# FURTHER EXPLORATION INTO THE DRUG-DRUG INTERACTION BETWEEN GEMFIBROZIL AND REPAGLINIDE IN RATS: UPTAKE TRANSPORT

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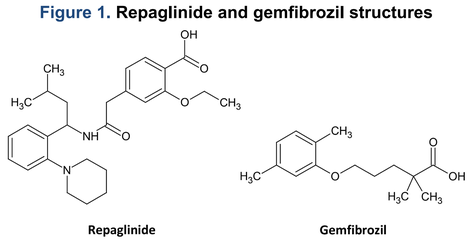
## INTRODUCTION

The clinically-relevant drug-drug interaction (DDI) between the dyslipidemia drug gemfibrozil and the insulin secretagogue repaglinide is well-documented throughout the literature. Coadministration of repaglinide with gemfibrozil causes a 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 and 2C8 and UGT1A1 and 1A3 (Bidstrup *et al.,* 2003; Säll *et al.,* 2012; **Figure 1** ). Gemfibrozil and its glucuronide metabolite inhibit CYP2C8 and UGT1A1 (Gan *et al.,* 2010), as well as the hepatic uptake transporter OATP1B1 (Kalliokoski *et al.,* 2008). These factors have been implicated in the clinical interaction.

**Figure 1. Repaglinide and gemfibrozil structures**

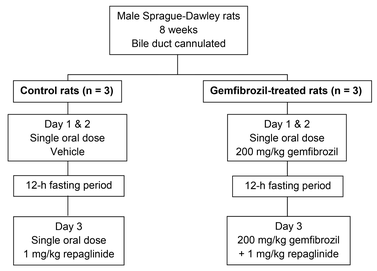



Recently, interest in animal models for DDI potential assessments has increased as utility has been demonstrated (Marathe and Rodrigues, 2010). A gemfibrozil / repaglinide DDI resulting in increased repaglinide and metabolite plasma exposure (>3-fold) and a vectoral shift in elimination pathways from biliary to urinary excretion following administration of gemfibrozil was previously established in male Sprague-Dawley rats (Barbara *et al.,* 2014). Rats were dosed according to **Scheme 1**. In that study, negligible effects on drug metabolizing liver enzyme activity were observed following gemfibrozil treatment, suggesting that the DDI in rats was unlikely to be caused by gemfibrozil altering drug metabolizing enzyme activity (e.g., by P450 or UGT inhibition). Repaglinide uptake in rat hepatocytes was recently characterized, with a derived active uptake clearance value of 299 µL/min/million cells, $K_m$ 2.98 µM, $V_{max}$ 804 pmol/min/million cells, and passive permeability clearance of 58.2 µL/min/million cells (Yabe *et al.,* 2011). In the present study, the potential role of hepatic uptake transporter proteins in the rat repaglinide / gemfibrozil interaction was explored *in vitro*.

## MATERIALS & METHODS

Experiments were performed to evaluate the effects of gemfibrozil and gemfibrozil glucuronide on repaglinide transport in sandwich-cultured cryopreserved rat primary hepatocytes.

**Scheme 1. Rat dosing regimen**




**Single dose experiment:** Hepatocytes were seeded on 24-well biocoat plates at approximately 1.2 million cells/mL. The hepatocytes were allowed to adapt for 48 h in culture with a Matrigel overlay. Cells were then treated with repaglinide (0.5, 5 or 50 µM) administered in the presence and absence of 100 µM gemfibrozil or gemfibrozil glucuronide. Repaglinide uptake was evaluated at 1, 3, 5, 10, 15 and 30 min.

**Pre-treatment experiment:** Hepatocytes were seeded on 24-well collagen-coated plates at approximately 1.2 million cells/mL. The hepatocytes were allowed to adapt for 48 h in culture with a Matrigel overlay, after which they were treated once daily for two consecutive days with vehicle control (0.1% DMSO) or 100 µM gemfibrozil or gemfibrozil glucuronide. On day 3, the hepatocytes were treated with repaglinide (0.5, 5 and 50 µM). The gemfibrozil-pretreated hepatocytes were treated with repaglinide coadministered with 100 µM gemfibrozil. The gemfibrozil glucuronide-pretreated hepatocytes were treated with repaglinide coadministered with 100 µM gemfibrozil glucuronide. Repaglinide uptake was evaluated at 3, 10, 30, 60 and 120 min.

At each time point, incubation media was removed, cells were washed once with 0.2% BSA in PBS, twice with PBS, lysed with organic solvent containing repaglinide ethyl-$d_5$ internal standard, and analyzed for repaglinide uptake by LC-MS/MS against a calibration curve (1 to 5000 nM).

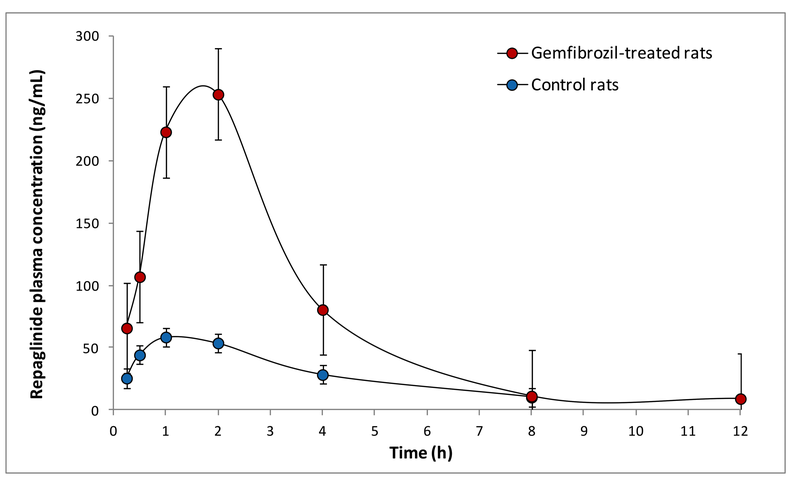
## RESULTS

In the previous study, marked changes in repaglinide AUC were observed in rats treated with gemfibrozil (**Figure 2**). 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 (**Table 1**). The observed plasma clearance and volume of distribution were both approximately 60% lower in gemfibrozil-treated rats than control rats (**Table 1**). Plasma levels of the selected metabolites M0-OH, M1, M2, M4, M5 and repaglinide acyl glucuronide were also greater in gemfibrozil-treated rats than control rats. Previously, inhibition of CYP or UGT enzymes was ruled out as a possible cause for the observed DDI using multiple experimental approaches (Barbara *et al.,* 2014).

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

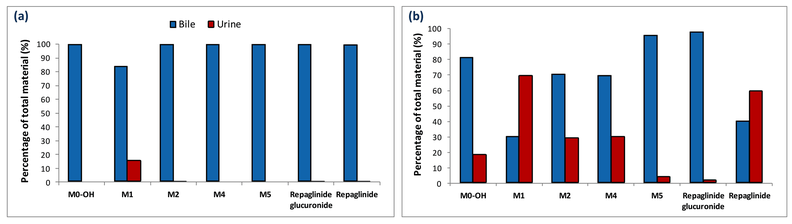
| Parameter | Control rats | Gemfibrozil-treated rats | % Change with gemfibrozil treatment |
|---|---|---|---|
| $C_{max}$ (ng $mL^{-1}$) | 66.2 ± 6.9 | 284 ± 85 | +329% (4.3 fold increase) |
| $t_{max}$ (h) | 1.2 ± 0.7 | 1.7 ± 0.6 | No significant change |
| $AUC_{0\text{-}12}$ (ng h $mL^{-1}$) | 243 ± 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 |

**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**




In control rats, the vast majority of the repaglinide-related material was excreted in bile (**Figure 3a**). The excretion profile for repaglinide and the monitored metabolites was markedly altered in the gemfibrozil-treated rats (**Figure 3b**). Following gemfibrozil treatment, more of both repaglinide and metabolite M1 was excreted in urine than in bile. Consistent with the control rat data, both repaglinide and M5 were still almost completely eliminated via biliary excretion. In contrast, approximately 20-30% of metabolites M0-OH, M2 and M4 were excreted in urine by the gemfibrozil-treated rats. Neither M0-OH nor M4 was detected in urine from the control rats.

**Figure 3. 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**

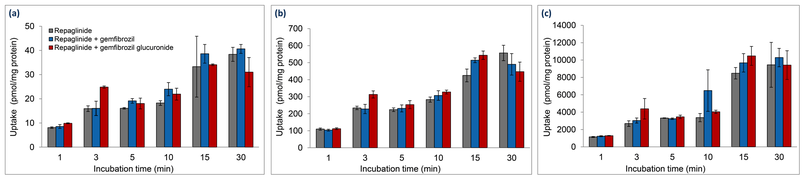



**Single dose experiment:** Repaglinide uptake into rat hepatocytes in the absence of gemfibrozil or gemfibrozil glucuronide was extensive, ranging from 1.9 to 9.1% at 0.5 µM, 2.6 to 13.1% at 5 µM, and 2.7 to 22.3 % at 50 µM, respectively, over 30 min incubation (**Table 2**; **Figure 4**). In general, repaglinide uptake into rat hepatocytes increased with both increasing concentration and time. At 50 µM repaglinide, uptake started to plateau at the longest 30 min time point. This may be indicative of saturation as it was not observed at either of the lower concentrations tested in the absence of gemfibrozil or its glucuronide.

**Table 2. Repaglinide uptake in rat hepatocytes treated once with repaglinide in the presence and absence of 100 µM gemfibrozil or 100 µM gemfibrozil glucuronide**

| Repaglinide concentration (µM) | Incubation time (min) | Uptake (pmol/mg protein) | | | Percent uptake | | |
|---|---|---|---|---|---|---|---|
| | | Repaglinide | Repaglinide + gemfibrozil | Repaglinide + gemfibrozil glucuronide | Repaglinide | Repaglinide + gemfibrozil | Repaglinide + gemfibrozil glucuronide |
| 0.5 | 1 | 8.08 ± 0.31 | 8.55 ± 0.79 | 9.85 ± 0.21 | 1.9 | 2.0 | 2.3 |
| | 3 | 15.9 ± 1.2 | 16.0 ± 3.0 | 24.8 ± 0.5 | 3.8 | 3.8 | 5.9 |
| | 5 | 16.1 ± 0.3 | 19.1 ± 1.0 | 18.0 ± 2.2 | 3.8 | 4.5 | 4.3 |
| | 10 | 18.3 ± 1.0 | 24.0 ± 2.7 | 21.9 ± 2.5 | 4.3 | 5.7 | 5.2 |
| | 15 | 33.3 ± 12.6 | 38.6 ± 3.9 | 34.0 ± 0.4 | 7.9 | 9.1 | 8.0 |
| | 30 | 38.4 ± 2.9 | 40.6 ± 1.9 | 31.0 ± 6.0 | 9.1 | 9.6 | 7.3 |
| 5.0 | 1 | 110 ± 6 | 103 ± 5 | 112 ± 6 | 2.6 | 2.4 | 2.6 |
| | 3 | 234 ± 10 | 228 ± 27 | 313 ± 21 | 5.5 | 5.4 | 7.4 |
| | 5 | 223 ± 11 | 231 ± 21 | 253 ± 24 | 5.3 | 5.4 | 6.0 |
| | 10 | 283 ± 15 | 307 ± 28 | 327 ± 11 | 6.7 | 7.2 | 7.7 |
| | 15 | 425 ± 38 | 514 ± 14 | 543 ± 25 | 10.0 | 12.1 | 12.8 |
| | 30 | 557 ± 45 | 490 ± 63 | 447 ± 57 | 13.1 | 11.6 | 10.5 |
| 50.0 | 1 | 1140 ± 50 | 1230 ± 60 | 1290 ± 30 | 2.7 | 2.9 | 3.0 |
| | 3 | 2680 ± 320 | 3040 ± 290 | 4390 ± 1180 | 6.3 | 7.2 | 10.4 |
| | 5 | 3320 ± 30 | 3240 ± 90 | 3460 ± 200 | 7.8 | 7.7 | 8.2 |
| | 10 | 3370 ± 470 | 6480 ± 2390 | 4040 ± 190 | 7.9 | 15.3 | 9.5 |
| | 15 | 8480 ± 660 | 9680 ± 1060 | 10500 ± 1100 | 20.0 | 22.8 | 24.7 |
| | 30 | 9450 ± 2590 | 10300 ± 1100 | 9420 ± 1660 | 22.3 | 24.3 | 22.2 |

**Figure 4. Repaglinide uptake in cryopreserved rat hepatocytes dosed once with (a) 0.5 µM (b) 5 µM or (c) 50 µM repaglinide in the presence and absence of 100 µM gemfibrozil or gemfibrozil glucuronide.**




Up to 15 min, the presence of gemfibrozil or gemfibrozil glucuronide had no effect on repaglinide uptake into rat hepatocytes, but effects were observed with both gemfibrozil (at 5 µM repaglinide) and gemfibrozil glucuronide (at 0.5 and 5 µM repaglinide) at 30 min incubation. In the presence of gemfibrozil, repaglinide uptake plateaued at 30 min at all three repaglinide concentrations. In the presence of gemfibrozil glucuronide, a decrease in repaglinide uptake was observed at 30 min at 0.5 and 5 µM repaglinide.

The observations noted in the coadministration experiment established that gemfibrozil and its glucuronide metabolite had a small impact on repaglinide uptake in rat hepatocytes after 30 min, but neither compound had any effect at the earlier timepoints. The observed results do not account for the 4-fold exposure increase or for the change in the vectoral elimination pathway observed in vivo. The results implicated a time-dependent effect. A long-lasting DDI between cyclosporine A and sulphobromophthalein observed in rats attributed to time-dependent oatp transporter inhibition has been previously reported in rats (Shitara *et al.,* 2011), so a longer term experiment was performed.

**Pretreatment experiment:** A similar experiment incorporating vehicle, gemfibrozil, or gemfibrozil glucuronide pretreatment for two days followed by administration of repaglinide alone or co-dosed with gemfibrozil or gemfibrozil glucuronide was performed. Repaglinide uptake in this three-day experiment was less extensive than that observed in the single day uptake experiment. Repaglinide uptake into rat hepatocytes in the absence of gemfibrozil or gemfibrozil glucuronide ranged from 0.8 to 2.3% at 0.5 µM, 1.5 to 2.8% at 5 µM, and 1.4 to 2.8% at 50 µM, respectively, over 120 min incubation (**Table 3**). Repaglinide uptake into rat hepatocytes initially increased with both increasing concentration and time, but saturation was observed at approximately 30 min (**Figure 5**).

**Figure 5. Repaglinide uptake in cryopreserved rat hepatocytes pretreated for 2 days with vehicle, 100 µM gemfibrozil or 100 µM gemfibrozil glucuronide and dosed once with (a) 0.5 µM (b) 5 µM or (c) 50 µM repaglinide in the presence and absence of gemfibrozil or gemfibrozil glucuronide.**

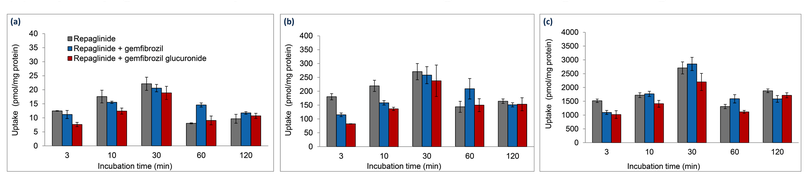


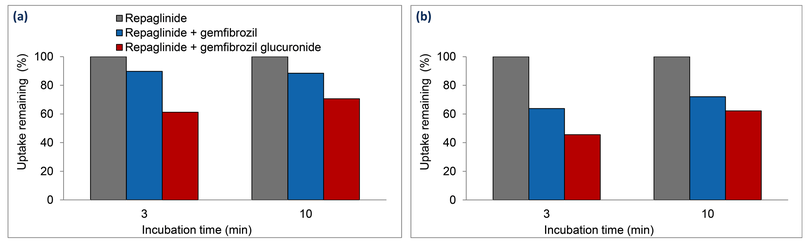

**Table 3. Repaglinide uptake in rat hepatocytes pretreated with vehicle, 100 µM gemfibrozil or 100 µM gemfibrozil glucuronide for two days and then treated with repaglinide in the presence and absence of 100 µM gemfibrozil or gemfibrozil glucuronide**

| Repaglinide concentration (µM) | Incubation time (min) | Uptake (pmol/mg protein) | | | Percent uptake | | |
|---|---|---|---|---|---|---|---|
| | | Repaglinide | Repaglinide + gemfibrozil | Repaglinide + gemfibrozil glucuronide | Repaglinide | Repaglinide + gemfibrozil | Repaglinide + gemfibrozil glucuronide |
| 0.5 | 3 | 12.5 ± 0.1 | 11.2 ± 1.4 | 7.63 ± 0.76 | 1.3 | 1.2 | 0.8 |
| | 10 | 17.6 ± 2.3 | 15.6 ± 0.4 | 12.4 ± 1.1 | 1.8 | 1.6 | 1.3 |
| | 30 | 22.1 ± 2.3 | 20.6 ± 1.3 | 18.9 ± 2.3 | 2.3 | 2.1 | 2.0 |
| | 60 | 8.04 ± 0.18 | 14.6 ± 0.7 | 9.07 ± 1.56 | 0.8 | 1.5 | 0.9 |
| | 120 | 9.65 ± 1.58 | 11.7 ± 0.5 | 10.7 ± 0.9 | 1.0 | 1.2 | 1.1 |
| 5.0 | 3 | 180 ± 11 | 115 ± 7 | 82.1 ± 0.7 | 1.9 | 1.2 | 0.9 |
| | 10 | 219 ± 20 | 158 ± 8 | 137 ± 6 | 2.3 | 1.6 | 1.4 |
| | 30 | 271 ± 29 | 258 ± 31 | 238 ± 57 | 2.8 | 2.7 | 2.5 |
| | 60 | 144 ± 20 | 209 ± 37 | 150 ± 23 | 1.5 | 2.2 | 1.6 |
| | 120 | 164 ± 8 | 151 ± 8 | 153 ± 23 | 1.7 | 1.6 | 1.6 |
| 50.0 | 3 | 1520 ± 60 | 1100 ± 70 | 1020 ± 140 | 1.6 | 1.1 | 1.1 |
| | 10 | 1720 ± 90 | 1770 ± 90 | 1410 ± 120 | 1.8 | 1.8 | 1.5 |
| | 30 | 2710 ± 220 | 2850 ± 240 | 2200 ± 310 | 2.8 | 3.0 | 2.3 |
| | 60 | 1310 ± 80 | 1590 ± 150 | 1120 ± 50 | 1.4 | 1.7 | 1.2 |
| | 120 | 1880 ± 70 | 1590 ± 120 | 1720 ± 90 | 2.0 | 1.6 | 1.8 |

Repaglinide uptake into rat hepatocytes occurs by both active transport and passive permeability. Active transport is the greater contributor at or below $K_m$, which has been reported as approximately 2.98 µM (Yabe *et al.*, 2011), so the 0.5 and 5 µM data were used to assess differences in repaglinide uptake in the pretreated hepatocytes. Repaglinide uptake at 3 and 10 min was compared for the vehicle control and treatment groups due to the saturation observed at longer timepoints.

Marked differences in repaglinide uptake were observed in rat hepatocytes following gemfibrozil and gemfibrozil glucuronide pretreatment (**Figure 6**). At 0.5 µM repaglinide, following 2 days of hepatocyte treatment with gemfibrozil, 10.2% and 11.5% repaglinide uptake inhibition at 3 and 10 min, respectively, was observed in rat hepatocytes co-dosed with repaglinide and gemfibrozil compared to vehicle-pretreated hepatocytes dosed with repaglinide alone. Similar gemfibrozil glucuronide pretreatment and co-dosing resulted in 38.8% repaglinide uptake inhibition at 3 min and 29.4% inhibition at 10 min. At 5 µM repaglinide, following 2 days of hepatocyte treatment with gemfibrozil, 36.2% and 27.9% repaglinide uptake inhibition at 3 and 10 min, respectively, was observed in rat hepatocytes co-dosed with repaglinide and gemfibrozil compared to vehicle-pretreated repaglinide-dosed hepatocytes. Gemfibrozil glucuronide pretreatment and co-dosing resulted in 54.4% repaglinide uptake inhibition at 3 min and 37.8% inhibition at 10 min.

**Figure 6. Repaglinide uptake inhibition in rat hepatocytes treated once with repaglinide in the presence and absence of 100 µM gemfibrozil or 100 µM gemfibrozil glucuronide after two days of pretreatment**




## CONCLUSIONS

- Coadministration of gemfibrozil and its glucuronide metabolite with repaglinide slightly reduced repaglinide uptake in rat hepatocytes after at least 30 min incubation.
- The effects observed with the single-dose experiment were minimal and disproportionate to the observed 4-fold repaglinide exposure increase and change in vectoral elimination pathway observed *in vivo*.
- Pretreatment of rat hepatocytes with gemfibrozil or gemfibrozil glucuronide for 48 h, along with coadministration, resulted in marked time-dependent repaglinide uptake inhibition.
- Gemfibrozil glucuronide pretreatment and coadministration caused more extensive repaglinide uptake inhibition than gemfibrozil pretreatment and coadministration.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. Brian Ogilvie for critical scientific review and discussion.

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