Exploring the Drug-Drug Interaction Between Gemfibrozil and Repaglinide in Rats: Metabolism and Transport

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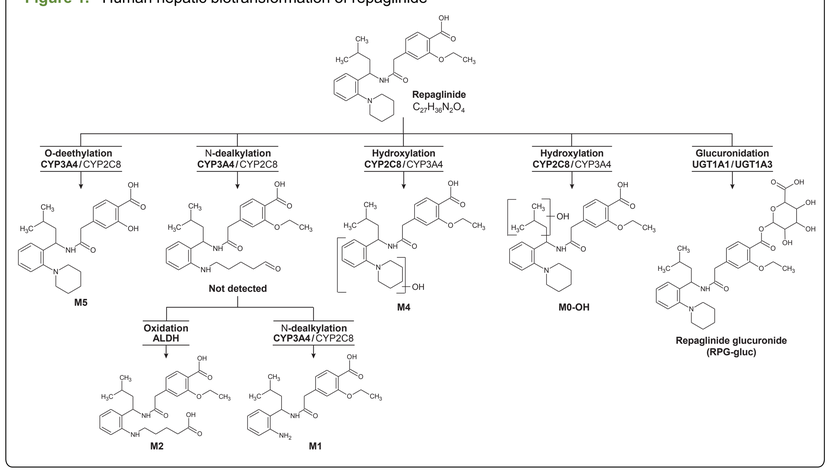
Introduction

Coadministration of the insulin secretagogue repaglinide with the dyslipidemia drug gemfibrozil has been established to cause a clinically-relevant drug-drug interaction (DDI) associated with an up to 8-fold increase in repaglinide plasma AUC resulting in severe, prolonged hypoglycemia (Backmann *et al.*, 2009). In humans, repaglinide is predominantly cleared by hepatic metabolism involving cytochrome P450 (P450) 3A4-mediated O- and N-dealkylation, 2C8-mediated hydroxylation, and UGT1A1- and 1A3-mediated glucuronidation (Figure 1). The P450 and UGT-based metabolism has been well characterized *in vitro* (Bidstrup *et al.*, 2003; Säll *et al.*, 2012), and a correlation between CYP2C8 polymorphism and repaglinide clearance has been demonstrated (Niemi *et al.*, 2003; Bidstrup *et al.*, 2006). The primary aromatic amine M1 and the dicarboxylic acid M2 are the major *in vivo* human metabolites formed following oral dosing, and more than 90% of the repaglinide and its metabolites are eliminated in the feces via biliary excretion with the remainder excreted in urine (van Heiningen *et al.*, 1999).

Gemfibrozil, through its glucuronide metabolite, is a potent, irreversible metabolism-dependent inhibitor of CYP2C8, and both gemfibrozil and the glucuronide cause time-dependent inhibition of UGT1A1, characteristics that have been proposed as key causes of the clinical DDI with repaglinide (Gan *et al.*, 2010). Additionally, gemfibrozil and gemfibrozil glucuronide are both established inhibitors of the human hepatic uptake transporter OATP1B1, proposed as a confounding factor in the interaction (Kalliokoski *et al.*, 2008; Kudo *et al.*, 2013). Recently, interest in animal models for DDI potential assessments has increased as utility has been demonstrated (Marathe and Rodrigues, 2010). In the present study, a mechanistic assessment of the complex gemfibrozil/repaglinide DDI was undertaken in male Sprague-Dawley rats to investigate both the drug-metabolizing enzyme and drug-transporter protein inhibition contributions.

Figure 1. Human hepatic biotransformation of repaglinide



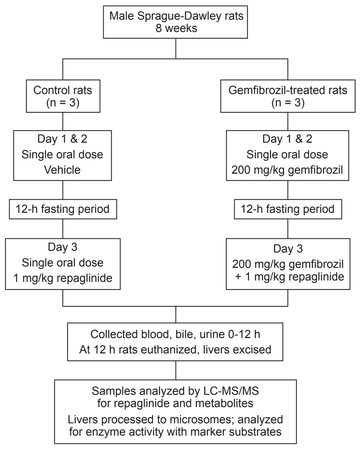
Materials & Methods

Repaglinide and gemfibrozil were purchased from Sigma-Aldrich (St. Louis, MO). Gemfibrozil 1-*O*-*β*-glucuronide and repaglinide-ethyl-d_5 were purchased from Toronto Research Chemicals (Ontario, Canada).

Animal dosing and sample collection

In-life work was performed by Xenometrics LLC (Stilwell, KS) as part of an IACUC-approved contract study. Repaglinide (0.1 mg/mL) and gemfibrozil (20 mg/mL) were suspended in 1.5% carboxymethylcellulose with sonication for dosing by oral gavage. Animals were dosed as shown in Scheme 1. Blood, bile and urine were collected for 12 h following repaglinide administration. Livers were excised and flash frozen in liquid nitrogen for microsome preparation.

Scheme 1. Rat dosing and sample collection scheme



Plasma samples (0, 0.25, 0.5, 1, 2, 4, 8 and 12 h) were analyzed individually for repaglinide quantitation. Samples were pooled across subjects and then further Hamilton pooled within each treatment group for metabolite profiling. The samples were prepared by protein precipitation with acetonitrile. Bile and urine samples were pooled across subjects within each treatment group and centrifuged.

Repaglinide quantitation

Rat plasma time-points were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) on a Shimadzu Nexera LC system (Columbia, MD) interfaced by electrospray ionization to an AB Sciex 5500 QTrap MS (Foster City, CA). A calibration curve range of 2-2000 ng/mL was employed with repaglinide ethyl-d_5 as an internal standard. The chromatographic gradient method employed 0.2% formic acid in water and acetonitrile mobile phases with a Phenomenex Kinetex XB-C18 column (2.6 µm, 2.1 x 100 mm). Repaglinide was detected with multiple reaction monitoring in positive mode using the mass transition 453/230 with 37 eV collision energy and 50 V declustering potential. This method was also used for relative quantitation of repaglinide in pooled liver microsomes for the repaglinide clearance assessments.

Metabolite profiling

Pooled plasma and 0-12 h urine and bile pools were analyzed by LC-MS/MS for metabolite profiling. Samples were analyzed on a Waters Acquity LC interfaced by electrospray ionization to either a Waters Synapt G2 High Definition Mass Spectrometry system quadrupole time of flight (QToF) mass spectrometer (Milford, MA) or an AB Sciex API4000 QTrap (Foster City, CA) operated in positive mode. Repaglinide and metabolites were separated on a Waters Acquity BEH column (1.7 µm, 2.1 x 100 mm) at 50°C with 0.1% formic acid in water and acetonitrile (0.4 mL/min) using a 25 min gradient method. Structural elucidation was performed manually.

Enzyme activity assays

Rat livers were pooled by treatment group and processed in-house to microsomal fractions. Enzyme activity assays were performed consistent with previously described procedures (Paris *et al.*, 2009). Briefly, selective LC-MS/MS assays were used to quantify formation of 6β-, 2α-, 16β- and 7α-hydroxytestosterone; 12-hydroxylauric acid; acetaminophen; 6-hydroxychlorzoxazone; 1′-hydroxymidazolam; 4′-hydroxydiclofenac; *N*-desethylamodiaquine; dextrorphan or estradiol-3β-D-glucuronide following 10 min incubation of cofactor-fortified pooled liver microsomes (0.01 mg/inc; 37°C; pH 7.4) at appropriate concentrations of each individual marker substrate.

Repaglinide clearance assays

Cofactor-fortified (NADPH and UDPGA) pooled liver microsomes (0.5 mg/mL) from control or gemfibrozil-treated rats were pre-incubated at 37°C, pH 7.4 for 30 min with gemfibrozil (100 µM) or gemfibrozil glucuronide (100 µM) or in the absence of inhibitor. Repaglinide (0.2 µM) was incubated in the pre-treated microsomes under the same conditions for up to 60 min.

Results

Gemfibrozil treatment greatly altered repaglinide clearance in rats (Figure 2; Table 1). Gemfibrozil-treated rats exhibited a 4-fold higher repaglinide C_{max} and a 3.5-fold greater $AUC_{0\text{-}12}$ than control rats, but the t_{max} (1.2 and 1.7 h) and $t_{1/2}$ (2.6 h) values were similar between treatment groups. The observed plasma clearance and volume of distribution were both approximately 60% lower in gemfibrozil-treated rats than control rats.

Figure 2. Mean plasma concentration time profiles in male Sprague-Dawley control and gemfibrozil-treated rats (n = 3/group) dosed once orally with 1 mg/kg repaglinide

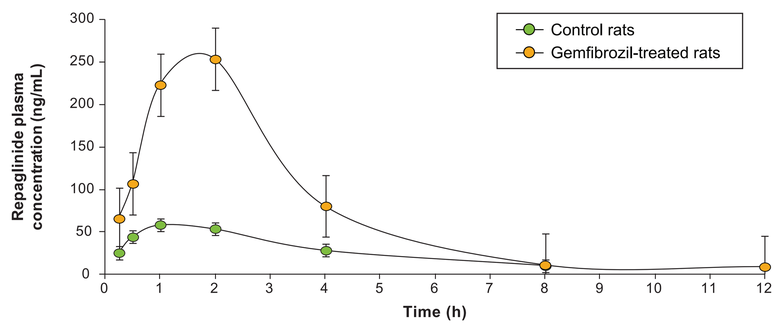
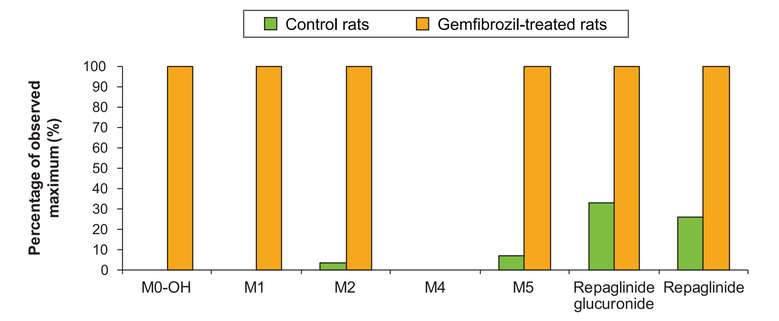


Table 1. Plasma pharmacokinetic parameters for male Sprague-Dawley control and gemfibrozil-treated rats (n = 3/group) dosed once orally with 1 mg/kg repaglinide

Parameter	Control rats	Gemfibrozil-treated rats	Percent change with gemfibrozil treatment
C_{max} (ng mL^{-1})	66.2 ± 6.9	284 ± 85	+329% (4.3 fold increase)
t_{max} (h)	1.2 ± 0.7	1.7 ± 0.6	No significant change
$AUC_{0\text{-}12}$ (ng h mL^{-1})	243 ± 33	854 ± 345	+251% (3.5 fold increase)
$AUC_{0\text{-}\infty}$ (ng h mL^{-1})	282 ± 44	838 ± 337	+197% (3.0 fold increase)
CL_{obs} (L h^{-1} kg^{-1})	3620 ± 590	1300 ± 523	-64%
VD_{obs} (L kg^{-1})	13200 ± 2480	5160 ± 3400	-61%
$t_{1/2}$ (h)	2.6 ± 0.6	2.6 ± 0.8	No significant change

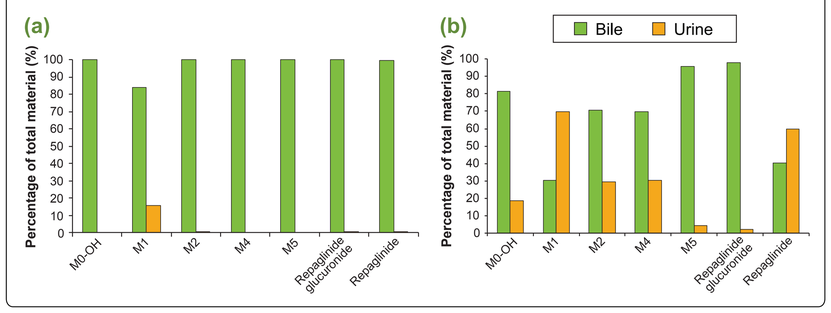
Pooled plasma samples were analyzed for repaglinide and repaglinide metabolites. Initially, data were processed to profile for repaglinide and selected metabolites of interest, specifically repaglinide glucuronide, M0-OH, M1, M2, M4 and M5 (Figure 3), although additional rat plasma metabolites were detected. Repaglinide, repaglinide glucuronide, M2 and M5 were observed in plasma from both control rats and gemfibrozil-treated rats. M0-OH and M1 were only observed in plasma from the gemfibrozil-treated rats. The hydroxylation metabolite M4 was not detected in plasma from either treatment group. Repaglinide and the selected metabolites observed were present at much higher abundance in plasma from gemfibrozil-treated rats than in plasma from the control rats.

Figure 3. Relative abundance of repaglinide and six metabolites of interest in pooled plasma from male Sprague-Dawley control and gemfibrozil-treated rats (n = 3/group) dosed once orally with 1 mg/kg repaglinide



In control rats, the vast majority of the repaglinide-related material was excreted in bile (Figure 4a). Metabolites M0-OH, M4 and M5 were solely detected in the bile, and only trace amounts of repaglinide, repaglinide glucuronide or metabolite M2 were present in the urine. Metabolite M1 was observed in both bile and urine, but the majority of M1 was observed in the bile. Following gemfibrozil treatment, the excretion profile for repaglinide and the monitored metabolites was markedly altered (Figure 4b). While repaglinide glucuronide and M5 were still almost completely eliminated in bile, more of both repaglinide and metabolite M1 was excreted in urine than in bile. Increased urinary excretion of repaglinide and all 6 selected repaglinide metabolites was observed in the gemfibrozil-treated rats compared to the control rats. The observations were consistent with inhibition of a hepatic transporter protein involved in uptake or efflux of repaglinide.

Figure 4. Biliary versus urinary excretion of repaglinide and six metabolites of interest in male Sprague-Dawley (a) control rats and (b) gemfibrozil-treated rats (n = 3/group) dosed once orally with 1 mg/kg repaglinide



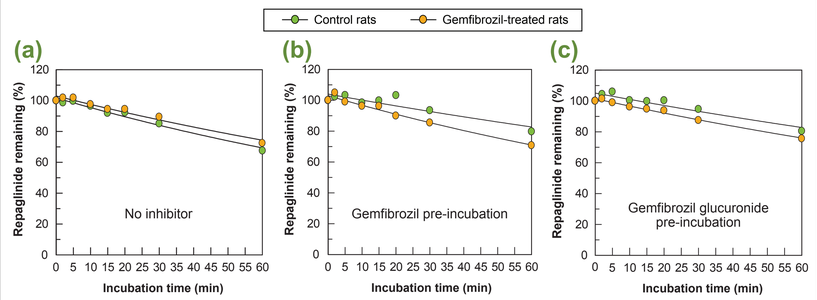
Determination of microsomal cytochrome P450 activity towards a panel of marker substrates revealed little difference between the gemfibrozil-treated and control rats (Table 2). No decreases in specific enzyme activities consistent with irreversible metabolism-dependent inhibition that could cause a 4-fold increase in plasma AUC were observed in liver microsomes from gemfibrozil-treated rats. The major difference between treatment groups was an increase in CYP4A1 activity in gemfibrozil-treated rats, most likely the result of the PPARα activation associated with gemfibrozil dosing.

Table 2. Enzyme activities in pooled liver microsomes from male Sprague-Dawley control and gemfibrozil-treated rats (n = 3/group) dosed once orally with 1 mg/kg repaglinide

Marker substrate	Marker metabolite	Human enzyme(s)	Rat enzyme(s)	Control rat liver microsome metabolite formation (pmol/mg protein/min)	Gemfibrozil-treated rat liver microsome metabolite formation (pmol/mg protein/min)
Testosterone	6β-Hydroxytestosterone	CYP3A4 CYP3A5	CYP3A2 CYP3A18	1320 ± 110	1070 ± 80
	2α-Hydroxytestosterone	NA	CYP2C11	459 ± 39	321 ± 40
	16β-Hydroxytestosterone	NA	CYP2B1	21.8 ± 3.8	54.9 ± 5.5
	7α-Hydroxytestosterone	NA	CYP2A1	170 ± 15	172 ± 15
Lauric acid	12-Hydroxylauric acid	CYP4A11	CYP4A1	1140 ± 20	2870 ± 120
Phenacetin	Acetaminophen	CYP1A2	CYP1A2	351 ± 13	350 ± 20
Chlorzoxazone	6-Hydroxychlorzoxazone	CYP2E1	CYP2E1 CYP1A2 CYP3A1	1020 ± 210	859 ± 177
Midazolam	1′-Hydroxymidazolam	CYP3A4 CYP3A5	CYP3A1 CYP3A2	240 ± 15	211 ± 1
Diclofenac	4′-Hydroxydiclofenac	CYP2C9	CYP2C6	481 ± 42	447 ± 31
Amodiaquine	*N*-Desethylamodiaquine	CYP2C8	NA	188 ± 14	173 ± 21
Dextromethorphan	Dextrorphan	CYP2D6	CYP2D2	409 ± 20	331 ± 12
Estradiol	Estradiol-3-β-D-glucuronide	UGT1A1	Unspecified	816 ± 19	992 ± 30

In vitro experiments with the control and gemfibrozil-treated rat liver microsomes revealed negligible repaglinide clearance differences in microsomes from control and gemfibrozil-treated rats (Figure 5). Even when a pre-incubation period with gemfibrozil or gemfibrozil glucuronide as potential cytochrome P450 or UGT inhibitors was incorporated into the experiment, little alteration in repaglinide clearance was observed in either treatment group. The results did not support a change in drug metabolizing enzyme activity or in microsomal clearance of repaglinide occurring as a direct result of gemfibrozil treatment in rats.

Figure 5. Repaglinide (0.2 µM) clearance in pooled liver microsomes (n = 3/group; 0.5 mg/mL) prepared from male Sprague-Dawley control and gemfibrozil-treated rats dosed once orally with 1 mg/kg repaglinide measured *in vitro* following 30 min pre-incubation with (a) no inhibitor, (b) gemfibrozil and (c) gemfibrozil glucuronide



Conclusions

- Gemfibrozil treatment altered the repaglinide pharmacokinetic profile in rats, significantly increasing repaglinide exposure.
- Gemfibrozil treatment resulted in a vectoral shift in repaglinide elimination pathways with increased urinary excretion of repaglinide and its metabolites.
- The drug metabolizing enzyme activity differences observed do not account for the repaglinide exposure or elimination alterations caused by gemfibrozil treatment.
- The PK data and altered excretion profile are consistent with hepatic transporter inhibition as the predominant cause of the gemfibrozil/repaglinide interaction in the rats.

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