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Metabolism and disposition of gemfibrozil in Wistar and multidrug resistance-associated protein 2-deficient TR$^-$ rats

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1. The roles of multidrug resistance-associated protein (Mrp) 2 deficiency and Mrp3 up-regulation were evaluated on the metabolism and disposition of gemfibrozil.

2. Results from *in vitro* studies in microsomes showed that the hepatic intrinsic clearance (CL_{int}) for the oxidative metabolism of gemfibrozil was slightly higher (1.5-fold) in male TR$^-$ rats, which are deficient in Mrp2, than in wild-type Wistar rats, whereas CL_{int} for glucuronidation was similar in both strains.

3. The biliary excretion of intravenously administered [^{14}C]gemfibrozil was significantly impaired in TR$^-$ rats compared with Wistar rats (22 versus 93% of the dose excreted as the acyl glucuronides over 72 h). Additionally, the extent of urinary excretion of radioactivity was much higher in TR$^-$ than in Wistar rats (78 versus 2.6% of the dose).

4. There were complex time-dependent changes in the total radioactivity levels and metabolite profiles in plasma, liver and kidney, some of which appeared to be related to the up-regulation of Mrp3.

5. Overall, it was demonstrated that alterations in the expression of the transporters Mrp2 and Mrp3 significantly affected the excretion as well as the secondary metabolism and distribution of [^{14}C]gemfibrozil.

Introduction

The transporter multidrug resistance-associated protein (Mrp) 2 was originally recognized in rat hepatocyte canalicular membranes and has been shown to mediate the ATP-dependent transport of glutathione, glucuronide and sulfate conjugates of lipophilic compounds, and several other endogenous and xenobiotic compounds (Jansen *et al.* 1985, Oude Elferink and Jansen 1994, Gerk and Vore 2002). The protein has been cloned from rat and human liver (Mayer *et al.* 1995) and also shown to be expressed in the renal proximal tubules, small intestinal villi and brain, as well as in the placenta (Schaub *et al.* 1997, St-Pierre *et al.* 2000, Mottino *et al.* 2001, Kusuhara and Sugiyama 2002).

Mrp2 deficiency has been implicated in the aetiology of Dubin–Johnson syndrome, a disease phenotype characterized by chronic conjugated hyperbilirubinemia and impaired hepatobiliary transport of many endogenous and

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xenobiotic compounds which, normally, are excreted into the bile via Mrp2 (Oude Elferink and Jansen 1994, Paulusma and Oude Elferink 1997). Several mutations in the Mrp2 gene have been characterized in patients with Dubin–Johnson syndrome (Paulusma *et al*. 1997, Wada *et al*. 1998, Mor-Cohen *et al*. 2001).

TR^- rats, a mutant strain that originated from albino Wistar rats, are characterized by a phenotype similar to the Dubin–Johnson syndrome. Hereditary chronic conjugated hyperbilirubinemia, caused by defective hepatic anion transport, has been reported in these rats (Jansen *et al*. 1985). Uptake of organic anions from plasma to liver is normal in the TR^- rats; however, transport of conjugated bilirubin, tetrabromosulfophthalein, glutathione conjugates and other organic anions from liver to bile is impaired severely (Jansen *et al*. 1985). A single-nucleotide deletion in the Mrp2 gene has been characterized in the TR^- rats (Paulusma *et al*. 1996).

Mrp3, another organic anion transporter, is expressed exclusively in the intestine but with much lower levels found in other tissues in Sprague–Dawley rats (Cherrington *et al*. 2002); however, the inducible nature of hepatic Mrp3 has been well characterized (Ogawa *et al*. 2000). In the normal rat, the expression of Mrp3 in the liver is induced by bile duct ligation (Hirohashi *et al*. 1998) as well as under cholestatic conditions in human (Konig *et al*. 1999). Furthermore, a large expression of Mrp3 was observed in Mrp2-deficient Dubin–Johnson patients (Konig *et al*. 1999) and a similar effect was observed in Eisai hyperbilirubinemic rats which also lack hepatic Mrp2 (Hirohashi *et al*. 1998). Mrp3 is localized to the basolateral membrane, where it is involved in the excretion of various organic anions from cells into the sinusoidal blood (Hirohashi *et al*. 1999, Renes *et al*. 2000). Therefore, it seems likely that Mrp3 is up-regulated to compensate for a deficiency of Mrp2.

Gemfibrozil (figure 1) is a fibric acid derivative widely used as a lipid-regulating agent. Even though its mechanism of action is not well established, gemfibrozil is believed to activate the peroxisome proliferator activated receptor-α resulting in an increase in lipoprotein lipase activity and a decrease in hepatic triglyceride production (Todd and Ward 1988).

Gemfibrozil undergoes extensive phase I and II metabolism in rats, hamsters, and humans (Okerholm and Keely 1976, Dix *et al*. 1999, Thomas *et al*. 1999). Phase I metabolism occurs primarily by hydroxylation of the phenyl ring (*para* to the side chain) and the meta-methyl group to give the *meta*-hydroxy methyl derivative, which is further oxidized to a benzoic acid derivative. Extensive glucuronidation reactions result in the formation of various ether and acyl glucuronide conjugates. The disposition of gemfibrozil is species and gender dependent. Biliary excretion as mainly acyl glucuronides is predominant in rats (50–90% of the dose) and dogs (62% of the dose), whereas in hamsters, monkeys and humans, most of the excretion occurs via the kidney (about 80, 62 and 66% of the dose, respectively). A significant gender difference has been reported in hamsters, where biliary excretion was about twofold higher in males than females (Okerholm *et al*. 1976, Dix *et al*. 1999). Enterohepatic circulation has been shown to play a significant role in the elimination of gemfibrozil in rats (Curtis *et al*. 1985).

The present investigation evaluated the role of the transporters Mrp2 and Mrp3 in the disposition of gemfibrozil in rats.