A synthetic farnesoid X receptor (FXR) agonist promotes cholesterol lowering in models of dyslipidemia

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Evans MJ, Mahaney PE, Borges-Marcucci L, Lai K, Wang S, Krueger JA, Gardell SJ, Huard C, Martinez R, Vlasuk GP, Harnish DC. A synthetic farnesoid X receptor (FXR) agonist promotes cholesterol lowering in models of dyslipidemia. Am J Physiol Gastrointest Liver Physiol 296: G543–G552, 2009. First published January 8, 2009; doi:10.1152/ajpgi.90585.2008—The nuclear hormone receptor farnesoid X receptor (FXR) plays a critical role in the regulation of bile acid, triglyceride (TG), and cholesterol homeostasis. WAY-362450 (FXR-450/XL335) is a potent synthetic FXR agonist as characterized in luciferase reporter assays and in mediating FXR target gene regulation in primary human and immortalized mouse hepatocytes. In vivo, WAY-362450 dose dependently decreased serum TG levels after 7 days of oral dosing in western diet-fed low-density lipoprotein receptor−/− mice and in the diabetic mouse strains KK-Ay and db/db comparable to that achieved with the peroxisome proliferator activated receptor-α agonist, fenofibrate. WAY-362450 treatment also reduced serum cholesterol levels via reductions in LDLc, VLDLc, and HDLc lipoprotein fractions that were not accompanied by hepatic cholesterol accumulation. This cholesterol lowering was dependent on FXR as demonstrated in a hypothyroid-induced hypercholesterolemia setting in FXR−/− mice. In fructose-fed models, WAY-362450 also decreased TG and VLDLc levels in rats and hamsters but significantly increased HDLc levels in rats while reducing HDLc levels in hamsters. The differential effect of WAY-362450 on HDLC is likely due to a murine-specific induction of endothelial lipase and scavenger receptor-BI that does not occur in rats. These studies demonstrate a consistent ability of WAY-362450 to lower both serum TG and cholesterol levels and suggest that synthetic FXR agonists may have clinical utility in the treatment of mixed dyslipidemia.

triglyceride; very low-density lipoprotein; high-density lipoprotein; low-density lipoprotein

FARNESOID X RECEPTOR (FXR, also referred to as NR1H4) is a member of the nuclear receptor superfamily of ligand-regulated transcription factors. Bile acids, the end product of cholesterol catabolism, are the physiological ligands for FXR and act to stimulate FXR-dependent gene expression. FXR thus functions as a bile acid sensor that impacts the regulation of genes involved in cholesterol, triglyceride (TG), and bile acid production to maintain lipid homeostasis (18).

Studies in both humans and animals show that modulation of the bile acid pool through binding resins or bile acid supplementation can have profound effects on plasma TG and cholesterol levels. Treatment with the bile acid chenodeoxycholic acid (CDCA) to patients for the dissolution of gallstones resulted in a concomitant decrease in circulating TG levels and VLDL production (26). Conversely, reducing the bile acid pool with bile acid sequestrants or in patients that have a defect in bile acid absorption in the ileum results in increased TG levels (1, 10). Cell-based experimentation and observations in FXR−/− mice established the role of FXR in TG homeostasis through its modulation of both TG clearance and synthetic pathways. Bile acids and synthetic FXR ligands have been demonstrated to regulate apolipoprotein CII (apoCII) and apolipoprotein CIII (apoCIII) cofactors involved in lipoprotein lipase (LPL)-mediated lipolysis and downmodulate sterol regulatory element-binding protein 1c (SREBP-1c), the master regulator of the TG synthetic pathway (5, 15, 33).

Although the regulation of TG levels by FXR is well defined, its impact on cholesterol homeostasis is less clear. Activation of FXR leads to the downregulation of Cyp7a1, the rate-limiting enzyme in bile acid synthesis resulting in reduced cholesterol catabolism. In contrast, bile acid sequestrants promote Cyp7a1-mediated catabolism of cholesterol resulting in lowering of LDL levels. Therefore, it has been postulated that an FXR agonist may elevate serum cholesterol levels, whereas an FXR antagonist would lower serum cholesterol levels. In support of this, the putative FXR antagonist, gugglesterone, was shown to exert hypolipidemic effects in mice and rats (8, 32). However, serum cholesterol lowering using the synthetic FXR agonist GW4064 has been observed in two different diabetic mouse strains (db/db) (4, 14, 35). Although none of these studies investigated the mechanism for the cholesterol lowering, it may be due to the induction of genes involved in reverse cholesterol transport and/or cholesterol biliary clearance. FXR activation has been shown to increase the abundance of LDL receptor (LDLR) and scavenger receptor-BI (SR-BI) involved in cholesterol uptake (16, 17, 21) as well as ATB-binding cassette (ABC) transporters ABCG5 and ABCG8, hepatic transporters that promote biliary cholesterol secretion (24). Therefore, the impact of FXR on cholesterol regulation and its effect on the balance between cholesterol catabolism and cholesterol clearance remained undefined.

Studies to investigate the role of FXR on cholesterol homeostasis in vivo by GW4064 have been limited because of its poor oral bioavailability. In this report, we have used the novel, orally bioavailable FXR ligand, WAY-362450 (FXR-450/XL335) that was in-licensed from Exelixis (3), that is, amenable to in vivo experimentation to begin to address this question.

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Specifically, we wanted to determine the TG lowering potential of FXR agonists compared with the clinical standard of care, fenofibrate, and to define the impact of FXR agonists on cholesterol homeostasis. To accomplish this, the FXR agonist WAY-362450 was tested in mouse, rat, and hamster models of dyslipidemia. WAY-362450 treatment resulted in a consistent lowering of serum TG levels comparable to that achieved with fenofibrate. Although the two agonists affect similar pathways, they clearly targeted unique gene sets to achieve the TG lowering observed. In addition, WAY-362450 treatment also resulted in a consistent lowering of serum cholesterol levels in all species. Interestingly, the cholesterol lowering was achieved by having differential affects on the lipoprotein fractions between the different species. Overall, these results demonstrate that FXR agonists offer a potential strategy for treating patients with mixed dyslipidemia.

MATERIALS AND METHODS

Reagents. Cholic acid and fenofibrate were purchased from Sigma. Wyeth chemists synthesized WAY-362450 and GW4064.

FXR, one hybrid assay. Human embryonic kidney (HEK293) stable clones expressing Gal4/hFXR-LBD fusion protein in assay medium (Phenol red-free, high-glucose DMEM with 10% fetal bovine serum, 1 mM sodium pyruvate, 100 U/ml penicillin, 1% glutamates, and 100 μg/ml streptomycin) were plated at 10,000 cells per well in 96-well plates in 50 μl assay medium. The cells were incubated at 37°C for ~2 h, and then 50 μl of each compound was added to each well. Cells treated with compounds were incubated for 24 h at 37°C. On day 2, the medium was removed and lysis buffer added, and the plates were analyzed for luciferase activity with luciferase assay reagent (Promega E1483) and read on Victor® V instrument (Perkin Elmer) with the use of the luciferase assay protocol.

Cell culture experiments. Mouse AML12 cells (American Type Culture Center catalog no. CRL-2254) were plated at 200,000 cells/well on the 24-well plate in 1 ml of growth medium [DMEM/F12 10% FBS, 1% penicillin and streptomycin, 1% insulin-transferrin-selenium-G supplement (ITS) (GIBCO catalog no. 41400-045), 0.1% dexamethasone (40 ng/ml, Sigma catalog no. D-8893)/well]. The cells were treated with increasing concentrations of WAY-362450 or GW4064 for 24 h. Total RNA was prepared and analyzed by real-time RT-PCR, and short heterodimer partner (SHP) expression was normalized to GAPDH and reported as fold induction vs. vehicle-treated cells. Preplated 24-well plates of human male primary hepatocytes with matrigel overlay were obtained from Cellz Direct. Cells were maintained in serum-free Williams medium E and supplemented with penicillin/streptomycin, dexamethasone, ITS, I-glutamine, and HEPES buffer. They were treated overnight with vehicle (0.01% DMSO) or varying concentrations of WAY-362450 or GW4064.

Mouse dyslipidemia models. Eight-week-old LDLR−/−, KK-Ay, db/db, and human apoAI transgenic mice were purchased from Jackson Laboratories and maintained on a chow diet. Some LDLR−/− mice were fed a western diet (AIN-76A, Purina Test Diets) as indicated. All mice (6/group) were treated by daily oral gavage with vehicle (90% corn oil/10% ethanol) or varying concentrations of WAY-362450 or fenofibrate as indicated for 7 days. CA at a concentration of 0.5% or 0.01% ezetimibe was also mixed in the diet as indicated. On the last day after the final dose, the food was removed to allow a 3-h fast, and serum and liver was harvested for analysis. Serum TG and cholesterol levels were determined using a Roche 912 clinical chemistry analyzer and expressed as mg/dl. Serum VLDL, LDL, and HDL cholesterol were determined by FPLC as previously described (23).

Mouse hypothyroid-induced hypercholesterolemia model. Male and female C57BL/6 mice or C57BL/6 FXR−/− mice (Jackson Laboratories strain no. 004144) aged 8–10 wk were fed either a...
control diet (TestDiet no. 5001) or a diet low in iodine supplemented with 0.15% propylthiouracil (TestDiet no. 95125) for 3 wk (6 mice/group). Throughout the final week, the mice received a 0.1-ml daily gavage treatment with vehicle (90% corn oil/10% ethanol) or 30 mg/kg WAY-362450. Additionally, for the final week the mice received a 0.1-ml daily intraperitoneal treatment with PBS/0.005 N NaOH vehicle or vehicle supplemented with thyroid hormone T3 (Sigma) to deliver a 1-µg/g dose. After the final treatment, mice were fasted for 4 h and euthanized, and serum was collected by cardiac puncture. Thyroid hormone status and serum lipid levels were determined by analysis of samples using a Hitachi 911 Clinical Chemistry analyzer.

**Liver TG and cholesterol assay.** Total lipids were prepared from rat liver using a modification of the Folch Extraction method (10a). Briefly, ~200 mg of liver was homogenized in chloroform/methanol (2:1) and shaken overnight at room temperature. Lipids were back extracted with water, and the organic phase was collected and filtered through cheesecloth. A sample (0.5 ml) of organic extract with or without internal spikes (cholesterol or triolein) was evaporated to dryness using a nitrogen stream. For TG determinations, dried lipids were resuspended and sonicated in 2.5 ml of polymonomonochloroform neutrophil buffer [30 mM piperazine-N,N'-bis(2-ethanesulfonic acid), pH 7.0, 60 mM MgCl2, 0.1% NP-40]. Samples were incubated for 1 h with or without lipase (Pseudomonas sp), and glycerol formation was enzymatically measured using Free Glycerol Reagent (Sigma). TG concentrations were deduced from a glycerol standard curve. The cholesterol assay was performed using the Amplex Red Cholesterol Assay Kit (Invitrogen). Total and free cholesterol were measured according to kit instructions. Both TG and cholesterol values were normalized (to yield mg lipid/g liver mass) after being adjusted for internal spike recovery.

**Fructose-fed models.** Eight-week-old male Sprague Dawley rats and Syrian Golden hamsters were fed a 60% fructose/0.15% cholesterol diet (Purina Test Diets). For the rat study, the rats were placed onto the diet for 2 wk and then treated by daily oral gavage with vehicle (80% polyethylene glycol/20% Tween) or varying concentrations of WAY-362450 (8/group) as indicated for 7 days (8 rats/group). The hamster study was conducted as a preventative treatment, so both the diet and drug treatments were initiated on day 1 and continued for 21 days (8 hamsters/group). On the last day after the final dose, the food was removed to allow a 3-h fast, and serum and liver were harvested for analysis as described above.

**RESULTS**

WAY-362450 is a potent FXR agonist. Human HEK293 cells expressing a Gal4/FXR chimera containing the human FXR ligand binding domain were used to assess the in vitro activity of WAY-362450 (Fig. 1). WAY-362450 potently induced luciferase reporter expression with an EC_50 value of 16 nM, comparable to that observed with the reference standard, GW4064. WAY-362450 and GW4064 were both potent stimulators of endogenous FXR gene activation in mouse AML12 cells (Fig. 1C) and in primary human hepatocytes (Fig. 1D). WAY-362450 was selective for human FXR since no activity was observed when tested against a panel of the other human nuclear receptors [i.e., androgen receptor, constitutive androstane receptor, estrogen receptor-α, estrogen-related receptor-α, glucocorticoid receptor, liver X receptor-α, peroxisome proliferator-activated receptor (PPAR)-α, progesterone receptor, pregnane X receptor, retinoic acid receptor-αβγ, retinoid X receptor-αβ, thyroid hormone receptor-αβ, and vitamin D receptor; Supplemental Fig. S1]. Supplemental material for this article is available at the American Journal of Physiology Gastrointestinal and Liver Physiology website.

To confirm its activity in vivo was also dependent on FXR; male and female FXR−/− and wild-type (WT) mice were treated orally daily with 30 mg/kg of WAY-362450 for 7 days. Gene expression analysis of FXR target genes was determined in both the liver and ileum. As shown in Fig. 2A, the hepatic induction of both SHP and bile salt export pump was observed with WAY-362450 treatment in the WT mice but not in the FXR−/− mice. A similar pattern was observed with ileum expression of SHP and fibroblast growth factor 15 (Fig. 2B). Therefore, these data demonstrate that WAY-362450 activity in vivo is dependent on FXR.

WAY-362450 is active in murine models of dyslipidemia. To address the role of FXR on lipid metabolism, WAY-362450 was tested in different in vivo models of mixed dyslipidemia. As shown in Fig. 3A, feeding a western diet to LDLR−/− mice for 7 days increased both circulating TG and cholesterol levels compared with chow-fed controls. Supplementation of 0.5% CA to the western diet further enhanced serum cholesterol levels and had no effect on TG levels, whereas daily oral treatment of WAY-362450 resulted in a significant dose-dependent reduction in both serum TG and cholesterol levels at doses as low as 3 mg/kg. The reduction in serum cholesterol and TG was not accompanied by their accumulation in the liver, and, in fact, both hepatic TG and cholesterol levels were

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**Fig. 2.** WAY-36250 in vivo activity is FXR dependent. Male FXR−/− and wild-type (WT) mice were treated by daily oral gavage with vehicle or 30 mg/kg WAY-362450 for 7 days (6 mice/group). Three hours following the final dose, liver and ileum were harvested and RNA prepared. A: hepatic expression of SHP and BSEP were determined by real-time RT-PCR and normalized to GAPDH. B: ileum expression of SHP and fibroblast growth factor (FGF)15 were determined by real-time RT-PCR and normalized to GAPDH. *P < 0.01 vs. vehicle-treated controls. F, female; M, male.
also decreased by WAY-362450 (Fig. 3 B). As a comparator, the PPAR-α agonist fenofibrate when tested at a dose of 30 mg/kg lowered serum TG comparable to that achieved with 10 mg/kg WAY-362450 but showed reduced efficacy in regards to lowering serum cholesterol levels. However, fenofibrate treatment resulted in the hepatic accumulation of TG while lowering hepatic cholesterol levels.

WAY-362450 was also tested in two murine genetic models of diabetes, KK-Ay and db/db mice that exhibit hyperinsulinemia, hyperglycemia, and hyperlipidemia. As shown in Fig. 4, A and B, although there was a trend toward a reduction in serum glucose levels with WAY-362450, it did not reach significance in either model. Together, these studies demonstrate that FXR activation is associated with beneficial effects on both circulating TG and cholesterol levels in diabetic models of dyslipidemia and reveal a differential mechanism of lipid regulation compared with fenofibrate.

WAY-362450 regulation of lipoprotein fractions. To determine which lipoprotein fractions were being modulated by WAY-362450, FPLC analysis was conducted. As shown in

![Diagram](http://ajpgi.org/)

Fig. 3. WAY-362450 regulates hepatic and serum triglycerides (TG) and cholesterol metabolism in LDL receptor (LDLR)−/− mice. Male LDLR−/− mice were fed a chow or western diet (WD) and supplemented with 0.5% cholic acid (CA) or treated by daily oral gavage with vehicle, 1–30 mg/kg (mpk) WAY-362450 or 30 mg/kg of fenofibrate (6 mice/group) for 7 days as indicated. Three hours following the final dose, serum was prepared and liver was harvested. A: serum total TG and cholesterol levels were determined using a clinical analyzer. B: hepatic TG and cholesterol (Chol) levels were determined from the chow- and western diet-fed mice treated with vehicle, 30 mg/kg WAY-362450 and 30 mg/kg fenofibrate. All data are an average of 2 independent experiments with *P < 0.01 vs. western diet-fed control mice.

![Diagram](http://ajpgi.org/)

Fig. 4. WAY-362450 regulation of hepatic and serum TG and cholesterol in murine diabetes models. Male KK-Ay (A) or db/db mice (B) were fed a chow diet and supplemented with either 0.5% CA or treated by daily oral gavage with vehicle, 30 mg/kg WAY-362450, or 30 mg/kg fenofibrate (Feno) (6 mice/group) for 7 days as indicated. Three hours following the final dose, serum total TG, cholesterol, and glucose levels were determined. C: hepatic TG and cholesterol levels were determined in the KK-Ay mice. *P < 0.01 vs. chow-fed control mice.

![Diagram](http://ajpgi.org/)

Fig. 5. WAY-362450 regulation of hepatic and serum triglycerides (TG) and cholesterol metabolism in LDL receptor (LDLR)−/− mice. Male LDLR−/− mice were fed a chow or western diet (WD) and supplemented with 0.5% cholic acid (CA) or treated by daily oral gavage with vehicle, 1–30 mg/kg (mpk) WAY-362450 or 30 mg/kg of fenofibrate (6 mice/group) for 7 days as indicated. Three hours following the final dose, serum was prepared and liver was harvested. A: serum total TG and cholesterol levels were determined using a clinical analyzer. B: hepatic TG and cholesterol (Chol) levels were determined from the chow- and western diet-fed mice treated with vehicle, 30 mg/kg WAY-362450 and 30 mg/kg fenofibrate. All data are an average of 2 independent experiments with *P < 0.01 vs. western diet-fed control mice.

![Diagram](http://ajpgi.org/)

Fig. 6. WAY-362450 regulation of hepatic and serum TG and cholesterol in murine diabetes models. Male KK-Ay (A) or db/db mice (B) were fed a chow diet and supplemented with either 0.5% CA or treated by daily oral gavage with vehicle, 30 mg/kg WAY-362450, or 30 mg/kg fenofibrate (Feno) (6 mice/group) for 7 days as indicated. Three hours following the final dose, serum total TG, cholesterol, and glucose levels were determined. C: hepatic TG and cholesterol levels were determined in the KK-Ay mice. *P < 0.01 vs. chow-fed control mice.
Fig. 5, A and B, both the VLDLc and LDLc fractions were significantly induced by the western diet in LDLR−/− mice, and these inductions were inhibited by WAY-362450 treatment. Moreover, HDLc levels were also significantly decreased by 30% with WAY-362450. Similar data was also obtained when the LDLR−/− mice were fed a high-fructose diet (data not shown). In the KK-Ay mice on a chow diet (Fig. 5, C and D), cholesterol is carried predominantly in the HDL fraction, and WAY-362450 treatment significantly lowered HDLc levels by 45% after 7 days of treatment. In addition, a 60% reduction in VLDLc and a nonsignificant 30% reduction in LDLc were also observed. Therefore, in two different models of dyslipidemia, FXR activation results in a consistent reduction in lipoprotein cholesterol concentrations.

WAY-362450 lowers serum cholesterol in a FXR-dependent manner. To confirm that the cholesterol-lowering effects associated with WAY-362450 were indeed due to activation of FXR, FXR−/− and WT mice were placed on a hypothyroid diet.
diet for 14 days to induce hypercholesterolemia (27) followed by a 7-day treatment with 30 mg/kg WAY-362450 or 1 mg/kg T3 as a positive control. The effectiveness of the diet to induce hypothyroidism was confirmed by measurement of T4 serum levels (Fig. 6A), and this resulted in a dramatic induction in serum cholesterol levels in both the WT and FXR−/− mice that corresponded with an elevation in both the LDLc and HDLc fractions (Fig. 6, B–D). No regulation in either serum TG (Fig. 6E) or VLDLc (data not shown) was observed with the diet. Treatment with WAY-362450 resulted in a significant reduction in serum cholesterol, LDLc, and HDLc in the WT but not the FXR−/− mice, whereas T3 treatment lowered all three parameters in both WT and FXR−/− mice. These results verify that the cholesterol lowering observed with WAY-362450 is indeed dependent on FXR.

WAY-362450 lowers serum cholesterol and TG in fructose/cholesterol-fed models. To determine whether the lipid-lowering effects could be observed in other species, WAY-362450 was tested in a fructose/cholesterol-fed rat model. Male Sprague Dawley rats were placed onto a high fructose/cholesterol diet for 14 days, and then drug treatment commenced for 7 days. As shown in Fig. 7, A and B, feeding the diet for 21 days resulted in a significant increase in both TG and cholesterol serum levels. Increasing concentrations of WAY-362450 resulted in a dramatic dose-dependent suppression of TG with a more modest lowering of cholesterol. The elevation in cholesterol on the fructose/cholesterol diet was due solely to a sevenfold increase in the VLDLc fraction (Fig. 7, C–E). The reduction in total cholesterol by WAY-362450 was due to a dramatic reduction in VLDLc levels (60%) that was partially off set by a significant increase in HDLc (30%).

Since it was previously demonstrated that CDCA supplementation reduces serum TG and cholesterol levels in fructose-fed hamsters via reductions in VLDL and LDL levels (2), the impact of WAY-362450 in this species was tested. Male Syrian hamsters were placed onto a chow or the high fructose/cholesterol diet and treated with vehicle or 30 mg/kg WAY-362450 for 21 days. As shown in Fig. 8, A–D, the fructose/cholesterol diet resulted in a dramatic induction in both serum TG and cholesterol levels, resulting in the elevation of all lipoprotein fractions. Treatment with WAY-362450 significantly reduced the diet-mediated elevations in serum TG as well as all lipoprotein fractions.

Therefore, in these fructose/cholesterol-fed models, WAY-362450 treatment exerts a dramatic repression in serum TG and VLDLc levels; however, it demonstrates a differential effect on HDLc levels. In rats, WAY-362450 results in an elevation in HDLc levels, whereas in hamsters it causes a reduction similar to that observed in mice.

Differential effect of WAY-362450 on HDLc levels in mice and rats is not due to apoAI regulation. The differential effect of FXR agonists on HDLc levels in mice and rats has been observed previously. Treatment of KK-Ay mice with 0.5% CA for 1 wk resulted in a marked decrease in HDLc levels (33), whereas GW4064 administration to rats increased circulating HDLc levels (34). It was suggested that FXR may decrease
HDL levels in mice via its downmodulation of apoAI expression (6, 30). To explore whether this is the mechanism responsible for the decrease in HDLc observed with WAY-362450, studies were conducted using the human apoAI transgenic mouse. As shown in Fig. 9A, HDLc levels were significantly reduced by WAY-362450 in both female and male human apoAI transgenic mice. As a positive control for FXR-mediated gene regulation, the hepatic induction of SHP gene expression by WAY-362450 was confirmed (Fig. 9B); however, no regulation of either endogenous mouse apoAI or human apoAI gene expression was observed (Fig. 9, C and D). These results suggest that the modulation of HDLc observed with WAY-362450 is likely not due to the regulation of apoAI gene expression. apoAI gene expression was also not regulated in the LDLR−/− mice or fructose/cholesterol-fed rats; however, the hepatic expression of SR-BI and endothelial lipase were both selectively induced by WAY-362450 in mice but not rats (Fig. 10) and may account for the differential effects observed on HDLc with WAY-362450 treatment.

DISCUSSION

FXR has been implicated in the regulation of TG and cholesterol homeostasis (18). Initial evidence for a role of bile acids in TG regulation came from patients who were treated with CDCA as a means to dissolve their gallstones. In a large multicenter, double-blind clinical trial, CDCA treatment resulted in >30% reduction of serum TG in 57% of patients (26). The mechanism for this was not understood until the identification that bile acids are the physiological ligands of FXR (20, 22, 29). Since then, through the use of bile acids, synthetic FXR ligands, and genetically modified mice, the role of FXR...
in TG regulation has been elucidated. There is now a multitude of genes involved in TG clearance, synthesis, and secretion that have been identified to be regulated by FXR (18).

We demonstrate that the novel FXR ligand WAY-362450 potently lowers serum TG levels and VLDLc in multiple models of dyslipidemia. Its efficacy in mice was comparable to that observed with the clinical standard of care, fenofibrate; however, WAY-362450 regulated a distinct subset of genes. In mice, WAY-362450 and fenofibrate both regulated genes involved in LPL-mediated lipolysis. WAY-362450 specifically upregulated apoCII and apoAV expression, whereas fenofibrate selectively downregulated the negative LPL cofactor apoCIII while inducing LPL expression (supplemental Fig. S2). FXR has also been shown to play a role in lipogenesis and VLDL secretion, whereas PPAR-α activation is involved in β-oxidation. Treatment of KK-Ay mice or hamsters with either GW4064 or CDCA, respectively, resulted in the reduction of hepatic TG secretion (2, 33) that appears to occur through a SHP-dependent downregulation of both TG synthesis mediated by SREBP-1c inhibition and VLDL secretion via microsomal TG transfer protein-1 repression (12, 33). This finding is consistent with the lack of GW4064-mediated TG lowering in SHP−/− mice (33). Although the repression of microsomal TG transfer protein-1 at the transcriptional level by WAY-362450 treatment was not observed, WAY-362450 administration did have a pronounced effect on genes involved in lipogenesis. Although the downregulation of SREBP-1c was not apparent after 7 days of treatment, consistent with previous observations (33), a robust downregulation of its downstream genes such as stearoyl-CoA desaturase-1 was observed for WAY-362450 but not fenofibrate (supplemental Fig. S3). Therefore, both fenofibrate and WAY-362450 treatment results in dramatic TG lowering, and mechanism of action of WAY-362450 appears to be through modulation of genes involved in both lipolysis and lipogenesis.

It has also been reported that FXR agonists can improve insulin sensitivity and glucose regulation (4, 35) and is consistent with the impaired glucose tolerance observed in FXR−/− mice (19). A nonsignificant 20% reduction in serum glucose levels was observed in the KK-Ay and db/db mice after WAY-362450 treatment for 7 days. However, formal studies will need to be conducted to assess the ability of WAY-362450 to modulate glucose tolerance or insulin sensitivity.

One important unanswered question regarding FXR is its overall impact on cholesterol homeostasis. Arguments have been made to suggest that either a FXR antagonist or FXR agonist could exhibit cholesterol-lowering properties. In studies of patients with gallstones, lowering of circulating TG levels by CDCA treatment was concomitant with an increase in LDLc (26), leading to the development of bile acid sequestrants as a strategy to lower LDLc levels (10). The bile acid sequestrants deplete the endogenous bile acid pool by ~40%, thereby stimulating increased bile acid synthesis via Cyp7a1-mediated catabolism of cholesterol. Therefore, approaches that maintain Cyp7a1 activity, such as FXR antagonists, are thought to hold promise as LDLc-reducing agents. In support of this, the putative FXR antagonist gugglesterone was shown to lower hepatic cholesterol levels in mice while elevating serum HDL levels in rats (8, 32). However, a randomized clinical trial in hypercholesterolemic patients with gugglesterone failed to result in an improvement in the plasma lipid profile and even led to a modest increase in LDLc (31). More recently, some limited preclinical data has been generated showing that the synthetic FXR agonist GW4064 lowers serum cholesterol levels in the db/db and ob/ob diabetic mouse strains (4, 14, 35) while raising HDLc levels in rats (34); however, the implication of these observations and the mechanism behind them has not been addressed.

Fig. 9. Differential effect of WAY-362450 on HDLc is not due to apolipoprotein AI (apoAI) regulation. Male and female human apoAI (hapoAI) transgenic mice were treated by daily oral gavage with 30 mg/kg of WAY-362450 (6 mice/group) for 7 days. Three hours after the final dose, HDLc (A) was determined by FPLC, and hepatic expression of SHP (B), mouse apoAI (mapoAI) (C), and human apoAI (D) were determined by real-time RT-PCR and normalized to GAPDH. *P < 0.01 vs. vehicle.

Fig. 10. WAY-362450 regulation of hepatic gene expression. Hepatic expression of apoAI, scavenger receptor-BI (SR-BI), and endothelial lipase (EL) levels from male LDLR−/− mice as described in Fig. 2 and fruc/chol-fed rats as described in Fig. 7 were determined by real-time RT-PCR and normalized to GAPDH. *P < 0.01 vs. western diet-fed control mice or fructose-fed control rats.
Previous investigations demonstrated that FXR regulates genes involved in reverse cholesterol transport and lipoprotein metabolism (16, 25, 35). As investigated here, activation of FXR by WAY-362450 resulted in a consistent lowering of serum cholesterol levels in multiple rodent models. However, the impact on the various lipoprotein fractions varied depending on the model. In models in which serum TG levels were elevated by dietary manipulation or genetic predisposition, WAY-362450 treatment resulted in a robust reduction in VLDLc levels. WAY-362450 treatment also resulted in reductions in LDLc levels and could be attributable to the ability of FXR to induce LDLR levels via stabilization of its protein levels through PCSK9 regulation as shown in human hepatocytes (17). However, no regulation in either LDLR or PCSK9 gene regulation was observed in either the KK-Ay or db/db mice (supplemental Fig. S4) and was consistent with the variable effect of WAY-362450 on LDLc levels. LDLc lowering was observed in models in which its levels were induced by dietary means (i.e., western and highcholesterol diet) but not on its basal levels such as in the KK-Ay and rat model. Therefore, the reduction of LDLc observed after WAY-362450 treatment may be secondary attributable to reductions in VLDLc levels.

In the mouse studies, WAY-362450 treatment also resulted in a consistent reduction in HDLc levels. Previous studies demonstrated that the CA-mediated repression of HDLc in C57BL/6 and human apoAI transgenic mice was attributable to the transcriptional repression of apoAI (6, 30). Similar studies performed with WAY-362450 did not demonstrate a regulation of either mouse or human apoAI gene expression even though the repression in HDLc levels was observed. Therefore, additional genes regulated by WAY-362450 were sought to explain the HDL lowering. The induction of SR-BI by FXR was confirmed (16), and a novel FXR-induced gene, endothelial lipase (EL), was identified. Both genes were selectively induced by WAY-362450 in mice but not in rats. The reason for this species-specific regulation is under investigation, but the regulation in mice is FXR dependent as demonstrated in FXR−/− mice (16; Supplemental Fig. S5). Therefore, the increased hepatic uptake of cholesterol esters from HDL via SR-BI and enhanced HDL metabolism by EL may account for the reduction in HDL observed in both mouse models. The regulation of hamster EL by WAY-362450 could not be determined since the gene has yet to be cloned, and, consequently, further experimentation is required to confirm this hypothesis.

Since the induction of SR-BI was not accompanied by an increased hepatic accumulation of cholesterol, there may also be active clearance of cholesterol via FXR activation. In support of this, the gene expression of the hepatic cholesterol transporters ABCG5 and ABCG8 was induced by WAY-362450 (Supplemental Fig. S6). Although these inductions are likely not mediated directly by FXR (24), their induction by WAY-362450 could be reproduced in primary human hepatocytes, suggesting that this regulation is conserved across species (Supplemental Fig. S7). Cholesterol synthesis is also likely impacted by FXR through the induction of SHP and insig-2, which have been shown to act as downmodulators of 3-hydroxy-3-methylglutaryl-coenzyme A reductase expression (9, 13). Therefore, a model emerges in mice in which FXR stimulates the reverse cholesterol transport system through the enhanced peripheral uptake of cholesterol by the liver via SR-BI that is then shuttled for excretion via ABCG5/G8. Simultaneously, to counteract the lack of cholesterol catabolism via the suppression of Cyp7a1 through SHP, it, along with insig-2, prevents the build up of hepatic cholesterol by inhibiting endogenous cholesterol synthesis.

In summary, WAY-362450 treatment elicited dramatic reductions in circulating TG in multiple rodent models with efficacy comparable to the PPAR-α agonist fenofibrate. In addition, FXR agonism also resulted in a consistent lowering in circulating cholesterol levels in mice, rats, and hamsters achieved through differential affects on the lipoprotein fractions. Overall, these results suggest that an FXR agonist may have therapeutic utility in the treatment of mixed dyslipidemia.

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