<td>N/A</td>
<td>Barbier et al. (2003)</td>
<td>34 (0.68)</td>
<td>Doser et al. (1996) (Mogi et al., 1995)</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td>1A4</td>
<td>&gt;200</td>
<td>125</td>
<td>Hagenauer (2001)</td>
<td>0.25</td>
<td>Rudek et al. (2003)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>&gt;200</td>
<td>520</td>
<td>Hamdoune et al. (1995)</td>
<td>10.6 (0.02)</td>
<td>Knadler et al. (1989)</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>1A8, 1A10a</td>
<td>7</td>
<td>N/A</td>
<td>Cheng et al. (1999)</td>
<td>3.3 (0.08)</td>
<td>Najib et al. (2003) (Pacifici et al., 1987)</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>&gt;200</td>
<td>82</td>
<td>Pruksaaranont et al. (2002)</td>
<td>132 (1.32)</td>
<td>Therapeutic Drugs (D, 1994)</td>
<td></td>
</tr>
<tr>
<td>Glipizide</td>
<td>N/A</td>
<td>150</td>
<td>N/A</td>
<td>Niemi et al. (2001)</td>
<td>0.35</td>
<td>Gu et al. (2002)</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>1A9</td>
<td>149</td>
<td>N/A</td>
<td>Green et al. (1998)</td>
<td>3.5</td>
<td>Gu et al. (2002)</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>1A4</td>
<td>&gt;200</td>
<td>5500</td>
<td>Furlan et al. (1999)</td>
<td>37 (15)</td>
<td>Ramsay et al. (1991) [Therapeutic Drugs (E, 1994)</td>
</tr>
<tr>
<td>Levotyphoxine</td>
<td>1A1c</td>
<td>4</td>
<td>N/A</td>
<td>Hasen et al. (2003) [Therapeutic Drugs (F, 1994)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>N/A</td>
<td>138</td>
<td>N/A</td>
<td>N/A</td>
<td>0.27 (0.16)</td>
<td><a href="http://www.fda.gov/cder/guidance/3645fnl.htm">www.fda.gov/cder/guidance/3645fnl.htm</a></td>
</tr>
<tr>
<td>Metonidazole</td>
<td>N/A</td>
<td>147</td>
<td>N/A</td>
<td>N/A</td>
<td>6.8 (6.8)</td>
<td><a href="http://www.fda.gov/cder/guidance/3645fnl.htm">www.fda.gov/cder/guidance/3645fnl.htm</a></td>
</tr>
<tr>
<td>Morphine</td>
<td>&gt;200</td>
<td>2300–4300</td>
<td>Court et al. (2003)</td>
<td>105 (0.1)</td>
<td>Therapeutic Drugs (H, 1994)</td>
<td></td>
</tr>
<tr>
<td>Naloxone</td>
<td>2B7b</td>
<td>&gt;200</td>
<td>N/A</td>
<td>Coffman et al. (2001)</td>
<td>11.5</td>
<td>Groeger and Inturrius (1987)</td>
</tr>
<tr>
<td>Naproxen</td>
<td>1A3</td>
<td>&gt;200</td>
<td>N/A</td>
<td>Green et al. (1998)</td>
<td>240 (0.26)</td>
<td>Toothaker et al. (2000) (Bertin et al. 1994)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>1A4d</td>
<td>&gt;200</td>
<td>227</td>
<td>Linnet (2002)</td>
<td>0.67</td>
<td>Bergeman et al. (2004)</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>&gt;200</td>
<td>43–303</td>
<td>Court et al. (2002)</td>
<td>1.7 (0.005)</td>
<td>Therapeutic Drugs (I, 1994)</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>1A1, 1A6, 1A9a</td>
<td>6000–5500</td>
<td>Court et al. (2001)</td>
<td>23 (18)</td>
<td>Therapeutic Drugs (J, 1994)</td>
<td></td>
</tr>
<tr>
<td>Propofol</td>
<td>1A9</td>
<td>&gt;200</td>
<td>190–280</td>
<td>Soars et al. (2003)</td>
<td>14 (0.03)</td>
<td><a href="http://www.fda.gov/cder/guidance/3645fnl.htm">www.fda.gov/cder/guidance/3645fnl.htm</a></td>
</tr>
<tr>
<td>Raloxifene</td>
<td>1A1, 1A8, 1A10d</td>
<td>97</td>
<td>8–58</td>
<td>Kemp et al. (2002)</td>
<td>0.003</td>
<td><a href="http://www.fda.gov/cder/guidance/3645fnl.htm">www.fda.gov/cder/guidance/3645fnl.htm</a></td>
</tr>
<tr>
<td>Tramadol</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.82 (0.65)</td>
<td><a href="http://www.fda.gov/cder/guidance/3645fnl.htm">www.fda.gov/cder/guidance/3645fnl.htm</a></td>
</tr>
<tr>
<td>Valdecoxib</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td><a href="http://www.fda.gov/cder/guidance/3645fnl.htm">www.fda.gov/cder/guidance/3645fnl.htm</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zidovudine</td>
<td>2B7b</td>
<td>&gt;200</td>
<td>510–1400</td>
<td>Court et al. (2003)</td>
<td>3 (1.08)</td>
<td><a href="http://www.fda.gov/cder/guidance/3645fnl.htm">www.fda.gov/cder/guidance/3645fnl.htm</a></td>
</tr>
</tbody>
</table>

N/A, information not available on www.rxlist.com or in the literature.

* * In Human Liver Microsomes unless specified.

* a Total (unbound + bound) $C_{max}$ concentrations reported.

* b Data from literature sources.

* c Data from www.rxlist.com.

* d $V_{max}$ not reached in human liver microsomes.

**TABLE 2**

High risk vs. low risk characteristics for moderate or large AUC/AUC ratios for metabolized drugs in the presence of inhibitor

<table>
<thead>
<tr>
<th>High Risk of Large AUC/AUC Ratio</th>
<th>Low Risk of Large AUC/AUC Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolized by single enzymea</td>
<td>Metabolized by multiple enzymesa</td>
</tr>
<tr>
<td>High $I/K_r$ ratioa</td>
<td>Low $I/K_r$ ratioa</td>
</tr>
<tr>
<td>High hepatic extractionb</td>
<td>Low hepatic extractionb</td>
</tr>
<tr>
<td>$K_{m}$ (equal to, or greater than liver drug concentrations at therapeutic doses)c</td>
<td>$K_{m}$ (much greater than liver drug concentrations at therapeutic doses)c</td>
</tr>
</tbody>
</table>

 segundos de deglucuronidación (IC$_{50}$) valores >200 μM), except for potent inhibition by the flavonoid hexamethoxyflavone. The potential clinical relevance (or lack thereof) of potent flavonoid inhibition of UGT1A1 will be discussed later.

As indicated in Fig. 1, UGT2B7 is the most commonly listed UGT for biotransformation of the most prescribed drugs. Using the established relationship between IC$_{50}$ and $K_i$ for this UGT2B7-catalyzed biotransformation, assuming competitive inhibition (which in many cases for UGT substrates would not be appropriate), would be approximately 100 μM. Assuming noncompetitive inhibition, the lowest $K_i$ would be approximately 200 μM. With notable exceptions (e.g., valproic acid), total (protein-bound and unbound) therapeutic plasma concentrations for most drugs are below 10 μM. The importance of the relationship between inhibitor concentration ($I$) and $K_i$ is discussed below, after consideration of the importance of $K_{m}$ in saturation of metabolism.

Figure 2 shows the results of a simulation of the effect of drug concentration in the liver on the percentage of maximal clearance for 2’-3’-dideoxythymidine; zidovudine) glucuronidation (Table 3): IC$_{50}$ values range between 186 and 4100 μM for a structurally diverse set of compounds including flurbiprofen, valproic acid, and the flavonoid 3’,4’,5’,6,7-hexamethoxyflavone (HMF). The same set of compounds also indicate low potency for inhibition of UGT1A1-catalyzed estradiol 3-glucuronidation (IC$_{50}$ values >200 μM), except for potent inhibition by the flavonoid hexamethoxyflavone. The potential clinical relevance (or lack thereof) of potent flavonoid inhibition of UGT1A1 will be discussed later.
metabolized drugs with an in vivo $K_m$ of either 3 μM or 300 μM (Fig. 2, bottom line), or 300 μM (Fig. 2, top line). This simulation, based on the following equation,

$$\text{Clearance}_{\text{hepatic}} = \frac{V_{\text{max}}}{K_m + [S]}$$

(1)

clearly shows the relationship between drug concentration and saturation of clearance. The in vitro $K_m$ for CYP3A-catalyzed midazolam 1'-hydroxylation is typically 2 to 5 μM in human liver microsomes and/or recombinant enzyme CYP3A enzymes. In general, in vitro $K_m$ values for CYP3A enzymes range between 5 and 200 μM (Williams et al., 2002b), although there are examples of $K_m$ that are below 1 μM or above 1000 μM (Ekins et al., 1999).

A $K_m$ value of 300 μM (or higher) is more typical for drug substrates of UGT enzymes (Table 1): $K_m$ values range between 8 and 55,000 μM. The mean in vitro $K_m$ value for the UGT substrates among the top 200 prescribed drugs in the United States listed in Table 1 is between 1725 μM and 7873 μM, depending on whether the lowest or highest values in a reported range are taken (the respective median values are 510 or 1400 μM). Assuming that in vitro $K_m$ values equate approximately to the in vivo situation for P450 and UGT substrates, for a drug with a $K_m$ of 300 μM (e.g., a UGT substrate), it can be seen that clearance is >95% of the maximal value at 15 μM.

![Diagram](image.png)

**TABLE 3**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>UGT1A1 $K_m$ μM</th>
<th>UGT2B7 $K_m$ μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Flurbiprofen</td>
<td><img src="image.png" alt="Structure" /></td>
<td>349</td>
<td>186</td>
</tr>
<tr>
<td>Propofol</td>
<td><img src="image.png" alt="Structure" /></td>
<td>626</td>
<td>314</td>
</tr>
<tr>
<td>Estradiol</td>
<td><img src="image.png" alt="Structure" /></td>
<td>N/A</td>
<td>300</td>
</tr>
<tr>
<td>3',5',6,7-Hexamethoxyflavone (HMF)</td>
<td><img src="image.png" alt="Structure" /></td>
<td>0.84</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Valproic acid</td>
<td><img src="image.png" alt="Structure" /></td>
<td>4100</td>
<td>&gt;4100</td>
</tr>
<tr>
<td>Zidovudine (AZT)</td>
<td><img src="image.png" alt="Structure" /></td>
<td>Stimulation</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Experiments were performed according to the previously described methodology (Court et al., 2003). HMF and valproic acid concentrations were taken to the limits of solubility. Data are means of triplicate incubations of three separate experiments for each compound. Stimulation of estradiol 3-glucuronidation increased with increasing AZT concentration to a maximal 174% of control activity at the highest AZT concentration tested (3 mM).*
drug concentration (Fig. 2). However, for a drug with a \( K_m \) of 3 \( \mu \)M (e.g., a P450 substrate), it can be seen that activity is reduced to 25% of the maximum at 10 \( \mu \)M concentration. As previously indicated, since the plasma concentrations of most marketed drugs are lower than 10 \( \mu \)M, a typical UGT substrate would therefore not be expected to saturate its own metabolism unless its hepatic concentration was in the hundreds of micromolar range. The concentrations of some UGT substrates are relatively high, e.g., valproic acid reaches plasma concentrations approaching 1 mM (Table 1). However, the corresponding substrates are relatively high, e.g., valproic acid reaches plasma concentrations of 300 to 1000 \( \mu \)M.

Although the intraluminal position of UGT enzymes differs from that of P450s, which are located on the cytoplasmic side, there are no significant differences in the pharmacokinetics (e.g., hepatic extraction ratios) of P450 and UGT substrates. Therefore, unless future evidence proves otherwise, it appears appropriate to assume that the same pharmacokinetic principles apply for both P450 and UGT substrates in vivo.

As indicated previously and in Table 2, other influencing factors on the magnitude of drug interaction include 1) the relationship between hepatic inhibitor concentration and \( K_i \), and 2) the fraction metabolized by a single enzyme. Equation 2 was developed (Rowland and Matin, 1973) incorporating these two components for metabolized drugs to provide a sensitivity analysis of AUCi/AUC ratio to these two independent variables (Fig. 3).

\[
AUC = \frac{1}{1 + (I/K_i)} + 1 - (f_m \cdot f_m^{UGT})
\]

where \( I \) is inhibitor concentration, \( K_i \) is a constant describing affinity of inhibitor for the enzyme, \( f_m \) is the fraction of drug cleared by metabolism (as opposed to renal or biliary excretion of unchanged drug), and \( f_m^{UGT} \) is the fraction of drug metabolized by a single UGT enzyme. When a drug’s clearance is solely mediated by metabolism (\( f_m = 1 \)) catalyzed by a single enzyme (\( f_m^{UGT} = 1 \)), eq. 2 (Rowland and Matin, 1973) can be simplified to eq. 3:

\[
\frac{AUC}{AUC} = 1 + \frac{I}{K_i}
\]

Based on eq. 2 (Rowland and Matin, 1973), the predicted magnitude of interaction (AUCi/AUC) increases as the contribution of a single enzyme to the metabolism of a drug increases from 0 to 1 (Fig. 3). Three scenarios, in which the \( I/K_i \) ratio is set at 0.1, 1, or 10, are presented in Fig. 3. If the \( K_i \) value is fixed at 10 \( \mu \)M, the corresponding liver concentrations, according to the three fixed \( I/K_i \) values, would be 1, 10, and 100 \( \mu \)M. A value of 10 \( \mu \)M is a conservative choice; most observed in vitro \( K_i \) values for UGT inhibitors are higher than 10 \( \mu \)M. This is exemplified by the data in Table 3, which lists a representative group of compounds with low inhibitory potency of UGT substrates for UGT2B7-catalyzed AZT glucuronidation and UGT1A1-catalyzed estradiol 3-glucuronidation. Potent in vitro inhibition by the flavonoid hexamethoxyflavone is an exception to the otherwise generally observed small effect, and will be discussed below.

For reference purposes, an AUC/AUC lower than 2 is considered to be within the bounds of natural interindividual variability of drug exposure in the absence of inhibitor. It can be seen from the simulation in Fig. 3 that when \( I/K_i \) is 0.1 or 1, the predicted AUC/AUC ratio does not rise above 2, even if a single enzyme is the only contributor to the clearance of a glucuronidated drug substrate. When \( I/K_i \) is 10, the predicted AUC/AUC only rises above 2 when the fraction metabolized by a single enzyme is greater than 0.6, and only rises above 3-fold when the fraction metabolized by a single enzyme rises above 0.8.

The sensitivity analysis presented in Fig. 3 is consistent with the observed pharmacokinetic findings showing low AUC/AUC ratios for glucuronidated drugs in the presence of inhibitor, as described below. It is also consistent with the observation that, dependent on methodology, multiple recombinant UGTs contribute to the in vivo glucuronidation of aglycones such as acetaminophen (Court et al., 2001; Goosen et al., 2004c), oxazepam (Court et al., 2002), zidovudine and codeine (Court et al., 2003), and CI-1027 (Bauman et al., 2004).

AZT can be taken as a case example for assessing potential extent of drug-drug interaction. AZT is an antiviral drug with a high plasma clearance mediated by UGT2B7-catalyzed glucuronidation. With regard to potential pharmacokinetic drug-drug interactions, in the most extreme scenario (Table 2), if AZT clearance were completely by hepatic glucuronidation, then, hepatic metabolic clearance would approach liver blood flow (20 ml/min/kg). The potential for a high AUC/AUC ratio would therefore be high, if a situation arose where the \( I/K_i \) ratio of a coadministered inhibitor was high (Fig. 3). However, observed drug-drug interactions indicate no greater than a 100% increase in exposure in AZT in the presence of an inhibitor. This result is consistent with the pharmacokinetic principles detailed above: 70% contribution of UGT2B7 and low in vitro inhibition potency for UGT2B7-catalyzed glucuronidation of AZT in vitro (see Table 3, and Trapnell et al., 1998). Furthermore, for AZT, since the in vitro \( K_m \) is in the 2 to 4 mM range and the therapeutic concentrations are an order of magnitude lower, assuming that in vitro \( K_m \) values roughly translate to in vivo \( K_m \) values and cofactor (UDP-glucuronic acid) levels are in excess, saturation of metabolism under conditions of UGT2B7 inhibition would be unlikely to occur and, thus, would not lead to a significant pharmacokinetic interaction (AUC/AUC < 2). Thus, using the data for valproic acid from Tables 1 (\( K_i > 4100 \) or 2050 \( \mu \)M, assuming noncompetitive or competitive inhibition, respectively; Cheng and Prusoff, 1973) and 3 (\( I_{total} = 805 \mu \)M or \( I_{unbound} = 113 \mu \)M) to plug into eq. 3, the greatest predicted AUC/AUC ratio would be, in any scenario, 1.4, which slightly underestimates the observed 2-fold increase in zidovudine AUC with concomitant valproic acid administration (Lertora et al., 1994).

II. Potent In Vitro Inhibitors of UGT Enzymes. The data presented in Table 3 and other recently published reports have indicated potent inhibition of UGTs in vitro by flavonoids such as hexamethoxyflavone (Table 3), tangeretin (Williams et al., 2002a), and...
silybin (Goosen et al., 2003, 2004c): IC50 values are in some cases reported to be less than 1 μM. However, there are no clinically relevant drug-drug interactions for UGT substrates after flavonoid consumption in healthy humans.

Flavonoids are universal components of citrus fruits, consumed in significant amounts by many individuals taking drugs primarily cleared by glucuronidation. Some hydroxylated flavonoids such as chrysin (Table 4) are extensively glucuronidated in the intestine. As a consequence, bioavailability and the potential for inhibition of glucuronidation is low. Silybin (Table 4), a major component of the herb supplement milk thistle, is a popular treatment for liver-related conditions. Some of these reports conclude that clinical relevance is likely, whereas others take the more conservative route of concluding that the clinical relevance is unknown (Goosen et al., 2004c). However, no observed clinically relevant (relating to efficacy or toxicity) interactions relating to UGT1A1 inhibition (e.g., reduction of bilirubin glucuronidation leading to jaundice) have been reported for healthy patients taking milk thistle.

Another recent study indicated that fatty acids are potent inhibitors of human kidney cortical microsome-catalyzed 4-methyl umbelliferone glucuronidation, with a reported Kᵢ of 0.15 μM (Tsoutikos et al., 2004). The speculated clinical relevance is that during periods of renal ischemia, fatty acids may impair renal drug glucuronidation, thus compromising the protective capacity of the kidney against drug-induced nephrotoxicity. However, the contribution of the kidney to systemic clearance by UGTs is thought to be low.

As with CYP3A enzymes (Ekins et al., 2003), accumulating evidence for the allosteric behavior of UGT1A1 (Williams et al., 2002a) and UGT2B7 (Stone et al., 2003) in vitro, and of overlapping sites for substrate binding for UGT1A1 (Rios and Tephly, 2002), suggests that extrapolation of in vitro modulation (inhibition and heterotropic activation) data to the in vivo situation for UGT substrates is becoming increasingly complex. Examples of heterotropic activation of UGT enzymes include ethinyl estradiol (Williams et al., 2002a) or AZT activation (Table 3) of estradiol 3-glucuronidation. There is an increased need to better understand the clinical relevance of in vitro UGT-ligand interactions.

### III. No “Poor Metabolizer Phenotypes” due to in Vivo Inhibition of UGT Enzymes.

There is a well established correlation between CYP2D6 genotype and phenotype: extensive and poor metabolizers of CYP2D6 substrates can be predicted from data on genetic polymorphisms (Sauer et al., 2003). The phenotype of extensive CYP2D6 metabolizers can also be altered to poor metabolizer status by coadministration of potent inhibitors of the CYP2D6 enzyme. UGT1A1 is the only UGT enzyme in which there is unequivocal evidence for a genotype/phenotype correlation (Miners et al., 2002). The severe Crigler-Najjar syndrome and the milder Gilbert’s syndrome are gene-based defects in the UGT1A1 gene that result in impaired

### Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>In Vitro IC50</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tangeretin</td>
<td><img src="image" alt="Tangeretin Structure" /></td>
<td>1</td>
<td>Williams et al. (2002)</td>
</tr>
<tr>
<td>Silybin</td>
<td><img src="image" alt="Silybin Structure" /></td>
<td>1b</td>
<td>Goosen et al. (2004)</td>
</tr>
<tr>
<td>Chrysin</td>
<td><img src="image" alt="Chrysin Structure" /></td>
<td>&lt;10</td>
<td>Williams et al. (2002)</td>
</tr>
<tr>
<td>Nobiletin</td>
<td><img src="image" alt="Nobiletin Structure" /></td>
<td>&gt;10</td>
<td>Williams et al. (2002)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td><img src="image" alt="Bilirubin Structure" /></td>
<td>&lt;30</td>
<td>Williams et al. (2002)</td>
</tr>
<tr>
<td>17α-Ethinylestradiol</td>
<td><img src="image" alt="17α-Ethinylestradiol Structure" /></td>
<td>Stimulation</td>
<td>Williams et al. (2002)</td>
</tr>
</tbody>
</table>

*Pooled human liver microsomes using estradiol 3-glucuronidation as a marker for UGT1A1 activity (Williams et al., 2002) unless indicated.

*Recombinant UGT1A1 using HFC as substrate (Goosen et al., 2004). Ethinylestradiol stimulation of estradiol 3-glucuronidation was maximal (180% of control activity) at the lowest tested ethinylestradiol concentration (5 μM).
biliarubin glucuronidation, leading to hyperbilirubinemia and even jaundice in some cases (Miners et al., 2002). However, as indicated above, in contrast to the situation with CYP2D6, there are no published observations of poor metabolizer UGT1A1 phenotype as a result of UGT1A1 enzyme inhibition in otherwise healthy patients.

Conclusions

Although speculation on the clinical relevance of in vitro observations of inhibition of glucuronidation is relatively common (e.g., Pueksaritanont et al., 2002), appropriate mechanistic studies attempting to link in vitro inhibition with influence on AUC in humans are rare. Trapnell et al. (1998) reported reasonable correlation between in vitro inhibition of AZT glucuronidation in human liver microsomes and inhibition in humans. Recent reports on the in vitro glucuronidation of statins speculate on the potential for pharmacokinetic drug-drug interactions. However, most statins are cleared primarily by UGT enzymes or nonmetabolic routes, and, as such, the fraction metabolized by (multiple) UGTs is likely to be small, indicating potential for a small change in AUC/AUC ratio as a result of in vivo inhibition of glucuronidation (Fig. 3). Based on low affinity for substrate to enzyme, low affinity of inhibitor to enzyme, multiple enzyme superfamilies (e.g., both P450s and UGTs) contributing to the metabolism of the drug, as well as the potential for multiple UGTs to metabolize the drug, it is anticipated that the likelihood of pharmacokinetic drug-drug interactions is low for drugs cleared by glucuronidation. This is consistent with observations in patients, where evidence of drug-drug interactions in which AUC/AUC ratios are >2 or there are clinically relevant drug-drug interactions are rare. It is important that these considerations be taken into account when speculating on the in vivo relevance of observed in vitro inhibition for drugs partly or primarily cleared by glucuronidation in humans.

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Address correspondence to: J. Andrew Williams, Pharmacokinetics, Dynam-ics and Metabolism, Pfizer Global Research and Development, Ann Arbor, Michigan. J. Andrew Williams is an Associate Research Fel-low in the Department of Pharmacokinetics, Dynamics and Metabolism at Pfizer Global Research and Development in Ann Arbor, Michigan. He received a B.Sc. in Genetics from the University of Wales, Swansea in 1989, a Master of Science in Toxicology from the University of Birmingham, England in 1991, and a Ph.D. in Pharmacology (Drug Metabolism) from the University of Aberdeen, Scot-land in 1995. Current research interests include pharmacogenomics of drug-metabolizing enzymes and transporters related to drug disposition and pharmacodynamics, ligand interactions with human CYP3A and UGT enzymes, and novel approaches to simulate and/or predict metabolic clearance and metabolic drug-drug interactions.