

**PHARMACEUTICALS AS EDCS - THE METABOLIC IMPACT
OF GEMFIBROZIL IN GOLDFISH**

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Pharmaceuticals are reported in the aquatic environment at concentrations that can exceed $\mu\text{g}\cdot\text{L}^{-1}$ post sewage treatment plant (Kolpin et al., 2002). Pharmaceuticals are designed to be bioactive, yet most of the literature available is limited to their occurrence rather than to their fate or effects on non-target organisms. This study was designed to test the bioactivity of a lipid regulator, gemfibrozil in the goldfish, *Carassius auratus*, a widely used model species in endocrine studies. This drug is reported in Canadian sewage treatment effluents at concentrations exceeding $2 \text{ ng}\cdot\text{L}^{-1}$ (Metcalf et al., 2002).

Gemfibrozil (GEM) is a lipid and cholesterol lowering fibrate drug that acts as a peroxisomal proliferator (PP), increasing cell peroxisome numbers and size through the activation of a nuclear receptor called the peroxisomal proliferator-activated receptor (PPAR) (Gonzalez et al., 1998). PPARs activate genes containing a PPAR responsive element, including those that code for many aspects of lipid catabolism (Kersten et al., 2000). Acyl-CoA oxidase (ACO), the first enzyme of the peroxisomal β -oxidation pathway, is one such enzyme that leads to hydrogen peroxide (H_2O_2) production and the potential for cellular oxidative stress. There are significant species differences in the sensitivities towards these fibrate drugs, with rodents hyper-responsive compared with humans. These differences may relate to the level of PPAR present (Gonzalez et al., 1998). Fibrate drugs induce peroxisomes in the trout less effectively than the rodent (Yang et al., 1990), but GEM does inhibit sex steroid production in the goldfish (G. Van Der Kraak, pers. commun.).

This study tests the hypothesis that GEM induces changes in lipid catabolism by acting as a PP in the goldfish. The objectives of this study are to examine the impact of GEM on 1) plasma lipid and carbohydrate levels, 2) tissue antioxidants and lipid peroxidation, and 3) induction of hepatic PPAR isoforms. Goldfish received intraperitoneal injections of dimethyl sulfoxide (DMSO; control) or 10 $\mu\text{g}\cdot\text{g}^{-1}$ (low dose, LDG) or 100 $\mu\text{g}\cdot\text{g}^{-1}$ (high dose, HDG; equivalent to a human therapeutic dose) GEM (in DMSO carrier) every other day for 8 days. Fish were sacrificed 48 h after the last dose, followed by blood sampling and tissue harvesting. Plasma metabolites, tissue enzymes and antioxidants were assayed using routine methods. PPAR α/γ cDNAs were cloned and sequenced based on other PPAR sequences available in GenBank.

Blood glucose levels significantly increased at both dose levels of GEM (DMSO, 0.4 ± 0.08 ; LDG, $0.8 \pm 0.06 \text{ mg}\cdot\text{mL}^{-1}$) while blood triacylglycerides decreased only in the HDG-treated group (DMSO, 3.3 ± 0.3 ; HDG, $1.9 \pm 0.24 \text{ mg}\cdot\text{mL}^{-1}$); no changes were noted in cholesterol or protein levels. Hepatic ACO activities increased ~ 60-fold when comparing the saline and LDG-treatments (0.014 ± 0.01 to $2.4 \pm 0.8 \text{ mU}\cdot\text{mg}^{-1}$ protein), while the HDG treatment was not significant. A number of tissue antioxidant enzymes were measured including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR). Only hepatic GPx significantly increased with HDG while all other enzymes were unaffected in the liver. Contrary to these stable values, hepatic total glutathione (GSH-eq) significantly increased at both GEM concentrations (DMSO, 0.2 ± 0.06 ; LDG, $1.6 \pm 0.35 \mu\text{mol}\cdot\text{g}^{-1}$) in the absence of changes in thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation. Thus, there was no indication of oxidative stress in the liver, as the antioxidants effectively overcame any change in H_2O_2 generated by the increased ACO activities. However, significant decreases in GPx, GST and GSH-eq were noted in the hearts of HDG-treated goldfish. Changes also occurred in interrenal tissue SOD activities.

Gemfibrozil increased PPAR α transcripts by 1.6-fold in the liver without changes in PPAR γ ; GEM is a known PPAR α ligand. In addition, serum cortisol values tended to decrease especially with HDG treatment. More interesting was the significant negative slope between cortisol level and body weight in the DMSO-treated goldfish and the positive slope for both the LDG- and HDG-treated fish. This dissociation between cortisol and body mass needs further study.

This study clearly indicates that GEM is bioactive in goldfish at the concentration used and that a low dose may be more effective than a high dose for some parameters tested. Apparent disruptions in both carbohydrate and lipid metabolism, and some imbalance in tissue oxidative status were observed. Whether this acute exposure is reflective of a more chronic impact of GEM is presently being investigated by using environmentally relevant exposures.

References

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