Hepatic Disposition of the Acyl Glucuronide 1-O-Gemfibrozil-β-D-glucuronide: Effects of Clofibric Acid, Acetaminophen, and Acetaminophen Glucuronide

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ABSTRACT
Glucuronidation of carboxylic acid compounds results in the formation of electrophilic acyl glucuronides. Because of their polarity, carrier-mediated hepatic transport systems play an important role in determining both intra- and extrahepatic exposure to these reactive conjugates. We have previously shown that the hepatic membrane transport of 1-O-gemfibrozil-β-D-glucuronide (GG) is carrier-mediated and inhibited by the organic anion dibromosulfophthalein. In this study, we examined the influence of 200 μM acetaminophen, acetaminophen glucuronide, and clofibric acid on the disposition of GG (3 μM) in the recirculating isolated perfused rat liver preparation. GG was taken up by the liver, excreted into bile, and hydrolyzed within the liver to gemfibrozil, which appeared in perfusate but not in bile. Mean ± S.D. hepatic clearance, apparent intrinsic clearance, hepatic extraction ratio, and biliary excretion half-life of GG were 10.4 ± 1.4 ml/min, 94.1 ± 17.9 ml/min, 0.346 ± 0.046, and 30.9 ± 4.9 min, respectively, and approximately 73% of GG was excreted into bile. At the termination of the experiment (t = 90 min), the ratio of GG concentrations in perfusate, liver, and bile was 1:35:3136. Acetaminophen and acetaminophen glucuronide had no effect on the hepatic disposition of GG, suggesting relatively low affinities of acetaminophen conjugates for hepatic transport systems or the involvement of multiple transport systems for glucuronide conjugates. In contrast, clofibric acid increased the hepatic clearance, extraction ratio, and apparent intrinsic clearance of GG (P < .05) while decreasing its biliary excretion half-life (P < .05), suggesting an interaction between GG and hepatically generated clofibric acid glucuronide at the level of hepatic transport. However, the transporter protein(s) involved remains to be identified.

Glucuronidation is a major conjugation pathway for the inactivation and detoxification of a wide variety of endogenous and exogenous compounds. Different types of glucuronide conjugates include C-, S-, N-, ether-, and ester-linked glucuronides. The ester or acyl glucuronides, which are formed from compounds possessing a carboxylic acid group, are chemically reactive metabolites due to the susceptibility of the ester linkage to nucleophilic substitution (Spahn-Langguth and Benet, 1992). Thus, depending on the attacking nucleophile, acyl glucuronides may form rearrangement isomers, hydroyze to the aglycone, or covalently bind to proteins (Spahn-Langguth and Benet, 1992) and probably also to DNA (Sallustio et al., 1997).

In general, glucuronide conjugates are ionized at physiological pH and are highly polar. Therefore, these conjugates may be subject to a diffusional barrier in their movement across biological membranes (Evans, 1996). Such movement, particularly between their major site of formation, the liver, and either the systemic circulation or bile, may thus depend on carrier-mediated transport systems that are present in the biological membranes separating these compartments (Keppler and Konig, 1997; Meier et al., 1997; Kusuhara et al., 1998; Muller and Jansen, 1998).

We have shown previously that in the rat isolated perfused liver preparation, the transport of the acyl glucuronide 1-O-gemfibrozil-β-D-glucuronide (GG) from perfusate into bile is a two-step concentrative process involving carrier-mediated systems at both the sinusoidal and canalicular membranes of hepatocytes (Sabordo et al., 1999). These transport processes were significantly inhibited by the organic anion dibromosulfophthalein (DBSP) (Sabordo et al., 1999), a substrate for both the sinusoidal organic anion-transporting polypep-

ABBREVIATIONS: GG, 1-O-gemfibrozil-β-D-glucuronide; DBSP, dibromosulfophthalein; oatp, organic anion transporting polypeptide; mdr, multidrug resistance-associated protein; fu, fraction unbound in perfusate; CL, total clearance; E, hepatic extraction ratio; CLint,app, apparent intrinsic clearance; t1/2,bile, biliary excretion half-life; CLint, intrinsic clearance.
tide(s) (rat oatp) (Takenaka et al., 1997; Ishizuka et al., 1998) and canalicular multispecific organic anion transporter (rat cmoat or mrp2) (Kusuhaara et al., 1998), suggesting that GG and nonbile acid organic anions may share the same hepatic sinusoidal and canalicular transport systems. Similarly, other studies of acyl glucuronides have reported that the hepatocellular uptake of bilirubin diglucuronide in rats is shared with nonbile acid organic anions (Adachi et al., 1990, 1991), and that the canalicular membrane transport of acyl glucuronides such as bilirubin mono- and diglucuronides (Jedlitschky et al., 1997), nafenopin glucuronide (Jedlitschky et al., 1994), grepafloxacin glucuronides (Sasabe et al., 1998), and glycyrrhizin (Shimamura et al., 1996) is mediated by rat mrp2, and therefore also shared with other organic anions.

For a number of ether glucuronides conjugates, carrier-mediated sinusoidal uptake (Iida et al., 1989; Takenaka et al., 1997) and canalicular transport (Takenaka et al., 1995; Niinuma et al., 1997) have also been demonstrated. Estradiol-17β-glucuronide is a substrate for a number of rat oatp isoforms (Meier et al., 1997; Noe et al., 1997), and estradiol-17β-glucuronide and the ether glucuronide conjugates of E3040, SN38 (a metabolite of irinotecan) and liquiritigenin are substrates for rat mrp2 (Shimamura et al., 1994; Keppeler and Konig, 1997; Niinuma et al., 1997; Kusuhaara et al., 1998). Furthermore, the hepatocellular uptake of E3040 glucuronide into isolated hepatocytes is inhibited by organic anions (Takenaka et al., 1997). At the canalicular membrane, the in vivo secretion of glycyrrhizin (Shimamura et al., 1996) and liquiritigenin glucuronides (Shimamura et al., 1994) into the bile of rats and the in vitro membrane vesicle transport of E3040 glucuronide (Takenaka et al., 1995; Niinuma et al., 1997) have been shown to be shared with nonbile acid organic anions. Given that acyl and ether glucuronides and other nonbile acid organic anions may share transporters, there is a potential for mutual competition.

In the present study, the rat isolated perfused liver was used to investigate the potential interactions between GG and other glucuronides at the level of hepatic membrane transport. Acetaminophen, acetaminophen glucuronide, and clofibric acid were used as potential inhibitors of the hepatic membrane transport of GG. In the rat isolated perfused liver preparation, acetaminophen is metabolized to ether glucuronide, phenolphthalein glucuronide, and sodium taurocholate were purchased from Sigma Chemical Co. (St. Louis, MO).

The hepatic disposition of GG was examined in six liver perfusions (controls) with GG added as a single bolus to the perfusion medium. For inhibition studies, either clofibric acid, acetaminophen glucuronide, or clofibric acid, all at an initial perfusate concentration of 200 μM. In each experiment, perfusion medium (1 ml) was collected from the reservoir before and at 1, 2, 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 90 min after addition of the drug, and bile samples were collected at 10-min intervals throughout the experiment. In perfusates with clofibric acid, perfusate and bile samples were stabilized with 15 μl of 1.5 M phosphoric acid and 100 μl of 1 M glycine buffer, pH 3.0, respectively. All samples were frozen and stored at −20°C until analysis.

The hepatic disposition of GG was examined in six liver perfusions (controls) with GG added as a single bolus to the perfusion medium reservoir to achieve an initial concentration of 3 μM. For inhibition studies, either clofibric acid (n = 6), acetaminophen (n = 6), or acetaminophen glucuronide (n = 6) was added to achieve an initial concentration of 200 μM, 10 min before addition of GG. Perfusion medium (1 ml) and bile samples were collected and stabilized as described above for the perfusion with clofibric acid. All acidic samples were frozen and stored at −20°C. The end of each perfusion, the liver was blotted dry, frozen, and stored at −80°C. On the next day, bile samples were thawed and diluted (1:100) in 1.0 M glycine buffer (pH 3.0). The diluted bile samples were stored at −20°C until analysis.

**Protein Binding of Gemfibrozil and 1-O-Gemfibrozil-β-D-gluco-ronide in Perfusate**. Protein-binding studies were carried out as previously described (Saborido et al., 1999). GG or gemfibrozil were added to perfusion medium at 37°C to achieve concentrations of 1.5 to 3 μM or 20 μM, respectively. For binding interaction studies, either clofibric acid (200 μM), clofibric acid glucuronide (15 μM), acetaminophen (200 μM), or acetaminophen glucuronide (200 μM) was added to the perfusate before addition of either GG or gemfibrozil. The binding of GG and gemfibrozil was determined at 37°C by rapid ultrafiltration of 1-ml aliquots, in quadruplicate, by using a micropartition filter (Centriffree; Amicon Corporation, Beverly, MA) centrifuged at 2000g for 10 min in an angled rotor. A 500-μl aliquot of ultrafiltrate was immediately stabilized by the addition of 50 μl of 0.3 M phosphoric acid and stored at −20°C until analysis. The fraction unbound (fu) was calculated as the rate of the concentration of GG or gemfibrozil in the ultrafiltrate to that in the unfiltered perfusion medium. Previous studies have demonstrated that there

**Experimental Procedures**

**Materials.** Clofibric acid, gemfibrozil, acetaminophen, acetaminophen glucuronide, phenolphthalein glucuronide, and sodium taurocholate were purchased from Sigma Chemical Co. (St. Louis, MO). GG was biosynthesized and purified as previously described (Sallus-tio and Fairchild, 1995) and a similar method was used to prepare clofibric acid glucuronide. Both glucuronides were stored at −20°C. Bovine serum albumin (Pentex, fraction V) was purchased from Miles Inc. (Kankakee, IL). All other reagents were of analytical grade.
was no nonspecific binding of GG or gemfibrozil to the ultrafiltration equipment (Sallustio et al., 1996).

Analytical Methods. Concentrations of GG and gemfibrozil in perfusion medium, bile, and ultrafiltrate were determined by direct HPLC analysis as previously described (Sallustio and Fairchild, 1995). Although this method was capable of quantifying the rearrangement isomers of GG, no quantifiable amounts were observed, consistent with previous studies (Sallustio et al., 1996; Sabordo et al., 1999). The limits of quantification for GG and gemfibrozil were 0.05 and 0.1 μM, respectively. Concentrations of GG and gemfibrozil in liver tissue at the end of each perfusion were determined as previously described (Sabordo et al., 1999). Ratios of GG concentrations in liver to perfusate (total and unbound) and bile to liver at the 90-min time point were calculated. Acetaminophen, clofibrate acid, and their conjugates did not interfere with the analysis of GG and gemfibrozil in perfusate, bile, or liver. In pilot studies, acetaminophen and acetaminophen glucuronide were quantified by HPLC based on a previously described method (Brouwer and Jones, 1990). Clofibrate acid and clofibrate acid glucuronide were measured by HPLC based on a method for GG (Sallustio and Fairchild, 1995) but by using phenolphthalein glucuronide as internal standard.

Pharmacokinetic Analysis. The half-life ($t_{1/2}$) of GG was determined by regression analysis of the terminal portion of the log perfusate concentration versus time profile. The area under the perfusate concentration versus time curve from 0 to 90 min [AUC$_{(90\text{ min})}$] was calculated by the trapezoidal method and was added to the extrapolated area to determine the area under the curve to infinite time [AUC$_{(\infty)}$].

For each liver perfusion experiment, the total clearance (CL) of GG was calculated as follows:

$$CL = \frac{D}{AUC_{(\infty)}} \tag{1}$$

where D is the dose of GG added to the perfusion medium.

The fraction of the eliminated dose of GG cleared unchanged via biliary excretion (fu) of GG was calculated as:

$$fu = \frac{B_{GG}}{A_{GG(0–90)}} \tag{2}$$

where $A_{GG(0–90)}$ is the amount of GG excreted in bile over 90 min.

The hepatic extraction ratio (E) of GG was calculated as the ratio of CL to perfusate flow rate (Q), and the apparent intrinsic clearance (CL$_{int}$) was calculated, assuming a well stirred model (Wilkinson and Shand, 1975), as follows:

$$CL_{int} = \frac{Q \cdot E}{fu \cdot (1 - E)} \tag{3}$$

where fu is the mean unbound fraction in perfusate.

The biliary excretion half-life ($t_{1/2,bile}$) of GG was calculated by regression analysis of the terminal portion of the log biliary excretion rate versus time profiles.

Statistical Analysis. All values are presented as mean ± S.D. Two-way ANOVA was used to test for differences in bile flow rates, oxygen consumption rates, and protein-binding data followed by Dunn’s test (Prism 2.0; GraphPad Software Inc., San Diego, CA) for post hoc comparisons. The nonparametric Kruskal-Wallis test was used for all other comparisons with post hoc analysis using Dunnett’s test (Prism 2.0). For all statistical tests, a P value less than .05 was taken to represent significance.

Results

With a mean fu of $0.171 \pm 0.041$ over the range of 1.5 to 3 μM, GG was less extensively bound to albumin than its aglycone, which had an fu of $0.021 \pm 0.002$ at a concentration of 20 μM (Table 1). In the presence of either 200 μM clofibrate acid, acetaminophen, or acetaminophen glucuronide, or 15 μM clofibrate acid glucuronide, the fu of GG was not significantly altered compared with the control values ($P > .05$; Table 1). In contrast, the presence of 200 μM clofibrate acid significantly increased the fu of gemfibrozil, whereas 200 μM acetaminophen significantly lowered it (Table 1). Acetaminophen glucuronide and clofibrate acid glucuronide did not alter the binding of gemfibrozil.

The viability of perfused livers was comparable between control, clofibrate acid, acetaminophen, and acetaminophen glucuronide experiments. Throughout all perfusions, bile flow rates and oxygen consumption rates remained greater than 5 μl/min and 10 μmol/min, respectively, and were not different between groups.

Representative perfusate concentration versus time profiles and biliary excretion rate versus time profiles from pilot studies with acetaminophen, acetaminophen glucuronide, and clofibrate acid are shown in Fig. 1. Acetaminophen exhibited biexponential kinetics with a terminal half-life of 24 min (Fig. 1). Perfusion concentrations of hepatically generated acetaminophen glucuronide reached a maximum of 7 μM at 90 min and 2.5% of the dose was excreted in bile as acetaminophen glucuronide. The concentrations of preformed acetaminophen glucuronide in perfusate remained relatively constant over 90 min with 0.4% of the dose excreted in bile at 90 min. Under similar conditions, clofibrate acid was slowly cleared from perfusate with a terminal half-life of approximately 91 min. Clofibrate acid was metabolized to its acyl glucuronide, which appeared in perfusate within 2 min, and in bile within the first 10 min. Concentrations of clofibrate acid glucuronide in perfusate reached approximately 3 μM by 90 min, and in bile, 15% of the dose of clofibrate acid was excreted as the acyl glucuronide. Approximately 1.6% of the dose was recovered as clofibrate acid in bile and may have been due to hydrolysis of clofibrate acid glucuronide in the biliary tract.

The perfusate concentration versus time profiles for GG and gemfibrozil and the biliary excretion rate versus time profiles for GG are shown in Figs. 2 and 3, respectively. The pharmacokinetic parameters describing the hepatic disposition of GG and gemfibrozil are shown in Tables 2 and 3, respectively. The liver-to-perfusate and bile-to-liver concentration ratios were all greater than unity (Table 2). Acetaminophen and acetaminophen glucuronide did not

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<td>The fu of GG and gemfibrozil (G) in perfusion medium containing 1% (w/v) albumin, in the absence (control) and presence of 200 μM acetaminophen (A), 200 μM acetaminophen glucuronide (AG), 200 μM clofibrate acid (CFA), or 15 μM clofibrate acid glucuronide (CG)</td>
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<tr>
<th>fu</th>
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<td>GG (1.5–3 μM)</td>
<td>0.171 ± 0.041</td>
<td>0.146 ± 0.039</td>
<td>0.150 ± 0.042</td>
<td>0.159 ± 0.048</td>
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<td>G (20 μM)</td>
<td>0.021 ± 0.002</td>
<td>0.013* ± 0.004</td>
<td>0.025 ± 0.003</td>
<td>0.055* ± 0.005</td>
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* P < .05 compared with the control value.
significantly alter any of the pharmacokinetic parameters describing the hepatic disposition of GG (Table 2). In the presence of clofibric acid, the CL, E and CL\textsubscript{int,app} of GG were significantly higher ($P < 0.05$) and the $t_{1/2,bile}$ was significantly lower than the control. The liver concentration of GG at the termination of the perfusion was lowered to 53% of the control value ($P < 0.05$, Table 2). However, other parameters for the disposition of GG and the liver-to-perfusate and bile-to-liver concentration ratios were not significantly altered (Table 2).

Discussion

The hepatic transport of organic anions has been studied extensively. In rats, sinusoidal uptake of many organic anions is mediated by the oatp proteins (oatp1 and oatp2) whose substrates include bile acids as well as nonbile acid organic anions (Meier et al., 1997; Muller and Jansen, 1998). Additionally, at least three other carrier systems may mediate sinusoidal uptake of nonbile acid organic anions, including another family of multispecific transporters (oat) (Sekine et al., 1998), bilitranslocase and bromosulfophthalein/bilirubin-
binding protein (Meier et al., 1997). Sinusoidal efflux of organic anions from the liver also has been shown to be carrier-mediated (De Vries et al., 1985; Evans et al., 1995). In this study, the appearance in perfusate of acetaminophen glucuronide and clofibrate acid during perfusion with the respective parent aglycones (Fig. 1) demonstrates sinusoidal efflux of heptatically generated ether and acyl glucuronides. Although the identity of the efflux transporter(s) is unclear, a number of mrp2 analogs have been identified at the hepatocyte basolateral membrane, including MRP3 in humans (Konig et al., 1999) and mrp6 in rats (Madon et al., 2000). Canaliculal transport of many organic anions is carried out by the ATP-dependent mrp2, whose known substrates include cysteiny1 leukotrienes, DBSP, glucuronide conjugates, glutathione conjugates, and the sulfate conjugates of bile acids (Keppler and Konig, 1997; Kusuhara et al., 1998; Muller and Jansen, 1998). Inhibition studies with DBSP and bromosulfophthalein, and studies with mutant TR/GY and Eisai hyperbilirubinemic rats, which have genetically defective mrp2, have provided evidence of common transporters for ether and acyl glucuronide conjugates, and other nonbile acid organic anions (Adachi et al., 1990; Sakuma-Sawada et al., 1997; Sabordo et al., 1999). Thus, potential competition between glucuronide conjugates and other organic anions for membrane transport systems is possible. Indeed, we have shown previously that the hepatic uptake and canaliculal transport of GG were significantly inhibited by DBSP at concentrations that saturated canaliculal transport (Sabordo et al., 1999). In the present study, the effect of a preformed glucuronide conjugate (acetaminophen glucuronide) and of drugs that generate conjugates in the liver (acetaminophen and clofibrate acid) on the hepatic disposition of GG was investigated.

Acetaminophen glucuronide administration did not significantly alter the pharmacokinetics of GG or the ratio of GG concentrations between the liver and perfusate, indicating a lack of effect on the sinusoidal uptake of GG. This is consistent with the relatively low affinity of acetaminophen glucuronide for sinusoidal uptake ($K_m = 20,000 \mu M$) (Iida et al., 1989) compared with high-affinity substrates such as bromosulfophthalein ($K_m = 2.1 \mu M$) (Blom et al., 1981), DBSP ($K_m = 7 \mu M$) (Scwenck et al., 1976), E3040 glucuronide ($K_m = 59 \mu M$) (Takenaka et al., 1997), and bilirubin glucuronide ($K_m = 68 \mu M$) (Adachi et al., 1990). Preformed acetaminophen glucuronide also had no effect on the ratio of GG concentrations between bile and liver tissue, indicating no significant alteration in the canaliculal transport of GG. This is consistent with its limited hepatic uptake as demonstrated in our pilot study (Fig. 1) and previous in vivo studies in the rat (Watari et al., 1983).

Acetaminophen also had no effect on the pharmacokinetics of GG and the concentration ratios of GG between liver tissue and perfusate and between bile and liver tissue. In the rat, acetaminophen is metabolized to a sulfate conjugate, a glucuronide conjugate, and an oxidized metabolite that is conjugated with glutathione (Hjelle and Klaassen, 1984). In the rat isolated perfused liver, hepatically generated acetaminophen glucuronide sulfate is recovered predominantly in perfusate, whereas acetaminophen glucuronide is preferentially excreted into bile with the extent of biliary excretion being dependent on dose and ranging from 0.3 to 23% of an acetaminophen dose (Mitchell et al., 1989; Studenberg and Brosnihan, 1991).

The lack of a direct effect of acetaminophen on the sinusoidal uptake of GG may reflect different hepatic uptake mechanisms for acetaminophen compared with GG, and is consistent with the large number of uptake proteins that have been identified at the basolateral membrane. In contrast, acetaminophen sulfate and GG may share common hepatic sinusoidal membrane transport systems because DBSP inhibits the hepatic sinusoidal uptake of both compounds (Sakuma-Sawada et al., 1997; Sabordo et al., 1999). However, previous studies have reported a relatively low affinity of acetaminophen sulfate for sinusoidal uptake ($K_m = 22,000 \mu M$) (Iida et al., 1989). Therefore, in the present study, its likely presence in perfusate was not expected to have an effect on the sinusoidal uptake of GG.

The lack of effect of acetaminophen administration on the ratio of GG concentrations between bile and liver tissue in-
indicates a lack of effect of the hepatically generated acetaminophen metabolites on the canalicul membrane transport of GG. This observation suggests that the intrahepatic concentrations of acetaminophen glucuronide, and the sulfate and glutathione conjugates, were below their $K_{i}$ for inhibition of GG canalicul transport. Alternatively, a multiplicity of canalicul transporters also may account for the lack of effect of the conjugates of acetaminophen on the transport of GG. This latter concept is consistent with observations that canalicul membrane vesicles from Eisai hyperbiliru-
binemic rats, which lack mrp2, still retain the transporter(s) for sulfate conjugates, as well as a low-affinity transporter for some ether glucuronides (Niinuma et al., 1997; Kusuhara et al., 1998). The presence of multiple canalicul transporters also has been proposed to explain the observation that the biliary excretion of liquiritigenin glucuronide was inhibited by DBSP but not by another organic anion, indocyanine green (Shimamura et al., 1994), and similarly, that the biliary excretion of estradiol-17β-glucuronide was inhibited by bromosulfophthalein but not by DBSP (Takikawa et al., 1996).

In contrast, clofibric acid administration significantly increased ($P < .05$) the CL, E and CLint,app of GG, and significantly decreased ($P < .05$) the $t_{1/2,\text{bile}}$ of GG. Clofibric acid is metabolized to an acyl glucuronide, clofibric acid glucuro-
nide, which is extensively excreted into bile. The increase in CL and E observed in this study was due to the observed increase in CLint,app rather than a change in fu because clofibric acid and clofic acid glucuronide did not alter the extent of binding of GG to albumin. Based on the well stirred model of hepatic disposition, CLint,app can be expressed as follows:

$$\text{CL}_{\text{int,app}} = \frac{\text{Pin} \cdot \text{CL}_{\text{int}}}{\text{CL}_{\text{int}} + \text{Pout}}$$  \hspace{1cm} (4)

where Pin and Pout represent the membrane permeability clearances of unbound ligand for the movement of substrate into and out of hepatocytes, respectively, and CLint represents the true intrinsic clearance (i.e., metabolism and biliary excretion) of the unbound ligand (Miyauchi et al., 1987). In this study, an additional complexity arises from the re-
versibility of acyl glucuronidation. Consequently, CLint depends on hydrolysis of GG to gemfibrozil, conjugation of gemfibrozil, and biliary excretion of GG. Therefore, as shown in Fig. 4, an increase in CLint,app may theore-
tically arise from a reduction in glucuronidation of gemfibrozil (step 5), a facilitation of hepatic hydrolysis of GG (step 4), an increase in canalicul secretion of GG (step 2), or an increase in the net sinusoidal influx of GG (net rate of combined steps 1 and 3). Although a reduction in conjugation (step 5) and/or increase in deconjugation (step 4) may lead to an increased CLint,app, it is not consistent with the lack of change in $B_{\text{GG}}$ (Table 2) and perfusate gemfibrozil concentrations (Table 3). Clofibric acid appeared to have no direct effect on the canalicul transport of GG (step 2) because there were no alterations in the ratio of GG concentrations between bile and liver tissue and there were no differences in bile flow rates between control and clofibric acid perfusions, indicating the absence of a choleretic effect. An increase in the net inward movement of GG into the liver (net rate of steps 1 and 3) is, therefore, the only other possible mechanism for the observed effects of clofibric acid. This is unlikely to be due increased sinusoidal uptake (step 1) but may rather be due to inhibition of sinusoidal efflux (step 3) by intracellular clofibric acid glucu-
rone. Such a mechanism would explain both the increase in CLint,app and the more rapid biliary excretion of GG. Inhibi-
tion of sinusoidal efflux has been demonstrated previously between the organic anion probenecid and morphine-3-gluc-
uronide (Evans et al., 1995) and between DBSP and harmol sulfate (De Vries et al., 1985). The apparent lack of effect of clofibric acid administration on the ratio of GG concentrations between the liver and perfusate may have been due to our inability to measure unbound tissue concentrations of GG because displacement of GG from intrahepatic-binding sites by clofibric acid glucuronide or clofic acid may have counteracted the effect of decreased efflux on the ratio of total tissue-to-perfusate concentration.

Further to our previous study demonstrating significant pharmacokinetic alterations due to inhibition of sinusoidal uptake and canalicul membrane transport of GG (Sabordo et al., 1999), the present study suggests that pharmacoki-
netic alterations also may result from inhibition of the sinusoidal efflux of GG. Inhibition of the sinusoidal uptake and canalicul transport of GG by DBSP increased hepatically generated gemfibrozil by shunting the elimination of GG to the hepatic hydrolysis pathway (Sabordo et al., 1999). In this study, inhibition of sinusoidal efflux did not result in in-
creased formation of gemfibrozil but rather led to a faster biliary excretion of GG. However, clofibric acid glucuronide did not appear to have a direct effect on the canalicul transport of GG, suggesting a lower affinity of clofibric acid glucuronide for the transporters compared with GG or a mul-
tiplicity of transport systems for glucuronide conjugates. Similarly, the lack of effect of acetaminophen conjugates on the sinusoidal and canalicul transport of GG may be due to lower affinities of these conjugates for the transport systems for GG or a multiplicity of transport systems for glucuronide conjugates.

References
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