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Exposure to gemfibrozil and atorvastatin affects cholesterol metabolism and steroid production in zebrafish (*Danio rerio*)☆

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ABSTRACT

The commonly used lipid-lowering pharmaceuticals gemfibrozil (GEM) and atorvastatin (ATV) are detected in the aquatic environment; however, their potential effects on non-target fish species are yet to be fully understood. This study examined the effects of GEM and/or ATV on female and male adult zebrafish after a 30 d dietary exposure. The exposure led to changes in several biochemical parameters, including reduction in cholesterol, triglycerides, cortisol, testosterone, and estradiol. Changes in cholesterol and triglycerides were also associated with changes in transcript levels of key genes involved with cholesterol and lipid regulation, including SREBP2, HMGCR1, PPARα, and SREBP1. We also noted higher CYP3A65 and atrogin1 mRNA levels in drug-treated male fish. Sex differences were apparent in some of the examined parameters at both biochemical and molecular levels. This study supports these drugs affecting cholesterol metabolism and steroid production in adult zebrafish. We conclude that the reduction in cortisol may impair the ability of these fish to mount a suitable stress response, whereas the reduction of sex steroids may negatively affect reproduction.

1. Introduction

Atherosclerosis is one of the leading causes of cardiovascular mortality and morbidity in North America (Choy et al., 2000; Boden et al., 2007), and several lipid-lowering pharmaceuticals are used in its treatment. Fibrates are a class of lipid-lowering pharmaceuticals that activate peroxisome proliferator-activated receptors (PPARs), primarily PPARα (Fig. 1A). The other PPAR-types are PPARγ, which is involved in adipogenesis, and PPARβ, whose role in lipid regulation is not fully understood (Bishop-Bailey, 2000). Activation of PPARα alters the expression of genes that regulate lipid metabolism (Mandard et al., 2004), which ultimately reduces plasma triglyceride levels and increases cholesterol content in high density lipoproteins (HDL) (Prindiville et al., 2011). Notably, HDL has an important protective role against cardiovascular disease; its protection is attributed to several mechanisms, including reverse cholesterol transport, improved endothelial function, and inhibition of LDL oxidation (Assmann and Gotto, 2004). The prescription rates for fibrates continue to increase, with gemfibrozil (GEM) being amongst the more popular fibrates (Prindiville et al., 2011).

Statins are another class of lipid-lowering drugs. In contrast to fibrates, statins specifically inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase (HMGCR; E.C. 1.1.1.34), an enzyme that converts HMG CoA to mevalonate, a cholesterol precursor (Fig. 1A). Statins compete with HMG CoA for the active site of the enzyme, altering its conformation and inhibiting its function, thereby decreasing cholesterol synthesis (Blumenthal, 2000). Prescription rates for statins continue to increase with atorvastatin (ATV, also known as Lipitor) being amongst the most prescribed statins in Canada (Cavallucci, 2007).

It is noteworthy that statins have few known side effects, but cerivastatin (another statin drug) was voluntarily removed from the US market because of its association with an increased risk for rhabdomyolysis, or the destruction of muscle. Rhabdomyolysis due to cerivastatin was reported most frequently when used in high doses and particularly, when used in combination with GEM (SoRelle, 2001).

Both fibrates and statins are detected in the aquatic environment. It was previously reported that Canadian wastewater treatment plant (WWTP) effluents contained detectable levels of fibrates and statins. The concentrations of GEM in surface waters and WWTP effluents were 1500 ng/L and 2100 ng/L, respectively (Metcalfe et al., 2004). ATV was the most prevalent statin, reaching concentrations of 15 ng/L in surface waters and 44 ng/L in WWTP effluents (Metcalfe et al., 2004).

The presence of these pharmaceuticals in aquatic environments raises concerns regarding their effects on non-target species. This is

☆ Contribution to a special issue celebrating the work of Dr. Thomas W. Moon on the occasion of his retirement after 45 years in comparative biochemistry and physiology.

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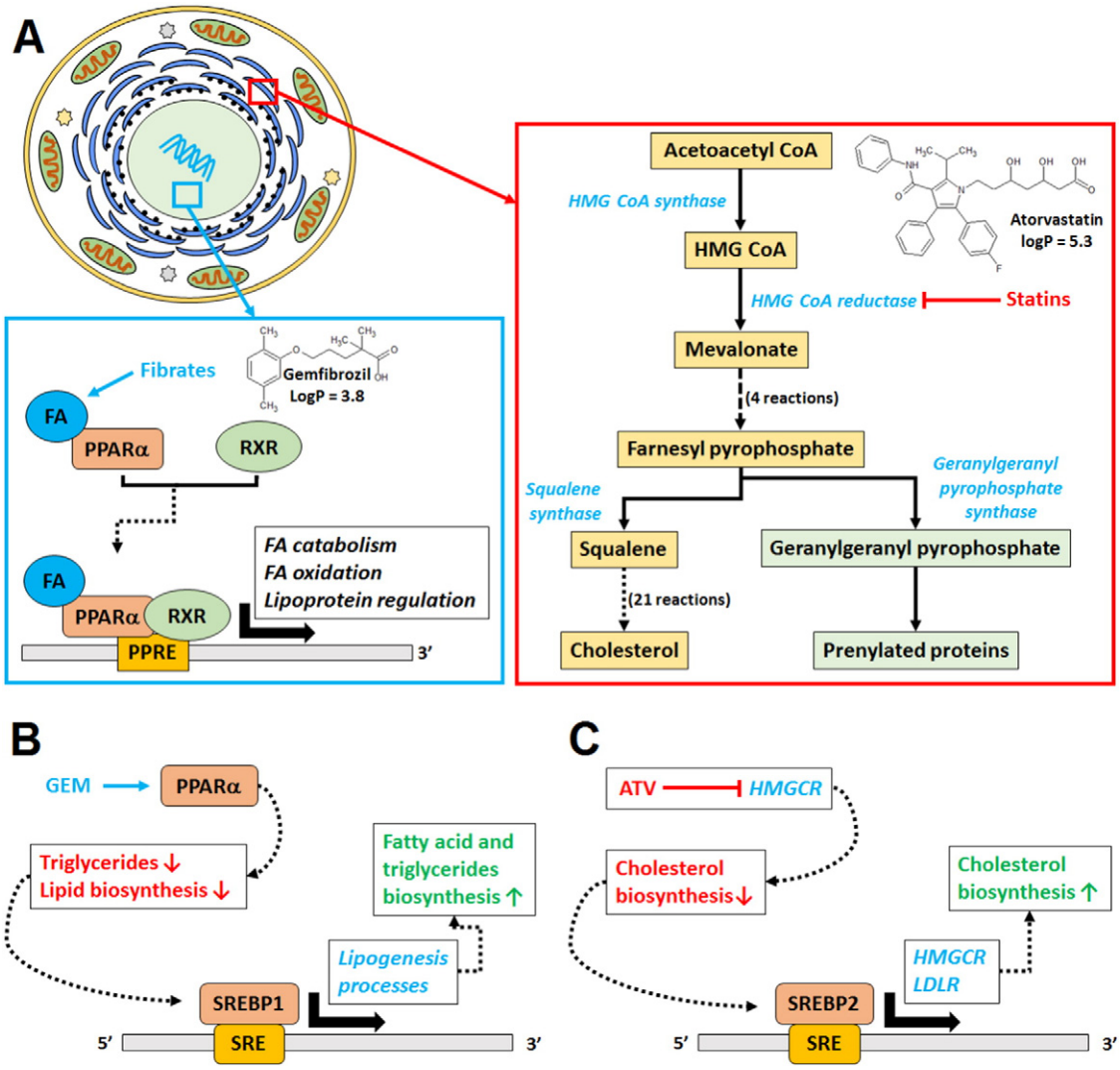

Fig. 1. (A) The modes of action of fibrate and statin drugs. Fibrates (e.g. GEM) mimic fatty acids (FA) and bind to PPARα, which then binds to retinoid X receptor (RXR) and together this heterodimer binds to peroxisome proliferator response elements (PPRE) in the promoter region of key genes involved in lipid metabolism; this occurs in the nucleus. Statins (e.g. ATV) compete with HMG CoA for the binding site on HMG CoA reductase thereby inhibiting its activity and reducing cholesterol synthesis; this occurs in endoplasmic reticulum. LogP values for GEM and ATV were calculated using Molinspiration.com online calculator. (B, C) The actions of GEM and ATV induce changes to counteract the decreasing concentrations of triglycerides and cholesterol: (B) GEM-driven reduction in triglycerides via PPARα activation increases the gene expression of sterol regulatory-element binding protein 1 (SREBP1) in order to facilitate lipid biogenesis. (C) ATV-driven reduction in cholesterol increases the gene expression of SREBP2 and its downstream targets – HMGCR and low-density lipoprotein receptor (LDLR) – in order to facilitate cholesterol biosynthesis. See text for further explanations.

especially relevant to teleost fishes where hypercholesterolemia is a normal condition in these vertebrates (Larsson and Fange, 1977). Fish and salmonids in particular, are susceptible to atherosclerotic lesions in coronary arteries, which are attributed not to hypercholesterolemia per se but to growth rate and sexual maturity (Saunders et al., 1992). In general, the plasma cholesterol concentration in most fish species is 2–6 times higher than that of mammals (Larsson and Fange, 1977; Babin and Vernier, 1989).

The toxicity of GEM has been addressed in a few studies. GEM exposure (5–28 d; 0.38 μg/L-15 mg/L) induced embryonic malabsorption syndrome in zebrafish larvae (Raldúa et al., 2008), led to genotoxic damage in adult zebrafish (Rocco et al., 2010), reduced plasma testosterone levels, decreased hepatic PPARβ mRNA levels, and induced several antioxidant defense enzymes in male goldfish (*Carassius auratus*) (Mimeault et al., 2005, 2006). In addition, GEM (2–21 d; 1.5–1500 μg/L) reduced plasma cholesterol levels, altered the abundance of genes involved with lipid metabolism and reduced fecundity, but did not appear to affect plasma triglycerides or sex steroid levels in fathead minnows (*Pimephales promelas*) (Skolness et al., 2012). Finally, GEM exposure (15 d; intraperitoneal injection of 100 mg/kg) modified plasma lipoprotein levels, size, and composition, increased lipoprotein lipase gene expression, but did not appear to activate PPAR pathways (Prindiville et al., 2011).

Several toxic effects of ATV are reported especially in zebrafish (*Danio rerio*) embryos. Exposure to ATV (24 h; ~6 mg/L) blocked primordial germ cell migration (Thorpe et al., 2004) and resulted in thicker yolk extension, kinked notochord, and midline unlooped hearts (D'Amico et al., 2007). In addition, ATV (48 h; 0.03–1 mg/L) affected blood vessel stability and induced hemorrhagic stroke (Gjini et al., 2011; Eisa-Beygi et al., 2013). Waterborne exposures to ATV (5–14 d; 0.2–10 μg/L) also induced genotoxic damage in adult zebrafish (Rocco et al., 2010) and upregulated the abundance of genes involved in membrane transport, oxidative stress response, and biotransformation in rainbow trout (*Oncorhynchus mykiss*) (Ellesat et al., 2012). Other statins are reported to induce the expression of atrogin1 (a biomarker of rhabdomyolysis) in skeletal muscle, and lead to sarcomere shortening, as reported for lovastatin exposures (12 h; ~0–4 mg/L) in zebrafish embryos (Hanai et al., 2007; Cao et al., 2009; Huang et al., 2011), and