Glucuronidation of Drugs by Hepatic Microsomes Derived from Healthy and Cirrhotic Human Livers

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Accepted for publication January 12, 1999

ABSTRACT

Pharmacokinetic studies demonstrated that the decrease in drug biotransformation in hepatic failure depends on the metabolic pathways involved. To test whether glucuronidation reactions supported by UDP-glucuronosyltransferases are differentially affected in such conditions, we investigated the in vitro glucuronidation of four selected drugs and xenobiotics (zidovudine, oxazepam, lamotrigine, and umbelliferone) by using microsomes from human healthy and unhealthy (cirrhosis, hepatitis) livers as enzyme sources. Theses substances are glucuronidated by several UDP-glucuronosyltransferase isoforms. Lidocaine N-deethylation activity measured concomitantly was used as a positive control, because the inhibition of this reaction in patients with hepatic diseases is well documented. The metabolic clearances of zidovudine and lidocaine were decreased significantly in liver cirrhosis (0.17 versus 0.37 µl/min/mg protein and 0.40 versus 2.73 µl/min/mg protein, respectively) as a consequence of a decrease of their corresponding V max of metabolism. By contrast, the metabolic clearances of oxazepam, umbelliferone, and lamotrigine glucuronidation remained unchanged. Previous studies reported that the in vivo oral clearances of zidovudine and lidocaine were decreased by 70% and 60%, respectively, in cirrhotic livers, whereas those of lamotrigine and oxazepam were not affected. Consequently, it is likely that the in vitro metabolic data, which support the in vivo results, therefore could contribute to reasonably predict the level of impairment of hepatic clearance in patients with liver cirrhosis.

It is commonly thought that phase II pathways of drug metabolism (conjugation reactions) are unaltered in liver dysfunction. The elimination as glucuronides of drugs slowly extracted by the liver (such as oxazepam) or highly extracted (such as morphine) has never been reported to be extensively impaired in patients with liver cirrhosis (Shull et al., 1976; Patwardhan et al., 1981). More recent investigations have shown that, indeed, this was not a general rule. Conjugation of 3-hydroxyantipyrine was reduced in patients with hepatic failure (Teunissen et al., 1984), and the oral clearance of zomepirac, a drug that undergoes extensive glucuronidation, was decreased by 50% in cirrhosis (Witassek et al., 1983). Zidovudine, which is excreted mainly as 5'-O-glucuronide in humans, presented a 70% decrease of oral clearance in patients with grade B or C cirrhosis (Taburet et al., 1990). Those values are in the same range as those mentioned for drugs eliminated through phase I pathways of biotransformation, with extensive extraction by liver, such as midazolam, nifedipine, or verapamil, whose clearance is decreased by 48, 60, and 65%, respectively (Howden et al., 1988).

The decrease of hepatic clearance of drugs has been explained by a reduction of the activity and expression of enzyme isoforms responsible for their metabolisms and by the presence of portosystemic shunts (Howden et al., 1988; Morgan and Mc Lean, 1995). Because glucuronidation is a main phase II metabolic pathway of drugs in humans, it is important to determine to what extent this reaction is sensitive to liver failure. Glucuronidation is supported by UDP-glucuronosyltransferases (UGT, EC 2.4.1.17). This multigenic family of enzymes catalyzes the binding of glucuronic acid, from the high-energy donor UDP-glucuronic acid (UDPGA), on the hydroxyl, carboxyl, amine, or thiol group of chemically unrelated substances. In this work, four drugs known to be excreted exclusively from the body as glucuronides were selected. Oxazepam and lamotrigine are cleared slowly by the liver, umbelliferone is highly cleared, and zidovudine is cleared at an intermediate rate (Ritschel et al., 1977; Greenblat, 1981; Collins and Unadkat, 1989; Rambeck and Wolf, 1993). The in vitro glucuronidation of these compounds by microsomes from healthy human and unhealthy liver was investigated. Oxazepam and umbelliferone are glucuronidated on the free hydroxyl group of the ring. Glucuronidation occurs on the 5'-hydroxyl end of the ribose moiety.
of zidovudine (Good et al., 1990), whereas lamotrigine forms a quaternary ammonium-linked glucuronide (Green et al., 1995). As a positive control, lidocaine N-deethylation supported by CYP3A was followed concomitantly. This reaction is known to be decreased in patients with liver disease (Bargetzi et al., 1989).

**Materials and Methods**

**Chemicals**

Zidovudine, its glucuronide, and 1-(3-azido-2,3-dideoxy-β-D-threopentofuranosyl) thymidine used as internal standard, and lamotrigine were provided by Wellcome (Issy les Moulineaux, France). The glucuronide of lamotrigine was synthesized as described previously (Magdalou et al., 1992). Oxazepam was supplied by Wyeth-Ayerst (France). Umbelliferone, 1-naphthol, and naphthyl-β-D-glucuronide were obtained from Sigma Chemical Co. (St. Louis, MO). Astra Laboratories (So¨derta¨lje, Sweden). UDPGA was purchased by CYP3A was followed concomitantly. This reaction is known to be decreased in patients with liver disease (Bargetzi et al., 1989).

Lidocaine and monoethylglycinexylidide (MEGX) were provided by Ayerst (France). Umbelliferone, 1-naphthol, and naphthyl-β-D-glucuronide were obtained from Sigma Chemical Co. (St. Louis, MO). Lodicaine and monoethylene glycol-xylidide (MEGX) were provided by Astra Laboratories (So¨derta¨lje, Sweden). UDPGA was purchased from Boehringer Mannheim (Mannheim, Germany). BSA and β-glucuronidase (Helix pomatia) were obtained from Sigma Chemical Co. The other chemicals were of the highest purity commercially available.

**Liver Specimens and Preparation of Microsomes**

Human liver samples were obtained from transplant donors (age range, 5–42 years) and recipients (age range, 0.5–60 years). Nineteen samples were obtained from normal livers donated for transplantation and surgically reduced for transplantation into young children. These healthy liver samples usually were perfused in situ with Belzer liquid at 4°C. Further details on the donors and recipients are given in Table 1. Sampling was made in accordance with French ethical and legal regulations. Thirteen unhealthy liver samples were obtained at the time of orthotopic liver transplantation. The detection limit of zidovudine glucuronide was 0.4 μg per 20 μl injected.

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**1-Naphthol**. Glucuronidation activity was measured using the modified method of Miners et al. (1988). The specific activity was calculated with 500 μM as a final concentration of 1-naphthol. Enzymatic assays contained 5 mM UDPGA, 50 μg microsomal proteins, 100 mM Tris-HCl buffer (pH 7.4), and 10 mM MgCl₂ in a total volume of 200 μl. 1-Naphthol was dissolved in dimethyl sulfoxide (DMSO), so that the final concentration of DMSO was 0.25% (v/v) in the incubation mixture. Incubations were performed for 10 min at 37°C. The reaction was stopped by the addition of 20 μl of 4 M trichloroacetic acid. 1-Naphthol glucuronide was assayed according to De Vries et al. (1989). Samples (20 μl) were injected into a RP18 reversed-phase HPLC system and eluted with 10 mM K₂HPO₄/acetoni-trile (75:35, v/v) pH 2.5, with a flow rate of 1.8 ml/min. Fluorometric detection was used with excitation and emission wavelengths of 290 nm and 330 nm, respectively. The detection limit was 7.5 ng per 20 μl injected, and the reproducibility of the assay was within 5% (n = 10).

**Lamotrigine**. Lamotrigine glucuronidation was assessed in vitro according to the method of Magdalou et al. (1992). Briefly, after incubation of microsomes with lamotrigine and UDPGA, the reaction concentrations were measured according to the method of Bradford (1976) by using BSA as standard. The red sirius coloration histological technique performed in all microsomal preparation according to James et al. (1990) indicated that proteins such as fibrinogen or collagen did not contaminate the microsomal proteins derived either from healthy or cirrhotic livers.

**Assay Procedures**

All experiments were performed under incubation conditions leading to linear reaction rates versus protein concentration and time. A high concentration of cofactor, UDPGA, was used in all incubation mixtures as described below. Control incubations were performed in the absence of UDPGA. At the end of the incubations, microsomal proteins were precipitated and discarded by centrifugation and the supernatants were analyzed by HPLC. Assay mixture composition and HPLC analysis for the four glucuronidated substrates and lidocaine are summarized below.

**Zidovudine**. Zidovudine glucuronidation was assessed according to the modified method of Haumont et al. (1990). Microsomes (0.5 mg of protein) were incubated in 100 mM Tris-HCl buffer (pH 7.4) at 37°C for 1 h in a final volume of 200 μl containing 10 mM MgCl₂, zidovudine (1–10 mM). The reaction started by the addition of 10 mM UDPGA and was stopped by 20 μl of 6 N HCl and 1 μl of internal standard [100 μl of solution, 10 μg/ml in methanol/water (50:50, v/v)] subsequently was added. The zidovudine glucuronide detected at 265 nm was separated by reversed-phase HPLC according to Haumont et al. (1990). The reproducibility of the assay was within 5% (n = 10).

**Table 1**

Clinicopathological characteristics of the subjects from whom liver samples were obtained

<table>
<thead>
<tr>
<th>Liver</th>
<th>Age</th>
<th>Histopathology</th>
<th>Pugh Classification</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.5</td>
<td>Cirrhosis</td>
<td>C</td>
<td>Cerebral vascular accident</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>Cirrhosis</td>
<td>B</td>
<td>Budd-Chiary syndrome</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>Cirrhosis</td>
<td>C</td>
<td>Biliary atresia</td>
</tr>
<tr>
<td>D</td>
<td>0.5</td>
<td>Cirrhosis</td>
<td>C</td>
<td>α-1-Antitrypsin deficiency</td>
</tr>
<tr>
<td>E</td>
<td>1.5</td>
<td>Cirrhosis</td>
<td>B</td>
<td>Biliary atresia</td>
</tr>
<tr>
<td>F</td>
<td>6</td>
<td>Cirrhosis</td>
<td>Unknown</td>
<td>Biliary atresia type II</td>
</tr>
<tr>
<td>G</td>
<td>60</td>
<td>Cirrhosis</td>
<td>Unknown</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>H</td>
<td>35</td>
<td>Cirrhosis</td>
<td>Unknown</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>I</td>
<td>58</td>
<td>Cirrhosis</td>
<td>Unknown</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>J</td>
<td>11</td>
<td>Cirrhosis</td>
<td>C</td>
<td>Autoimmune hepatitis</td>
</tr>
<tr>
<td>K</td>
<td>20</td>
<td>Cirrhosis</td>
<td>C</td>
<td>Autoimmune hepatitis</td>
</tr>
<tr>
<td>L</td>
<td>28</td>
<td>Hepatitis</td>
<td>Unknown</td>
<td>Fulminans hepatitis (unknown etiology)</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>Hepatitis</td>
<td>Unknown</td>
<td>Fulminans hepatitis (toxic)</td>
</tr>
</tbody>
</table>
was stopped by addition of 6 N HCl. After centrifugation, lamotrigine glucuronide was measured in the supernatant by RP18 reversed-phase HPLC. The limit of quantification was 10 pg per 20 μl injected, and reproducibility of the assay was within 9% (n = 10).

**Umbelliferone.** Umbelliferone glucuronidation was performed at 37°C in a total volume of 300 μl containing 100 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, umbelliferone (5–330 μM), UDPGA (1 mM), and microsomal proteins (0.1 mg). After 30-min incubation, the reaction was stopped by the addition of 20 μl of 60% HClO₄. In the absence of glucuronide standard, glucuronide formation was calculated from the difference between the amount of substrate in the incubation mixture at time 0 (before UDPGA addition) and that remaining at the end of incubation. Umbelliferone concentration was measured using the modified method of Tan et al. (1976). After centrifugation, supernatant was injected into RP18 reversed-phase HPLC column and eluted with 10 mM K₂HPO₄/acetonitrile (90:10), pH 2.9. Fluorescence was measured at excitation and emission wavelengths of 370 and 450 nm, respectively. The reproducibility of the assay was within 4.2% (n = 10). The lowest detectable amount of umbelliferone was 10 pmol per 20-μl injection mixture.

**Oxazepam.** Microsomal proteins (0.9 mg) were incubated with UDPGA (5 mM), oxazepam (20–600 μM), 10 mM Tris-HCl (pH 7.4), and 50 mM MgCl₂ in a total volume of 500 μl. Oxazepam was dissolved in DMSO in the incubation mixture so that the DMSO final concentration was 0.05% (ν/ν). Incubations were performed for 90 min at 37°C. The reaction was stopped by heating for 10 min. The amounts of oxazepam and its two diastereoisomer glucuronides were determined by RP18 reversed-phase HPLC and UV detection (λ = 254 nm), using the modified method of Saint-Pierre and Pang (1987). The mobile phase contained 0.067 M K₂HPO₄, pH 3.0/methanol (60:40, ν/v) with triethylamine (0.05%), and the flow rate was 1 ml/min. In the absence of an oxazepam glucuronide standard, the amount of the parent drug glucuronidated in the incubation mixture was assessed as follows. The two diastereoisomeric glucuronides of oxazepam were quantitated using the peak area corrected by a factor taking into account the ratio of extinction coefficient between oxazepam and its glucuronides. This factor was calculated from urinary oxazepam glucuronides issued to a patient treated by Seresta. The ratio of peak area of the amount of oxazepam yielded after glucuronide hydrolysis by β-glucuronidase to the sum of peak area of detected glucuronides was found to be 0.87. The limit of detection was 2 ng of oxazepam glucuronides per 20 μl injected, and reproducibility of the assay was 7% (n = 10).

**Lidocaine N-Deethylation.** Composition of the incubation mixture allowing determination of lidocaine deethylation and the quantification of MEGX formed were described by Bargelzi et al. (1989). Formation of MEGX was determined for concentrations of lidocaine of 0.05 to 5 mM. Because MEGX formation showed biphasic characteristics of Michaelis-Menten kinetics, the sum of low- and high-affinity components was calculated.

**Kinetics and Statistic Analysis**

The apparent Michaelis-Menten kinetic constant $K_m$ and the $V_{max}$ were determined from the double-reciprocal plot representation (Lineweaver-Burk). Lines were plotted by a method of weighed linear regression as recommended by Dowd and Riggs (1965). The weighing factor of the reciprocal velocity (1/ν) was $ν^2$. $V_{max}/K_m$ ratios were determined as a rough calculation of metabolic clearance. All results were expressed as means ± S.D. Examination of the frequency distribution of activities or enzyme parameters showed that some data were not normally distributed. Therefore, apparent differences between groups were analyzed using either Student's t test (normal distribution of data) or Welch's t test. Correlations between enzyme activities were performed using standard regression analysis. Data were analyzed on a digital computer using a commercial statistical software (Graphpad Software Instat II, version 2.0; Macintosh, San Diego, CA).

**Results**

Mean rates of metabolite formation with increasing substrate concentration are shown in Fig. 1 for all healthy and unhealthy liver microsomes. The four substrates of UGT exhibited monophase glucuronidation kinetics in healthy or unhealthy microsomes. The rate of glucuronidation of lamotrigine, oxazepam, and umbelliferone remained unaltered in cirrhosis microsomes. In contrast, there was a marked decrease in the rate of zidovudine glucuronidation. The magnitude of this phenomenon was close to that observed with lidocaine oxidation. All substrates underwent reduced rates of metabolism by microsomes from fulminans hepatitis. Parameters related to these enzyme kinetics are listed in Table 2. Liver diseases did not alter the $K_m$ value for the glucuronidation of drugs. Maximal velocity of zidovudine glucuronidation and lidocaine deethylation were decreased significantly in samples from liver cirrhosis (0.31 ± 0.16 versus 1.05 ± 0.46 nmol/min/mg of protein and 1.10 ± 1.40 versus 6.35 ± 2.88 nmol/min/mg of protein, respectively). $V_{max}$ values of umbelliferone, oxazepam, and lamotrigine were in the same range whether measured with healthy or unhealthy (cirrhosis) microsomes. The rate of 1-naphthol glucuronidation was unchanged whether measured in healthy or cirrhosis liver microsomes (7.22 ± 2.76 nmol/min/mg of protein versus 8.07 ± 4.47 nmol/min/mg of protein, respectively). As expected, $V_{max}$ values of substrates were decreased in microsomes prepared from liver with fulminans hepatitis.

Individual metabolic clearances are depicted in Fig. 2. It should be pointed out that the interindividual variability was quite large: a 10-fold variation in metabolic clearance measured in healthy livers was observed for all substrates considered, except umbelliferone and lamotrigine. A decrease in $V_{max}$ of lidocaine and zidovudine in cirrhosis led to a significant drop in their metabolic clearances. $V_{max}$ values for umbelliferone, oxazepam, and lamotrigine in cirrhosis were in the same range as those measured in healthy livers.

**Discussion**

As suggested from previous pharmacokinetic studies (Hoyumpa and Schenker, 1991), our in vitro data clearly show that the glucuronidation pathway is not always preserved in hepatic failure. If the glucuronidation rate of umbelliferone, oxazepam, or lamotrigine was not altered in cirrhosis, the metabolic clearance of zidovudine, calculated from the in vitro kinetics, was decreased in such a pathological condition. This drop was similar to that observed for lidocaine deethylation. As expected, rates of glucuronidation, whatever the substrate used, were barely measurable with microsomes prepared from fulminans hepatitis.

Glucuronidation of drugs and xenobiotics containing a wide range of acceptor groups has been reported, including phenols (umbelliferone, 1-naphthol), alcohols (zidovudine, oxazepam), and aliphatic amines (lamotrigine). This illustrates the large variability of acceptor groups that can be conjugated to glucuronic acid in humans. Although the multiplicity of UGTs is now well established (Mackenzie et al., 1997), specific marker substrates or inhibitors of isoforms are still lacking, because of the well known overlapping substrate specificity of these proteins. Therefore, several structurally unrelated substances presenting different pharmacokinetic
properties and different in vitro glucuronidation rates were arbitrarily chosen in an attempt to follow the overall glucuronidation reaction in hepatic diseases. A pharmacokinetic study performed with coumarin suggested that the main hydroxyl metabolite (7-hydroxycoumarin) was glucuronidated extensively and excreted rapidly as glucuronide (Ritschel et al., 1977). This result was in accordance with the very high rate measured in vitro leading to mean metabolic clearance value of about 100 ml/min per mg of protein. Planar phenolic substances, such as 7-hydroxycoumarin or 1-naphthol, generally are glucuronidated much faster than bulkier substances. Zidovudine or lamotrigine were metabolized at a lower rate, despite the fact that glucuronidation is the main metabolic pathway of these drugs. The specific activity measured with microsomes of healthy livers was similar to that reported previously (Haumont et al., 1990; Magdalou et al., 1992). We are not aware of in vitro glucuronidation data for oxazepam, but it is considered as a model drug for low hepatic extraction, and its in vitro hepatic clearance is, on average, 5000 times lower than that of umbelliferone. It should be pointed out that in vitro metabolic clearances of zidovudine and lamotrigine are in the same range, whereas systemic clearance of zidovudine in healthy volunteers is 80 times higher than that of lamotrigine (Yuen and Peck, 1988;

![Fig. 1](image1.png)

**TABLE 2**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Healthy Livers</th>
<th>Cirrhosis</th>
<th>Fulminans Hepatitis (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
<td>K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>nmol/min/mg</td>
<td>mM</td>
<td>nmol/min/mg</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>6.35 ± 2.88</td>
<td>2.97 ± 2.02</td>
<td>1.10 ± 1.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(n = 19)</td>
<td>(n = 19)</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>1.05 ± 0.46</td>
<td>3.72 ± 2.00</td>
<td>0.31 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 12)</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>Umbelliferone</td>
<td>32.4 ± 27.2</td>
<td>0.36 ± 0.26</td>
<td>21.5 ± 14.1</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 12)</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>0.0034 ± 0.0009</td>
<td>0.30 ± 0.23</td>
<td>0.0028 ± 0.0001</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 9)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>0.96 ± 0.38</td>
<td>5.49 ± 5.15</td>
<td>0.85 ± 0.54</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 12)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
</tbody>
</table>

<sup>a</sup>P < .01 versus healthy livers.
Taburet et al., 1990). These data suggest that the liver is not the only site of zidovudine metabolism. Furthermore, another metabolic pathway involving cytochrome P-450 and leading to the formation of the toxic AMT (3'-amino-3'-deoxythymidine) metabolite has been demonstrated (Placidi et al., 1993).

Lidocaine metabolism catalyzed by CYP3A, the major cytochrome P-450 in human liver, was used as a positive control. The apparent kinetic constants measured in microsomes of healthy livers were similar to those of Bargetzi et al. (1989). Interestingly, a considerable interindividual variation in lidocaine deethylase activity or metabolic clearance was observed, probably in relation to variation in the expression level of the CYP3A4 protein (Lemoine et al., 1993). In liver cirrhosis, the deethylation rate was decreased significantly. These findings were in agreement with pharmacokinetic studies that showed a reduced clearance in individuals with alcohol cirrhosis or chronic hepatitis (Colli et al., 1988).

Despite large interindividual variations in glucuronidation rates, our data clearly show that glucuronidation, depending on the substrate used, can be impaired in liver disease to the same extent as lidocaine oxidation. Liver cirrhosis decreased zidovudine glucuronidation, but not that of umbelliferone, oxazepam, and lamotrigine. The disease affected the apparent $V_{\text{max}}$, but not $K_{\text{m}}$, suggesting that the level of expression was impaired. These results could reveal the different susceptibility of isoforms involved in the glucuronidation of these drugs toward the disease. Indeed, the drugs used in this study appear to be glucuronidated by distinct UGT isoforms belonging to family 1 or 2. The isolation of cDNAs encoding human UGT isoforms and their expression in heterologous cells are invaluable in determining the substrate specificity of each isolated protein (Fournel-Gigleux et al., 1990). Umbelliferone is glucuronidated mainly by UGT1A6, which catalyzes the glucuronidation of planar and short phenols (Ebner and Burchell, 1993). On the other hand, UGT1.4 isozyme has been found to be very effective in the formation of quaternary ammonium-linked glucuronide from lamotrigine (Green et al., 1995). UGT1A6 and UGT1.4 are products of the same UGT1 gene locus by alternate splicing. This gene also encodes for UGTs active toward bilirubin. In contrast, no human UGT isoform isolated so far has shown to glucuronidate zidovudine to an appreciable extent. Indirect evidence using alternate substrates as competitors or inducers, therefore, is a useful tool to try to identify the isoforms involved in zidovudine glucuronidation. Rajaonarison et al. (1991) suggested that the drug was glucuronidated preferentially by family 2 UGT isoforms because its glucuronidation was impaired in vitro by substances that are mainly substrates of such isoforms. Recently, Coffman et al. (1998) showed that UGT2B7Y and UGT2B7H react poorly with oxazepam. The susceptibility of zidovudine glucuronidation in cirrhosis and fulminant hepatitis indicates that this drug would be substrate of a selective UGT isoform undiscovered...
until now whose expression is sensitive to the physiopathological state. By contrast, the overall glucuronidation level of the other drugs not affected by the diseases would suggest the implication of several, differentially sensitive UGT isoforms. The same situation stands for cytochrome P-450. George et al. (1995) reported recently that the expression of P-450 isoforms is selectively altered in severe chronic diseases. Some isoforms are profoundly decreased, others are decreased to a lesser extent, and some are not affected at all. These authors also pointed out the same situation in the presence or absence of cholestasis in patients with cirrhosis severe enough to require transplantation. Six of our 11 livers with cirrhosis were cholestatic. Analysis of the data as a function of identification of cholestasis failed to show any difference either in the rate of N-deethylation of lidocaine, presumably by CYP3A, or in the rate of glucuronidation of the four substrates studied. Therefore, cholestasis is unlikely to be a major factor explaining the effect of cirrhosis on glucuronidation.

Extrapolation of in vitro metabolic clearance to measure in vivo metabolic clearance is difficult because of protein losses during preparation. A “correcting” factor was proposed (Hoener, 1994) but was not found to be reliable possibly because endogenous substances can induce or inhibit drug-metabolizing enzymes. Hepatic clearance also depends not only on metabolic clearance but also on the fraction unbound to plasma proteins and on hepatic blood flow. Intrinsic clearance in vitro depends on protein binding in the incubation; this binding was considered identical in healthy and unhealthy incubation mixtures and assumed to be zero. Each of these factors affects low or high hepatic drug extraction differently according to the model proposed by Rowland et al. (1973). However, assuming that absorption is not a limiting factor, oral clearance is directly related to metabolic clearance for both low and high hepatic drug extraction.

Pharmacokinetic parameters of the drugs measured in volunteers and patients with liver cirrhosis were compared with in vitro metabolic clearance (Table 3). It appears clearly that there is a close relationship between in vitro and in vivo data for lidocaine and zidovudine. For lamotrigine, the in vivo data have shown an insignificant trend to decrease in glucuronide production in patients with severe liver cirrhosis (De Bony et al., 1997). It should be noted that protein binding is not a limiting factor for lidocaine, which is highly extracted, and that protein binding of zidovudine and lamotrigine can be neglected. Two pharmacokinetic studies with oxazepam led to controversial data that could be explained by the severity of the disease of the patients (Shull et al., 1976; Sonne et al., 1990). Furthermore, oxazepam is highly protein bound, and the increased free plasma fraction in cirrhosis could counterbalance the decrease in metabolic clearance in some patients with mild to moderate hepatic failure (Shull et al., 1976).

Different theories have been proposed to account for the effects of chronic liver disease on hepatic drug clearance (Morgan and McLean, 1995). None of them has been able to predict the extent of clearance impairment of a given drug. Whatever the mechanism of spared glucuronidation of some drugs relative to others, this study demonstrates that the comparison of in vitro metabolic clearance in healthy liver and liver with cirrhosis could be a useful tool to predict whether or not the rate of glucuronidation will be extensively impaired in patients with cirrhosis. As in the field of drug-drug interactions, such in vitro studies could help to set up properly designed clinical trials.

Acknowledgments

We thank Professor J. Valayer (Department of Surgery, Bicêtre Hospital) and Dr. A. Lemoine (Department of Biology, P. Brousse Hospital) for providing fragments of human livers and Dr. R. Bidauld from Glaxo-Wellcome (France) for providing data on lamotrigine pharmacokinetics in patients with cirrhosis.

References

Dragacci S, Thomassin J, Magdalou J, Souhaili EL, Amri H, Boissel P and Siest G

<table>
<thead>
<tr>
<th>Substrates</th>
<th>In Vivo Oral Clearance*</th>
<th>In Vitro Intrinsic Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy subjects</td>
<td>Cirrhotic patients</td>
</tr>
<tr>
<td></td>
<td>ml/min</td>
<td>µl/min/mg</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>1614 ± 432</td>
<td>700 ± 390b</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>2562 ± 816</td>
<td>686 ± 243b</td>
</tr>
<tr>
<td>Umbelliferone</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>156 ± 17</td>
<td>156 ± 31</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>26 ± 8</td>
<td>24 ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 ± 8</td>
</tr>
</tbody>
</table>

* Values from literature (Colli et al., 1988; Taburet et al., 1990; Shull et al., 1976; Sonne et al., 1990; De Bony et al., 1997).

b P < .01, cirrhosis versus control.

P Moderate liver cirrhosis.

Severe liver cirrhosis.

P Severe liver cirrhosis + ascites.


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