

LXR Ligand Lowers LDL Cholesterol in Primates, is Lipid Neutral in Hamster, and Reduces Atherosclerosis in Mouse.

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Abbreviated title: WAY-252623 reduces plasma LDL cholesterol in primate.

Abbreviations: ABCA1, ATP binding cassette transporter A1; ALT, alanine aminotransferase; AST, aspartate aminotransferase; apoE, apolipoprotein E; cyp7a1, cholesterol 7 α hydroxylase; CETP, cholesteryl ester transfer protein; FAS, fatty acid synthase; FDR, false discovery rate, FXR, farnesoid X receptor; FGF19, fibroblast growth factor 19; LBD, ligand binding domain; INSIG, insulin induced gene 1; IVUS, intravascular ultrasound; LXR, liver X receptor; PLTP, phospholipid transfer protein; RCT, reverse cholesterol transport; SCD1, stearoyl CoA desaturase-1; SHP, small heterodimer partner; SREBP, sterol-response element binding protein; TG, triglyceride.

ABSTRACT

Liver X receptors (LXRs) are ligand-activated transcription factors that coordinate regulation of gene expression involved in several cellular functions but most notably cholesterol homeostasis encompassing cholesterol transport, catabolism and absorption. WAY-252623 (LXR-623), is a highly selective and orally bioavailable synthetic modulator of LXR, that demonstrated efficacy for reducing lesion progression in the murine LDLR^{-/-} atherosclerosis model with no associated increase in hepatic lipogenesis either in this model or Syrian hamsters. In non-human primates with normal lipid levels, WAY-252623 significantly reduced total (50-55%) and LDL-cholesterol (LDLc) (70-77%) in a time and dose dependent manner as well as increased expression of the target genes ABCA1/G1 in peripheral blood cells. Statistically significant decreases in LDLc noted as early as 7d reached a maximum by 28d and exceeded reductions observed for simvastatin alone (20 mg/kg). Transient increases in circulating triglycerides and liver enzymes reverted to baseline levels over the course of the study. Complementary microarray analysis of duodenum and liver gene expression revealed differential activation of LXR target genes and suggests no direct activation of hepatic lipogenesis. WAY-252623, displays a unique and favorable pharmacological profile suggesting synthetic LXR ligands with these characteristics may be suitable for evaluation in patients with atherosclerotic dyslipidemia.

Supplemental key words: cynomolgus monkey, LDLR^{-/-}, ABCA1, ABCG1, FGF-19

INTRODUCTION

Atherosclerosis is the major cause of cardiovascular disease and its incidence is on the rise due to its tight relationship to obesity and diabetes. Therapeutic interventions targeted at reducing elevated plasma low-density lipoprotein cholesterol (LDLc), the primary risk factor for development of atherosclerosis, do not eliminate cardiovascular risk particularly in several high-risk subpopulations. The statin class of drugs, achieve dramatic reductions in LDLc, yet reduce heart attack risk only 33% per 1.5 mmol/L reduction in LDL (1). As statins primarily limit disease progression through the inhibition of endogenous cholesterol synthesis, newer treatment modalities directed at reversing established atherosclerotic plaque are likely to provide additional benefit and can have important clinical implications for disease management. This is exemplified by the exploratory clinical studies targeting the enhancement of high density lipoprotein (2). In this study, intravenous infusion of apoA-1 milano facilitated coronary atherosclerosis lesion regression as measured by intravascular ultrasound (IVUS) in individuals with acute coronary syndrome suggesting that enhanced cholesterol efflux via the ABCA1 pathway may be a viable mechanistic strategy to provide incremental pharmaceutical benefit.

Liver X receptors are ligand-inducible transcriptional activators involved in the coordinate regulation of cholesterol and lipid metabolism and are attractive drug targets. Discovery of oxysterols, as potential endogenous ligands and subsequent identification of the first generation of non-steroidal agonists have provided insight into the role of LXR nuclear receptor signaling in different peripheral tissues and are the topic of several recent reviews (3, 4). The therapeutic potential of such ligands for the prevention of atherosclerosis was deduced from their roles in 1) mediating cholesterol efflux from macrophages via activation of ABCA1, ABCG1 and apoE, 2) promoting HDL cholesterol elimination via the reverse cholesterol transport (RCT) process and 3) modulation of cholesterol absorption through intestinal activation of ABCA1 or ABCG5/G8

cholesterol transporters. However, the therapeutic benefits afforded by these ligands extend beyond cholesterol homeostasis since LXR nuclear receptors also modulate chronic inflammatory processes of the arterial intima. Consistent with this phenotype, the literature reports indicate suppression of atherosclerosis in apoE^{-/-} & LDLR^{-/-} murine atherosclerosis models (5-8) and lesion regression (7, 9) upon ligand activation.

Unfavorable side effects of LXR stimulation include increases in circulating plasma triglycerides hepatic steatosis and the elevation of LDLc in species expressing cholesterol ester transport protein (CETP) such as nonhuman primates, which have negatively impacted progress in this area. Efforts to minimize side-effects through selective targeting of the β -isoform of LXR (LXR β) are based on the premise that LXR α is the predominant subtype expressed in liver responsible for observed increases in hepatic lipogenesis (10-12). Recent studies provide confirmation that selective LXR β activation reverses atherosclerosis in a murine model lacking apoE and LXR α (13). However, crystallography modeling initiatives aimed at the rapid identification of potent orally bioavailable agonists have been less useful for isolating subtype-selective ligands, as the LXR ligand-binding domain (LBD) sequence is highly conserved. Only a single conservative amino acid change (Ile 277(LXR β)/Val263(LXR α)) differentiates the ligand binding pocket for the two LXR subtypes (14). Several recent reports document antiatherosclerotic effects for novel, potent synthetic steroidal LXR agonists in LDLR^{-/-} mice (15, 16). While steroidal ligands minimize the hepatic lipogenesis there remain significant deficiencies that may limit this class of compounds as drugs including poor pharmacokinetic properties (15-17). Tissue selectivity with lesion activity has also been achieved for a nonsteroidal agonist (18), but there is no data on the effect of these compounds in higher species (15, 16, 18).

The design and preliminary characterization of a series of indazole-based LXR modulators having improved drug-like properties and efficacy in murine models of atherosclerotic lesion progression was reported recently (19). In this report, the lead compound from this series, WAY-252623 (LXR-623), a novel partial LXR agonist, is profiled more extensively in several animal models including a nonhuman primate. This unique LXR ligand exhibits an improved therapeutic index that diverges from that described previously for other LXR ligands in species expressing CETP, including hamster and cynomolgus monkeys (20, 21). A direct comparison of LXR-mediated effects with a statin cholesterol-lowering drug and combination therapy are also illustrated in the primate model.

METHODS

Compounds and Reagents

Compound, WAY-252623 2-(2-chloro-4-fluorobenzyl)-3-(4-fluorophenyl)-7-(trifluoromethyl)-2H-indazole was synthesized at Wyeth Research in Collegeville, PA (19). LXR reference ligands TO901317, GW3965, and WAY-254011 were prepared as described (17, 22) and the FXR ligand 3-(2,6-dichlorophenyl)-4-(3''-carboxy-2-chloro-stilben-4-yl) oxymethyl-5-isopropyl-isoxazole (23), GW4064) was synthesized at Wyeth. The HMG-CoA reductase inhibitor, simvastatin was purchased from Krebs Biochemical and Industries, LTD (Hyderabad, India).

Multiplex LXR α / β cofactor peptide interaction assay

Each unique fluorescently coded low capacity avidin modified Luminex bead (RADIX Biosolutions, Georgetown, TX) was incubated overnight with one of 50 biotinylated peptides (Anaspec, San Jose, CA) at 12.5 μ M (24, 25). The beads were washed twice with multiplex buffer (50mM TRIS pH 8.0, 50mM KCl) and incubated with 100 μ M D-biotin (Invitrogen, Carlsbad, CA) for 30 min at room temperature to block any remaining unbound avidin sites. Following two additional washes, the beads were pooled and resuspended in multiplex buffer to make a 2X concentrated stock containing approximately 40,000 of each unique bead per milliliter. The assay was performed for two hours at room temperature in 100 μ l volume consisting of multiplex buffer, 10nM GST LXR LBD protein (Invitrogen, Carlsbad, CA), 0.1% BSA, 2mM DTT, 0.8 μ g/ml anti-GST phycoerythrin antibody (Martek, Columbia, MD) and 1x peptide beads along with the test compounds. The plates were read on the Luminex instrument using standard settings with a minimum of 50 events quantified for each bead per well. Data were normalized by subtraction of the mean background fluorescence intensity (determined with vehicle alone) from the mean fluorescence intensity obtained with each compound treatment. Heat maps were generated in Spotfire DecisionSite 8.1.1 (TIBCO, Somerville, MA).

Mice and Diets for Lesion Study

All animal studies and experimental procedures were performed according to protocols approved by the Wyeth Institutional Animal Care and Use Committee (Collegeville, PA). 4-7 week old Male LDLR knockout mice on a C57BL/6 background were obtained from Jackson Laboratories (Bar Harbor, ME.). Mice were randomized by body weight and group housed 3 per cage in plastic cages with ad libitum access to water and diet (Purina Rodent Chow 5001). Animals were allowed to acclimate for 1 week under standard conditions with a 12:12-h light/dark cycle (lights on at 0600 h) with temperature controlled at $22\pm 2^{\circ}\text{C}$. Following acclimation for 1 week, animals were divided into groups (n=10 per group) and fed high fat, high cholesterol (HFHC) Purina diet #47431 (TestDiet, Richmond, IN) containing 1.25% cholesterol and 17% fat ad libitum with or without WAY-252623. Body weight and food intake were measured at regular intervals. The HFHC diet was supplemented with WAY-252623 in order to deliver a dose of 15 mg/kg/day or 50 mg/kg/day per animal (assuming 4 g chow/day for 20 g mouse). Following 8 weeks of treatment, animals were anesthetized with isoflurane. Blood was obtained and liver and duodenum samples collected and flash frozen in liquid nitrogen for gene expression analysis. The heart and aorta were perfused with 0.9% saline followed by 10% neutral buffered formalin prior to excision and placed in vials containing 10% neutral buffered formalin for analysis.

Quantification of lesions in the aorta

The thoracic aorta was collected from the aortic root to the diaphragm and adventitial tissue carefully removed. The aorta was opened longitudinally, pinned on a black wax surface and stained for lipid deposition with Oil Red-O. Images of the open luminal surface of the aorta were captured using a dissecting microscope (Nikon SMZ 800) equipped with a digital video camera (Nikon DXM 1200). Oil Red-O stained atherosclerotic plaque area was quantified and expressed as a percentage of total luminal surface area of the aorta (22).

Experimental protocol for Golden Syrian Hamster studies

Male Syrian Golden Hamsters, 5-6 weeks in age (90-100g, Harlan, Indiannapolis, IN) were randomized by body weight and housed 2 per cage in plastic cages with ad libitum access to water and diet (Purina Rodent Chow 5001) as described previously (19). Briefly, animals were allowed to acclimate for one week under standard conditions with a 12:12-h light/dark cycle (lights on at 0600 h) with temperature controlled at $22\pm 2^{\circ}\text{C}$. Animals were divided into treatment groups and compounds were administered by gavage. Following 7 days of treatment, animals were weighed, anesthetized with isoflurane and blood samples collected prior to sacrifice. Livers were collected and weighed at sacrifice.

Experimental protocol for Monkey Studies

Cynomolgus monkeys (old world) (non-naïve) supplied by Covance Research Products, Inc., (Alice, Texas 78333) were used for this study. The monkeys were housed individually in stainless steel cages (30"x23"x30"). In the first experiment, 12 monkeys were randomized into three groups (4 per treatment group; two males and two females, 6-8 kg, 6-10 yrs in age) and treated orally with vehicle or WAY-252623 at doses of 15 and 50 mg/kg/day once daily for 28 days. The diet given to these monkeys was Certified High Protein Diet (LabDiet #5048) and drinking water was available ad libitum via automatic watering system. The water source is United Water Company, Spring Valley, NY, and meets the water quality standards set by the New York State Department of Health and the US Environmental Protection Agency. Additionally, water quality is periodically monitored for microbial and chemical contaminants by BioResources. The vehicle used for the study contains 2% polysorbate 80, NF, 0.5% methylcellulose (4000cps), and purified (Type I water). WAY-252623 was prepared in this vehicle and doses were adjusted based on the most recent scheduled body weights. In second study, 16 male monkeys were randomized into 4 groups (4 per treatment group, 5-8 kg) received

vehicle, 20 mg/kg of simvastatin, 50 mg/kg of WAY-252623, or a combination of simvastatin and WAY-252623 (20 and 50 mg/kg doses respectively) dosed once daily for 28 days. In both studies, an initial blood sample was obtained the day prior to initiation of dosing (predose baseline), followed by postdose samples on study days 7, 14, 21, and 28 for analysis of clinical parameters. All animals were fed immediately following dosing. Treatment of animals was in accordance with standard procedures for veterinary care and in compliance with USDA and animal welfare guidelines.

Clinical Parameters and Lipoprotein Determinations

Whole blood was collected into serum separator tubes and serum was analyzed for total cholesterol, triglycerides, ALT and AST using enzymatic methods with commercially available kits (Roche Diagnostics, Indianapolis, IN) and a Roche Hitachi 911 clinical chemistry analyzer. Separation and quantitation of serum lipoproteins were performed using FPLC gel filtration chromatography. Briefly, 50 μ L of serum was diluted 1:1 with saline and filtered through a 0.45 μ m spin filter. 15 μ L of the diluted solution was injected onto Superose 12 HR 10/30 and Superose 6 HR 10/30 columns (Amersham Biosciences, Piscataway, N.J.) connected in series and eluted at a constant flow rate of 0.5 ml/min with 0.15M NaCl. The eluant was enzymatically treated with a commercially available cholesterol reagent (Roche Diagnostics) utilizing a post-column reactor system. The area under the curve of each peak representing VLDL, LDL and HDL was integrated using Waters Millennium software 4.0 (Waters Corp., Milford, MA) and each lipoprotein fraction was quantified by multiplying the total cholesterol value by the relative percent area of each respective chromatographic peak. Hepatic lipids, cholesterol and triglyceride concentrations in liver were determined by Analytics (Gaithersburg, MD) for primate studies or internally at Wyeth using methods previously described (19).

RNA isolation from whole blood and tissues

Peripheral blood was drawn in PAXgene tubes for RNA purification according to manufacturer's recommendations (Qiagen, Valencia, CA). Total cellular RNA was isolated from whole blood using the PAXgene Blood RNA kit (Qiagen, Valencia, CA). On-column DNase digestion was performed during RNA purification and DNase removed during subsequent wash steps. RNA concentrations were quantitated by RiboGreen assay, #R-11490 (Molecular Probes, Eugene, OR) for qRT-PCR analysis or with an Eppendorf BioPhotometer 6131 for Gene chip analysis. RNA quality was assessed using an Agilent BioAnalyzer with the RNA Nano-chip (Agilent, Santa Clara, CA).

Gene expression analysis

Quantitative measurement of primate ABCA1 and ABCG1 mRNA was performed by qRT-PCR on an ABI Prism 7700 Sequence detection system (Applied Biosystems, Foster City, CA). Whole blood RNA was assayed using Quantitect Reagents for one-step RT-PCR (Qiagen, Valencia, CA) and the standard curve approach. Primate specific primer and probe sets for ABCA1 and ABCG1 were designed with Primer Express Software (Applied Biosystems, Foster City, CA). The ABCG1 probe, FAM-CTGGTGACGAGAGGCTTCCTCAGTCC, for-5'-GGCAGAATTTAAACTGCAACACA-3' and rev-5'-GGTGCCTGGTACTAAGGAGCAA-3', primers were generated using Rhesus macaque nucleotide sequence (GenBank Accession BV209042). Human ABCA1 qRT-PCR reagents, reported previously (17) were used for ABCA1 quantitation following their validation using total RNA from cynomolgus monkey liver and results were normalized to human 18S rRNA (Applied Biosystems, Foster City, CA) following validation for monkey RNA. FGF19 probe-primer sets were designed from Rhesus macaque sequence for use following validation on monkey RNA. qRT-PCR was performed with human FGF19 sequences for cell studies: For-5'-GGGCCACTTGGAATCTGACAT-3', rev 5'-TCCGGTGACAAGCCCAAAT-3' and probe 6-FAM-TCTTCGCCCTGGAGACCGACA.

Microarray analysis of cynomolgus monkey liver and duodenal RNA

Each RNA sample was amplified and labeled using the Ovation Biotin Labeling and RNA Amplification System (NuGEN, San Carlos, CA). The labeled RNA was then used for microarray analysis using the GeneChip® Rhesus Macaque Genome array (Affymetrix, Santa Clara, CA). Expression profiling was performed on the GeneChips® as described previously (26). Statistical analysis was performed using the Genedata Expressionist software package (Basel, Switzerland). For each gene represented on the microarrays, hybridization signal intensities of probe sets representing that gene were measured for individual samples in each cohort group. An average signal intensity for that gene was then calculated for each cohort, and compared to the average signal values from other cohorts. Genes were judged to be changed significantly by treatment if the change in the mean hybridization signal intensity for the probe set(s) representing that gene were ≥ 2 fold higher or lower in the treatment group than in the control group, with a false discovery rate (FDR) < 0.05 as determined by the method of Benjamini and Hochberg (27).

Determination of plasma drug levels of WAY-252623

Plasma concentrations of WAY-252623 were determined by mass spectrometry using the HPLC system, Micromass Ultima (Advanced Chemistry Development, Inc., Ontario, Canada) with a Chromolith SpeedROD monolithic 20 μ l C18 column (EM Science, Gibbstown, NJ). Samples were extracted by protein precipitation and quantification performed by comparison to a standard curve with an internal standard.

Cell Culture and Treatment of Caco2 cells

Caco2 cells were obtained from ATCC and maintained according to its depository. Confluent cells were treated with vehicle (DMSO) or ligands as described in figure legends. RNA was isolated 24-48 hrs later using RNeasy (Qiagen, Valencia, CA) and qRT-PCR performed.

Statistical Analysis

Values are presented as means \pm standard error of the mean (SEM). Statistical significance was analyzed by one-way analysis of variance (ANOVA) with least significant difference (LSD) tests using SAS Statistical Analysis Software (Cary, NC). Repeated-measures analysis was performed for the primate studies to determine significance on the response parameters for each treatment group using SAS® (Cary, NC).

RESULTS

In vitro characterization of WAY-252623 as a partial agonist have been described recently (19). Briefly, WAY-252623 preferentially binds LXR β (IC₅₀=24 nM) vs LXR α (IC₅₀=179 nM) and demonstrates full-agonism for upregulating macrophage ABCA1 gene expression and cholesterol efflux. In contrast, WAY-252623 is less potent for inducing SREBP1c and associated triglyceride accumulation in liver cells when compared with more potent dual agonist ligands such as T0901317 or WAY-254011 (19). WAY-252623 exhibits selective properties when analyzed using co-activator recruitment analysis which models co-activator/co-repressor exchange associated with LXRE-bound LXR-RXR heterodimers induced upon ligand binding (Fig. 1). This approach can be useful in defining ligand-induced conformational differences not easily described by a simple separation in binding potency for LXR subtypes due to the high degree of homology between the two LBD sequences. The WAY-252623 fingerprint in multiplex co-recruitment analysis revealed reduced affinity for the LXR α subtype with little affinity for CBP1 and p300 peptides (Fig. 1). Its beta-selective peptide recruitment profile was comparable that of WAY-254011 which shares similar activity for activation of LXR α/β isoforms (Fig. 1B). This peptide-based approach for probing conformational subtleties has not yet evolved to predict pharmacologic outcomes, however, it may suggest a unique conformation consistent with the distinct pharmacology of WAY-252623 versus several other ligands and supports subtype-selectivity associated with reduced liability on the induction of triglyceride synthesis in hepatocytes.

WAY-252623 reduces lesion progression in murine model of atherosclerosis

WAY-252623, possesses favorable pharmacokinetic properties following oral administration that allowed *in vivo* evaluation in the LDLR $^{-/-}$ murine model of atherosclerosis. LDLR $^{-/-}$ mice were fed HFHC diets for 8 weeks and lesion development was measured in aortic arches by Oil Red O

staining in mice treated with 15 and 50 mg/kg doses of WAY-252623 or 10 mg/kg GW3965. Treatment with GW3965 or WAY-252623 (15 mg/kg) resulted in a significant reduction of atherosclerosis (28 and 37% for GW3965 and WAY-252623, respectively) (Fig. 2A). Similar observations were made in a repeat experiment in which two different doses of WAY-252623 were used (Fig. 2B). Compared to high fat fed controls, inclusion of WAY-252623 resulted in dose-dependent decreases (47 and 66%) in lesion progression for 15 and 50 mg/kg doses, respectively. In the same experiment, GW3965 treatment resulted in 41% reduction in lesion progression. Significantly, serum cholesterol, triglycerides and liver lipids were unchanged or decreased in samples collected at the end of the study II when aortic arches were removed for lesion analysis (Table 1).

WAY-252623 exhibits little lipogenic potential in Syrian Hamster.

The lipogenic potential of WAY-252623 was characterized in Syrian Golden hamsters, a species expressing CETP and possessing LDL metabolism more similar to man. Hamsters were dosed for 7 days with vehicle or 20, 60 and 120 mg/kg/day of WAY-252623 by oral gavage. Doses were selected to achieve plasma exposures of approximately 4-6 fold over the concentration (EC₅₀) for ABCA1 induction in CHOK1 cells (data not shown). As shown in Table 2, dose-dependent increases in plasma drug levels for WAY-252623 had no effect on serum cholesterol, triglyceride, hepatic enzymes (ALT and AST) or liver weights approximately 2 hrs following dosing. FPLC analysis confirmed that VLDL, LDL and HDL cholesterol fractions were unchanged in treated samples (data not shown). Hepatic lipids were measured in a separate study where hamsters dosed with 100 mg/kg WAY-252623 for 7 days achieved blood levels of 3598 ng/ml. Liver triglyceride and cholesterol concentrations were not increased in hamsters treated with WAY-252623 (supplemental Fig. 2). Thus, the LXR modulator, WAY-252623 displays neutral lipid effects in this CETP-expressing species.

Primate serum total cholesterol and LDL cholesterol are diminished by WAY-252623 treatment.

The findings for WAY-252623 warranted further preclinical assessment in the nonhuman primate, with the hope of extending the characterization of this compound in a species closer to humans. In the first of two independent studies, 12 cynomolgus monkeys were randomized as described in the experimental methods and treated orally with vehicle or WAY-252623 at doses of 15 and 50 mg/kg/day once daily for 28 days. The second study was designed to compare WAY-252623 (50 mg/kg) with the HMG-CoA reductase inhibitor simvastatin (20 mg/kg) alone and in combination. A significant reduction in serum total cholesterol was observed at weeks 2, 3, and 4 following an initial lag phase in Study I (Fig. 3A). The administration of WAY-252623 caused a time- and dose-dependent decrease in serum total cholesterol of 28-34 and 45-57% for the 15 and 50 mg/kg/day doses, respectively. In the second study, reductions were observed as early as 7d following the initiation of dosing. WAY-252623-mediated effects exceed those observed for simvastatin and the combination treatment provided no additional lipid lowering benefit over WAY-252623 monotherapy (Fig. 3B).

Decreases in LDLc followed a similar temporal pattern to total serum cholesterol (Fig. 4A-B). Maximal reductions in LDLc of 42 and 77% with 15 and 50 mg/kg/day treatments respectively were reached by the end of the study on day 28 (Fig. 4A). Significant LDLc reductions were observed for WAY-252623 and the combination treatment in the second study, while simvastatin at the dose tested (20 mg/kg) failed to produce significant LDLc lipid lowering.

Total HDL cholesterol (HDLc) concentrations were largely unaltered with two exceptions that reflect significant reductions as indicated (Fig. 4C-D). Insignificant trends in HDLc suggest there was significant intra-subject variability in this parameter and thus, it is hard to draw any firm conclusions for the significance of this observation. Moreover, as a consequence of the low

serum VLDL-cholesterol concentrations in non-human primates, chromatographic quantification was deemed unreliable for this lipoprotein subfraction and results were not reported.

Effects of WAY-252623 on plasma triglycerides, liver enzymes and hepatic lipid accumulation in primates.

Plasma triglyceride measurements were not consistently elevated over the course of either study following WAY-252623 administration (Fig 3C-D). In the first experiment, triglycerides were transiently increased on day 7 in the 15 mg/kg/day treatment group after which concentrations decreased towards vehicle control levels (Fig. 3C). There was however, no significant difference between animals receiving the vehicle and the higher dose of WAY-252623. In the second study, a transient elevation in triglycerides at 7 days in combination treatment group did not persist, resolving potentially as homeostasis was achieved. Serum concentrations of the liver enzymes, alanine amino transferase (ALT) and aspartate amino transferase (AST), markers of hepatic toxicity and/or injury, were also relatively unchanged over the course of the study compared to baseline. As shown in the supplemental data (Fig. 2), the 50 mg/kg- dose of WAY-252623 increased serum ALT and AST on day 7 after which both concentrations decreased towards pre dose levels by day 28 (Fig. 2 A, C Supplemental Data). In the second study, the WAY-252623 + simvastatin (combination) treatment increased serum ALT on day 14, after which levels decreased to control concentrations by day 28 (Fig. 2 B, D Supplemental Data). There were no significant changes in AST enzyme levels.

Since the second study was a terminal study, liver tissues were obtained from this study for quantification of hepatic cholesterol and triglycerides. Measurements of lipid levels indicated accumulation of cholesterol (2.5-fold) and triglycerides (5-fold) in WAY-252623 treatment group in contrast to the simvastatin treatment group where there was no increase in hepatic lipid levels suggesting a specific effect of WAY-252623 (Table 3). It should be noted that no lipid

accumulation was observed at a lower dose of 15 mg/kg in a subsequent study where liver biopsies were analyzed (Supplemental, Fig. 3).

WAY-252623 induces ABCA1 and ABCG1 gene expression in primate peripheral blood cells.

To establish LXR target engagement, transcript levels for LXR target genes, ABCA1 and ABCG1, were measured in peripheral blood samples obtained from the non-human primate studies. There was a dose-dependent increase in ABCG1 expression starting on day 7 that was sustained throughout the length of each study (Fig. 5A and B, left panels). More variable results were displayed for ABCA1, potentially due to the smaller magnitude of ABCA1 induction (2-3 fold). Significant increases in ABCA1 mRNA observed at 7 and 14d time-points returned to control levels later in the first study (Fig 5A, right panel). Simvastatin by itself did not have any effect on ABCG1 or ABCA1 expression (Fig. 5B). The gene expression profile for combination treatment was very similar to that obtained for WAY-252623 alone indicating a compound specific effect on LXR-mediated target gene transcription.

WAY-252623 displays tissue-selective effects in primate liver vs. duodenum.

RNA profiling was utilized to gain a mechanistic understanding of *in vivo* gene expression changes in monkey liver vs. duodenum that might differentiate the observed pharmacology of WAY-252623 from other LXR ligands in this species. Microarray results reflect a subset of genes selected with relevance to LXR activation including those related to hepatic lipogenesis or the observed reduction in LDLc following WAY-252623 exposure to confirm the responsiveness of the tissues. Relative abundance is displayed graphically to illustrate tissue-specific expression differences.

Global gene expression changes in the duodenum revealed the induction of a number of well-defined LXR target genes associated with RCT and lipid metabolism following treatment with WAY-252623 (Fig. 6). ABCG1 increased 10-fold whereas the magnitude of ABCA1 stimulation was more moderate, in the range of 3.1-3.9 fold for WAY-252623 treatment alone or in combination with simvastatin. Modest effects on intestinal SREBF1 or its target genes, INSIG, LDLR, PCSK9, and SCD suggest the lipogenic axis is intact in this tissue. By contrast in liver, no obvious LXR target genes were stimulated by WAY-252623. In addition, WAY-252623 had no PXR or CAR crossreactivity that might explain the induction of CYP3A7. As reported, it shows >40-fold less potent agonist activity for PXR than LXR β (19) and in corecruitment crossreactivity screens for a panel of nuclear receptors, WAY-252623 had no agonist or antagonist activity for CAR up to 50 mM. CYP3A7 is not a known target gene of LXR. Divergent repression of lipogenic gene transcripts FAS, LDLR, PCSK9, SREBF, and SCD however did associate with hepatic lipid accumulation observed at the 50 mg/kg dose of WAY-252623 (Fig. 6 and Table 3). Simvastatin at the dose tested induced few changes in either tissue with the exception of a decreased the relative abundance of the LDL receptor (40%) in the gut whereas hepatic HMG-CoA reductase inhibition paralleled reductions in hepatic SCD, SREBF and ABCG1 mRNA in livers from the same animals. Expression profiling confirms LXR activation in the duodenum where the regulation of ABC cholesterol transporters correlates with the induction of these biomarkers in peripheral blood.

LXR-mediate transcriptional activation of FGF19 in primates.

The largest magnitude of change observed in the duodenum was for the fibroblast growth factor 19 (FGF19) gene, induced 43-fold by WAY-252623 treatment alone and 14-fold by the combination of WAY-252623 and simvastatin (Fig. 6). In contrast, FGF19 expression levels were unchanged in liver by either WAY-252623 or simvastatin treatment. Quantitative analysis confirmed the large magnitude of duodenum FGF19 activation in the duodenum (Fig. 7A). In

humans, FGF19 production by the intestine is strongly induced by bile acids (BA) (28) through a farnesoid X receptor (FXR) mediated pathway (29). However, WAY-252623 has no significant cross-reactivity for human FXR in nuclear receptor screens (19). Moreover, the expression of the canonical FXR target gene, small heterodimer partner (SHP), was not induced in the duodenum of monkeys treated with WAY-252623 (data not shown). These results suggest FGF19 induction mediated by WAY-252623 is not likely due to increased BA biosynthesis or flux resulting in FXR activation.

To verify that FGF19 is a direct target of LXR, human Caco2 intestinal cells were treated with four structurally diverse LXR ligands (T0901317, GW3965, WAY-252623, and WAY-254011) or the FXR agonist GW4064. T0901317, GW3965 and WAY-254011 significantly induced FGF19 expression (Figure 7B,C), as seen for WAY-252623 in the monkey duodenum. Further, the EC50 for induction of ABCA1 and FGF19 expression were nearly equivalent for either WAY-252623 (445 nM) or WAY-254011 (23 nM, Figure 7C). Finally, numerous published studies and our own data (not shown) have not identified the mouse FGF15 gene (the homolog of the human FGF19 gene) as a target gene for LXR regulation.

A genomics search for potential LXR transcription factor binding sites (LXREs) was performed for FGF19 homologues of all three species spanning the regions including 5kb upstream and 5kb downstream of each gene. There are 22 predicted LXR response elements within the human FGF19 gene locus, 19 were identified for the rhesus monkey, and 20 for the mouse. Of these, only a subset of six were conserved in both sequence and position as defined by our search criteria and all six identities were primate-specific between monkey and man, with no similar identities found in mouse.

DISCUSSION

The present report describes the pharmacological characterization of WAY-252623 which had been previously described (19) focusing on the unique biologic response profile in nonhuman primates where total cholesterol and LDLc were reduced by 50-55% and more than 70%, respectively. These findings for WAY-252623 differ from those previously published data for GW3965 and SB742881 (20) and two structurally distinct LXR ligands (ie., WAY-254011 or WYE-061) that were previously characterized in this species (21). An important inference from these results is that the previously described elevations of LDLc in non-human primates implied a class effect of LXR ligands on LDLc levels that may not be accurate (20).

Furthermore in these studies, functional efficacy as denoted by the activation of LXR target gene expression (i.e., ABCG1, ABCA1) in peripheral blood monocyte/macrophages and duodenum was achieved without increasing steady state HDL cholesterol levels. The upregulation of these important cholesterol transporters is requisite for effective reverse cholesterol transport that mobilizes cholesterol removal from peripheral tissues, including macrophage foam-cell rich lesion sites back to the liver for eventual elimination. The findings in non-human primates are consistent with previous studies suggesting the contribution of macrophage ABCA1 expression to circulating plasma HDL levels as minimal (30) as well as showing macrophage specific ABCA1 expression mediates antiatherosclerotic effects in LDLR^{-/-} mice in the absence of significant plasma lipid changes (31, 32). Furthermore, although intestinal-specific induction of ABCA1 expression may raise circulating plasma HDL in mice (33), the available data, in species expressing CETP (hamster and primates) suggests reduced effects on steady state HDL levels due to the enhanced return of HDL cholesterol through VLDL/LDL via CETP (20). In the current study, reductions in HDL likely reflect efficient HDL cholesterol transfer to LDL for clearance by

the liver via the RCT process however additional studies are needed to delineate the mechanisms behind the unique lipoprotein changes.

A functional consequence of ABCA1/G1 induction by WAY-252623 appears to have been the dose-dependent reduction in lesion progression in LDLR^{-/-} murine model of atherosclerosis. Antiatherosclerotic effects in this established model have been described for several other LXR ligands (5, 6, 8) and were achieved for WAY-252623 without stimulation of hepatosteatosis or hypertriglyceridemia (Table 1). LXR-mediated effects on lesion progression were observed in the absence of plasma cholesterol lowering and in this respect the mouse model fails to mimic or predict the lipid-lowering observed in primates. It is interesting to note that statins are efficacious in lowering cholesterol in man but do not necessarily do so in apoE^{-/-} and LDLR^{-/-} mice (34). For this reason, the monkey findings may be more predictive of LDLc outcomes in man.

This report represents the first description of combination statin/LXR agonist therapy in a higher species. Simvastatin did not stimulate ABCA1 or G1 gene expression in peripheral macrophages (Fig. 5B), despite a recent report suggesting that HMG-CoA reductase inhibitors (atorvastatin and simvastatin) induce ABCA1 and CE in human THP-1 macrophages (35). Furthermore, it was not as effective at lowering LDL cholesterol and seemed to provide little advantage over WAY-252623 alone. Statin drugs block the production of apo-B containing lipoproteins via inhibition of cholesterol and lipid biosynthesis. Therefore the combination of statins and LXR agonists might be useful in atheroprotection. Mechanistically statins reduce LDL cholesterol and decrease cholesterol influx into plaque but may not directly enhance cholesterol removal from arterial walls. LXR agonists may offer a pathway for regression of established lesions and advantages for prevention and treatment of atherosclerosis in patients with isolated low HDL or those that do not respond adequately to statin treatment. In the current studies, WAY-252623 and simvastatin combination treatment did not appear to compromise animals and literature exists suggesting that

LXR agonists may actually prevent some adverse statin effects associated with intracellular cholesterol balance and depletion of oxysterol pools (36).

The impact of WAY-252623 on lipogenesis and triglycerides was explored in several species. The findings for WAY-252623 and the close structural analog reported earlier (19) suggests a reduced triglyceride liability in hamsters for these partial agonist indazoles relative to more potent dual agonist ligands (20). GW3965 (30 mg/kg), in contrast to WAY-252623 (Table 2), induced significant hyper-triglyceridemia and -cholesterolemia in the hamster model. In the reported literature, plasma triglycerides, VLDL-c and LDL-c were increased 230, 270, and 180% respectively relative to control hamsters. GW3965 surprisingly had no effect on liver triglyceride content whereas, SB742881, a close structural analog increased liver triglycerides at 10 and 30 mg/kg doses, the latter in distinct contrast to WAY-252623 (supplemental Fig. 1).

Although the evidence for WAY-252623 administration in primates documents a possible trend toward increased plasma triglycerides and liver enzymes at early timepoints, these transient effects resolved with time. Increased plasma triglyceride does not appear to be a significant issue in this species (20) in accordance with an earlier report for GW3965 and SB742881. This may reflect species-specific differences in VLDL production or catabolism, however, liver lipid accumulation was not assessed in earlier non-terminal monkey studies (20). In the present NHP studies, increases in hepatic cholesterol and triglyceride observed in WAY-252623-treated animals (Table 3) associate with hepatic downregulation of several lipogenic genes: FAS, LDLR, INSIG1 and SREBF1, consistent with the inhibition of SREBP proteolysis. In fact, a number of well-defined LXR target genes associated with RCT or lipid metabolism fail to be induced in liver (Fig. 6) upon WAY-252623 administration. Based on these findings we postulate that hepatic accumulation may simply reflect lipid returning to the liver as a result of increased RCT, rather than direct lipogenic effects resulting from LXR-mediated *de novo* lipid synthesis. The

downregulation of LDL receptor and perhaps primate *Cyp7a1* gene lacking an LXR-response element is consistent with this explanation. Unclear from these initial findings observed at study termination is whether homeostasis might be reached over a longer timeframe. It is notable that in a subsequent monkey study performed with a lower dose of WAY-252623 (15 mg/kg) there was no evidence for steatosis based on liver triglyceride measurements (supplemental, Fig. 3).

Gene chip studies support tissue-selective activation of LXR targets in the duodenum of primates treated with WAY-252623. WAY-252623 dosed alone or in combination with simvastatin was associated with robust increases in intestinal ABCA1, ABCG1, SREBF1, INSIG1 and SCD mRNA. The intestine represents a crucial organ for generating HDL formation through ABCA1 activation at the basolateral surface of the enterocyte (33, 37) however less is known about a specific role for ABCG1 in this tissue. LXR agonists stimulate cholesterol recycling from the apical surface of enterocytes to the intestinal lumen through the activation of ABCG5/G8 (38). No significant regulation of ABCG5/G8 was observed in liver or gut in monkeys at the 28d terminal timepoint despite adequate ABCG5 and G8 expression levels (Fig. 6).

A surprising result from the WAY-252623 studies was transcriptional activation of the gene encoding primate FGF19. To our knowledge, no literature exists describing LXR regulation of either FGF19 or the rodent ortholog FGF15. It is known that the FXR nuclear receptor can regulate FGF15 expression to influence lipid metabolism and that FGF19 induces a signaling cascade, through activation of FXR to repress *CYP7A1* expression gene in liver providing a mechanism for feedback repression of BA biosynthesis (39). It appears that LXR activation may provide an additional means for increasing plasma FGF19 protein levels and for triggering signaling through its hepatic receptor FGFR4.

Discrete regulation of FGF19 by LXR-specific ligands in human Caco2 cells provided the most definitive evidence for direct LXR-mediated effects, as established for several structurally distinct LXR agonists (Fig. 7). Direct modulation of FXR can probably be discounted as no FXR NR crossreactivity has been established for WAY-252623 (19) and no distinct FXR activity was demonstrated in primate tissues (liver or duodenum). In addition to the SHP findings described in the results section, there was no hepatic regulation of direct FXR targets such as ABCB11 (BSEP) involved in bile acid secretion into bile, apoCII or PLTP (Fig. 6). Although duodenal FGF19 effects might reflect negative feedback regulation by recirculating bile acids upon activation of FXR in the primate experiments, bile acid reabsorption occurs in the colon or ileum rather than the duodenum. Finally, while the spatial conservation of six predicted LXRE-binding sites in the human and rhesus FGF19 genes and their apparent absence from the murine FGF15 homolog may correlate with observed species-specific LXR regulation, detailed promoter studies are needed.

The complexity of FGF biology is underscored by recent discoveries of FGF19 participation in 1) the maintenance of physiological homeostasis (40), 2) metabolic rate increases concurrent with increases in fatty acid oxidation, and reversal of dietary and leptin deficient diabetes (41) and 3) repression of hepatic CYP7A1 expression (39). One is tempted to ascribe the repression of *cyp7a1* in the current studies to FGF19 signaling via its hepatic receptors and intestinal specific FGF19 gene activation may provide a mechanistic explanation for plasma lipid lowering effects. Recent genetic studies in mice suggest that hepatocyte FGFR4 plays an essential role in systemic lipid homeostasis, providing protection against hyperlipidemia and hypercholesterolemia to the detriment of liver lipid accumulation (42). However, our data indicates that several LXR ligands can activate human FGF19 *in vitro* and two of these (GW3965 and WAY-254011) ligands actually raised LDLc *in vivo*. The significance of LXR-mediated FGF19 regulation is an interesting topic for future study that lends itself to transgenic approaches due to species

specificity and the restriction of gene responsiveness to primates and man. The FGF19 findings provide new mechanistic insight that may extend the therapeutic opportunities to impact dyslipoproteinemia and accelerated atherosclerosis related to diabetes/insulin resistance and metabolic disease via LXR modulation.

In summary, WAY-252623 distinguishes itself by 1) reducing both total plasma cholesterol and LDLc in monkeys and 2) remaining lipid neutral in the hamster, without significant effects on triglycerides/VLDLc or LDLc. This is contrary to previously reported LXR ligands which raise LDLc in species expressing CETP (20, 21), or elevated TG/VLDLc in hamsters despite favorable anti-atherosclerotic activity in genetic models of lesion progression. Lastly, although not proven, the findings for WAY-252623 suggest that reductions in LDL steady state levels by receptor uptake may occur more efficiently in the absence of direct hepatic lipogenesis and enhanced VLDL biosynthesis and secretion. Unfortunately, these favorable plasma effects were counterbalanced by hepatic lipid accumulation potentially due to the lack of LXR stimulation in this tissue. The latter induction of hepatic steatosis in the absence of LXR activation is not without implications for the design and preferential targeting of tissue or β -selective LXR ligands and underscores how little is known about ligand effects in higher species where *cyp7a1* activation is not under direct LXR control.

WAY-252623 with limited effects on TG biosynthesis or LDL generation provides a useful tool for additional proof of concept studies in hyperlipidemic monkeys or preclinical models of lesion assessment such as cholesterol-fed rabbits where its selective properties can be further characterized. The current studies suggested a path forward for WAY-252623 into the clinic where it was recently tested in phase I human clinical trials to be described in a separate publication (26). Based on its distinct favorable pharmaceutical profile, WAY-252623 appears superior to many synthetic LXR ligands for therapeutic modulation of LXRs as a possible

treatment strategy to correct dyslipidemia, accelerated atherosclerosis or metabolic disease associated with diabetes or obesity.

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FIGURE LEGENDS

Figure 1

Effect of WAY-252623 on recruitment of cofactor peptides on human LXR α and LXR β LBD protein. The LXR multiplex assay was performed with 10 μ M WAY-252623 or WAY-254011 for comparison and panel A) represents LXR α and panel B) LXR β . The mean fluorescence intensity for treatments was plotted as a heat map for 42 of the peptides tested.

Figure 2

Treatment of LDLR $^{-/-}$ mice with synthetic WAY-252623 ligand reduces atherosclerotic lesion formation. Male LDLR $^{-/-}$ mice (8 wks of age) were fed an atherogenic diet or diet admixed with WAY-252623 or GW3965 as indicated for 8 wks. Study I (A) and II (B): Analysis of atherosclerotic lesions quantified by enface analysis of aortas stained with oil red O. Data represents mean \pm SEM, n = 10 mice per treatment group, *p < 0.05, **p < 0.01 by Dunnett's test.

Figure 3

Serum lipid effects in primates treated with WAY-252623, simvastatin or combination therapy. Cynomolgus monkeys were maintained on chow diets and administered compounds orally by gavage for 28 days. **A + B**) represents total serum cholesterol and **C + D**) triglyceride concentrations measured at time zero, 7, 14, 21, and 28 d. In study I (A + C), animals (n = 4 per group) receive vehicle, 15 or 50 mg/kg doses of WAY-252623 (623-15 and 623-50, respectively). In study II (B + D), monkeys received vehicle, 20 mg/kg simvastatin (SIM), 50 mg/kg WAY-252623 (LXR-623) or a combination of simvastatin and WAY-252623 at 20 and 50 mg/kg doses respectively (623+SIM). Significance was determined by repeated measures analysis: **A**) *p <

0.05, both treatment groups, **B**) $**p < 0.01$, all treatment groups, **C**) $*p < 0.05$ 15 mg/kg treatment group (623-15), **D**) $*p < 0.05$, LXR-623 treatment and combination groups.

Figure 4

Serum lipoprotein profiles in primates treated with WAY-252623, simvastatin or combination therapy. Cynomolgus monkeys were maintained on chow diets and administered compounds orally by gavage for 28 days. **A + B**) represent LDL-cholesterol and **C + D**) HDL-cholesterol determined by FPLC in serum samples obtained 2 hr following dosing on day 0, 7, 14, 21, and 28. In study I (**A + C**), monkeys receive vehicle, 15 or 50 mg/kg doses of WAY-252623 (623-15 and 623-50, respectively). In study II (**B + D**), monkeys receive vehicle, 50 mg/kg WAY-252623 (LXR-623), 20 mg/kg simvastatin (SIM) or a combination (623+SIM). Significance was determined by repeated measures analysis: **A**) $*p < 0.05$, both 623 treatment groups, **B**) $**p < 0.01$, 623 and combination groups, **C**) $*p < 0.05$, 50 mg/kg group, **D**) $*p < 0.05$, SIM and combination groups.

Figure 5

WAY-252623 effects on ABCA1 and ABCG1 target gene expression in peripheral blood. Monkeys were maintained on chow diets and dosed daily by gavage for 28 days. **A:** study 1, monkeys receive vehicle and WAY-252623 (15 or 50 mg/kg), **B:** study 2, vehicle, WAY-252623 (50 mg/kg), Simvastatin (20 mg/kg), or a combination of WAY-252623 (50 mg/kg) and Simvastatin (20 mg/kg) (623+sim). Blood samples were drawn 2 hr after last dose to quantify ABCA1/G1 gene expression by real-time PCR as described in the methods. Data represents mean \pm SEM, (n = 4 monkeys per group) and statistical significance was determined using ANOVA. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ represent significance compared to vehicle treatment, presented as relative expression (panel A) or fold change from vehicle control for each timepoint (panel B).

Figure 6

Subset of transcriptional changes in cynomolgus liver and duodenum following administration of WAY-252623. Duodenum and liver were harvested from study II at termination (28d) and RNA purified from these primate tissues for profiling using the Affymetrix Rhesus macaque Genome Array. Study II compares WAY-252623 (50 mg/kg/day), Simvastatin (20 mg/kg/day), or the combination to vehicle cohorts. Shown is a list of genes that confirm the responsiveness of the tissues and provide information on candidate genes involved in the LDL effect seen for WAY-252623 in this species. Values represent mean \pm SEM (n = 4 monkeys per group), *p < 0.05, **p < 0.01 compared to vehicle treatment as determined by Welch test.

Figure 7

WAY-252623 treatment reveals FGF19 as a potential LXR target gene in higher species. **Panel A:** Confirmation of microarray profiling results for FGF19 using quantitative RT-PCR. Comparison of FGF19 gene expression in duodenum vs. liver of primates treated for 28 days with WAY-252623 (50 mg/kg) (LXR623), Simvastatin (20 mg/kg) or the combination as indicated (LXR623+Sim). Data represent mean \pm SEM for treatment vs control cohorts. **Panel B:** Induction of FGF19 in human intestinal epithelial cells (Caco2) by LXR agonists. Caco2 human intestinal epithelial cells were grown to confluence and differentiated for 17 days. Cells were then treated with DMSO vehicle, FXR agonist (1 μ M GW4064) or LXR agonists (1 μ M T0901317 or 1 μ M GW3965) for either 24 or 48 hours. **Panel C:** Dose-dependent induction of FGF19 mRNA in human intestinal epithelial cells (Caco2) treated with LXR agonists. Caco2 cells were treated with GW4064, WAY-252623, and WAY-254011 for 24hrs and ABCA1, SHP and FGF19 expression quantified by real time-PCR. The data is representative and similar to results at 48 hrs.

Table 1

Plasma and liver lipid, liver weight, lesion area and serum drug concentrations in LDLR^{-/-} mice maintained for 8 weeks on an atherogenic diet in the presence or absence of GW3965 and WAY-252623 (LXR-623).

	Vehicle	GW3965 10 mg/kg	LXR-623 15 mg/kg	LXR-623 50 mg/kg
Serum Cholesterol (mg/dL)	1293 ± 5.8	1227 ± 17.0	1227 ± 25.5	1066 ± 56.0
Serum Triglyceride (mg/dL)	455 ± 42	564 ± 55	508 ± 40	450 ± 46
% Liver Weight	5.57 ± 0.13	5.06 ± 0.08	5.40 ± 0.10	5.47 ± 0.06
Liver Cholesterol (mg/gm)	12.5 ± 0.69	9.0 ± 0.71*	7.7 ± 0.40*	7.8 ± 0.39*
Liver Triglyceride (mg/gm)	35.4 ± 4.0	38.9 ± 5.1	19.7 ± 1.5*	23.2 ± 1.4
Lesion Area		-40.7*	-46.9*	-65.8*
Serum Drug Conc. (ng/ml)		811	1038	2427

Data are expressed as mean ± SEM. *p < 0.05, n = 10 mice per treatment group.

Table 2
Effects of WAY-252623 in Golden Syrian Hamsters on serum cholesterol, triglycerides, and liver enzymes, and liver weights.

Doses (mg/kg/day)	Serum TC mg/dL	Serum TG mg/dL	Serum AST U/L	Serum ALT U/L	Liver Weight % of Body Wt	Blood levels ng/ml
Vehicle	104 ± 6	193 ± 8.9	69.4 ± 23.9	91.1 ± 12.8	4.05 ± 0.1	N/A
20	101 ± 2.3	197 ± 9.1	57.0 ± 6.9	86.5 ± 11.8	4.20 ± 0.1	1076 ± 345
60	106 ± 2.3	182 ± 10.0	47.6 ± 5.1	77.1 ± 5.6	4.10 ± 0.1	1670 ± 1422
120	103 ± 2.7	205 ± 10.1	44.5 ± 3.3	82.9 ± 3.6	4.40 ± 0.1	5001 ± 1343

Doses were administered once daily by oral gavage for 7 d and blood collected 2 h post dose on the last day of the study. Data are expressed as mean ± SEM. * $p < 0.05$, $n = 7$ hamsters per treatment group.

Table 3.**Liver Cholesterol and Triglyceride (Study II)**

	Cholesterol mg/gm liver	Triglyceride mg/gm liver
Vehicle	3.0 ± 0.0	9.7 ± 0.7
WAY-252623	7.7 ± 0.6*	52.0 ± 5.5*
Simvastatin	3.3 ± 0.6	9.0 ± 1.1
Combination	6.0 ± 2.3*	47.7 ± 19.2*

Liver recovered at study termination (28 d). Results represent mean ± SEM, *p < 0.05,

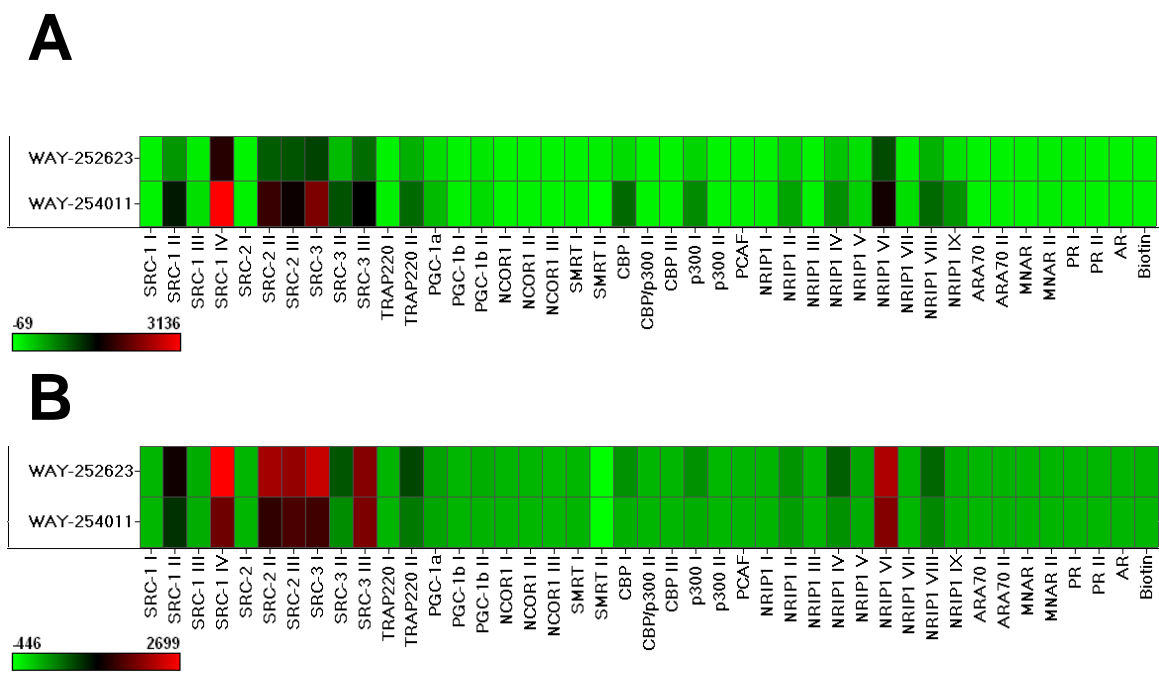


Figure 1

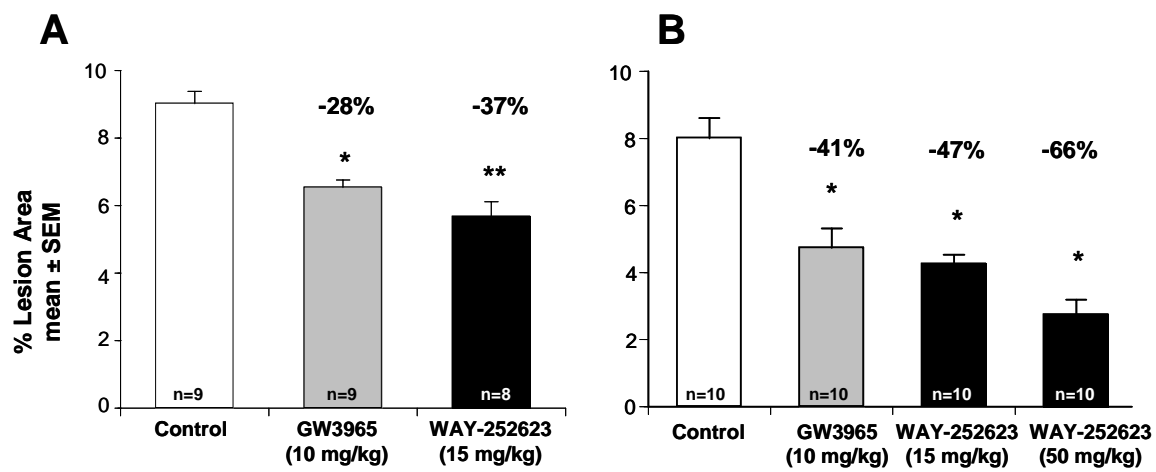


Figure 2

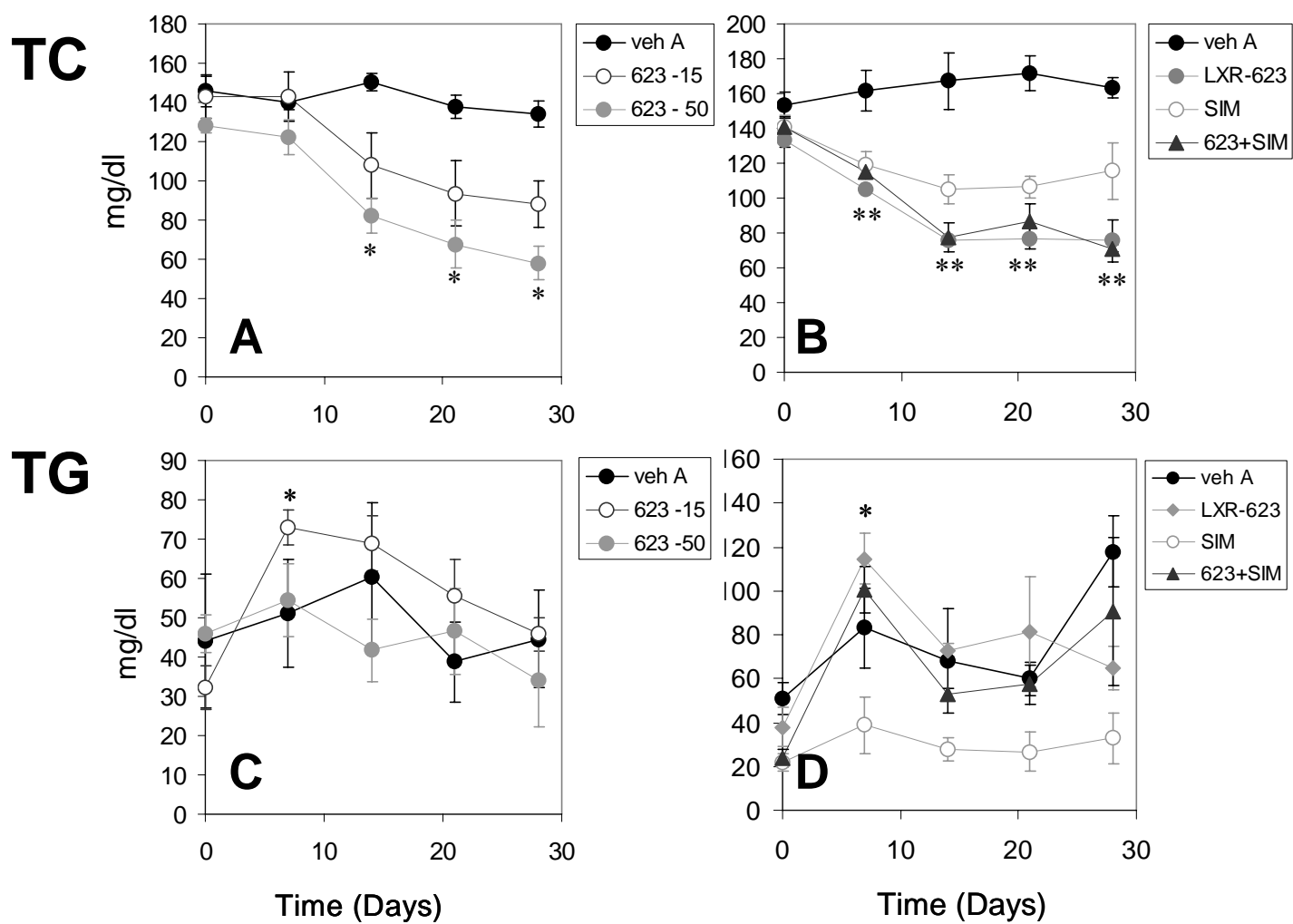


Figure 3

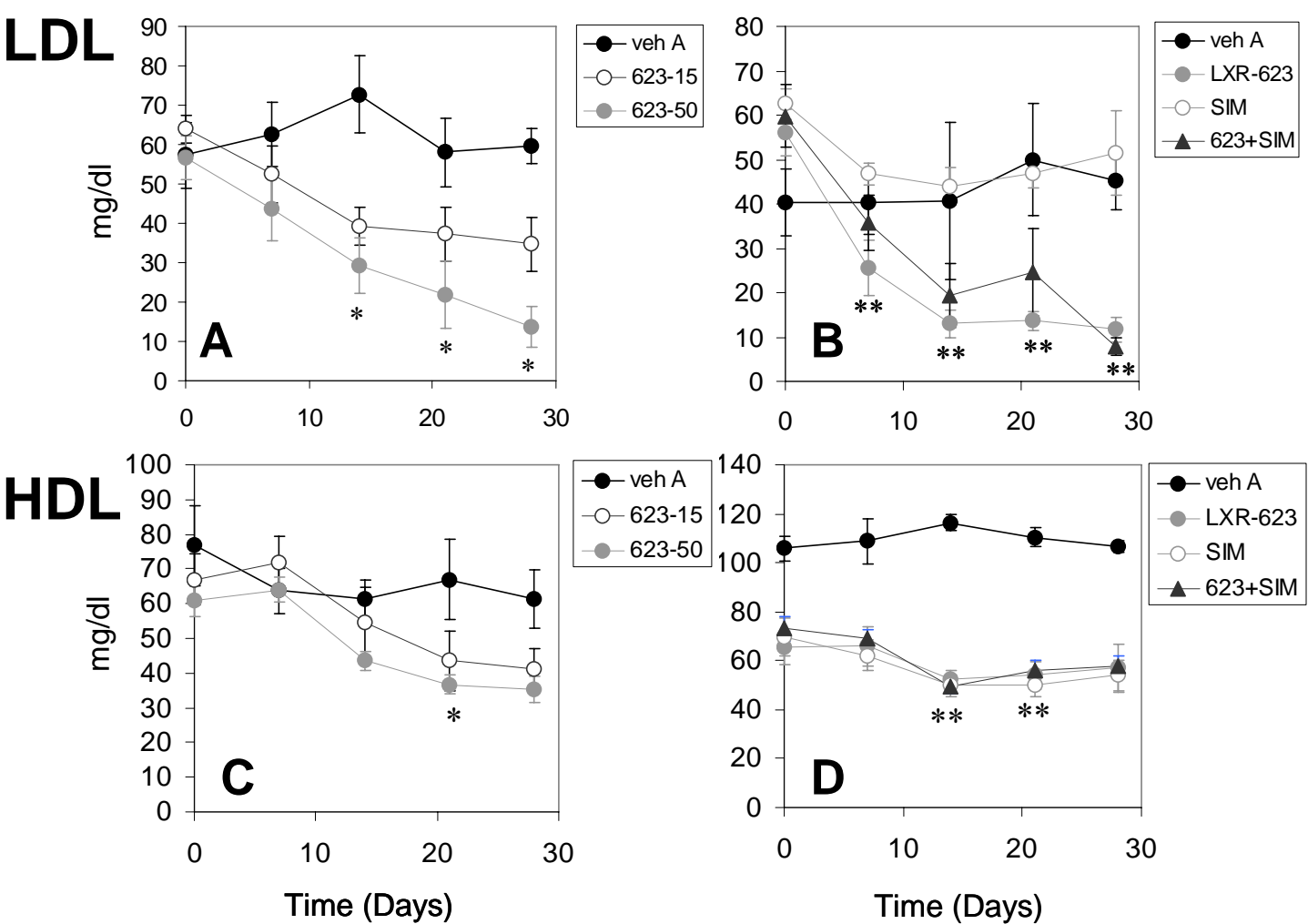


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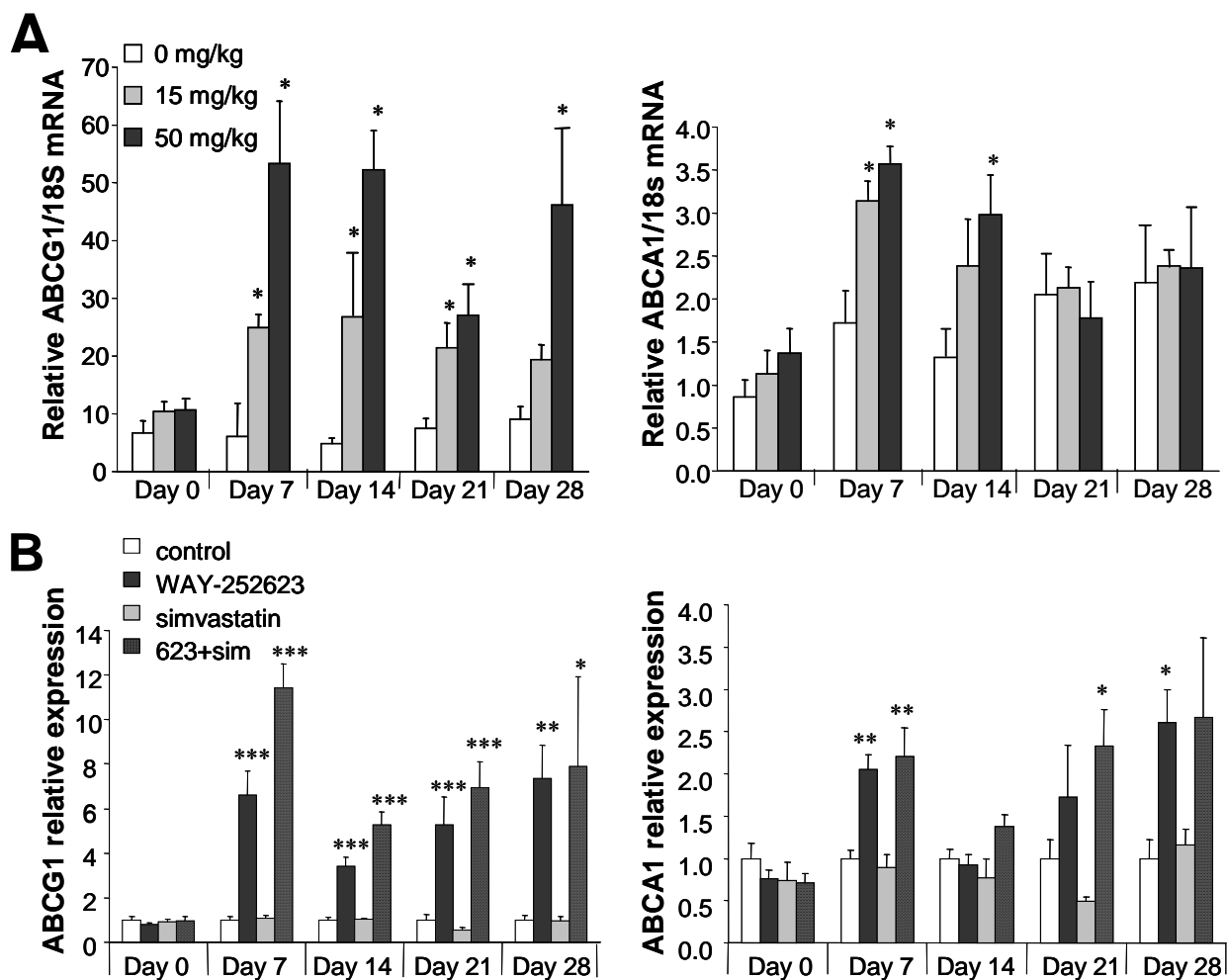


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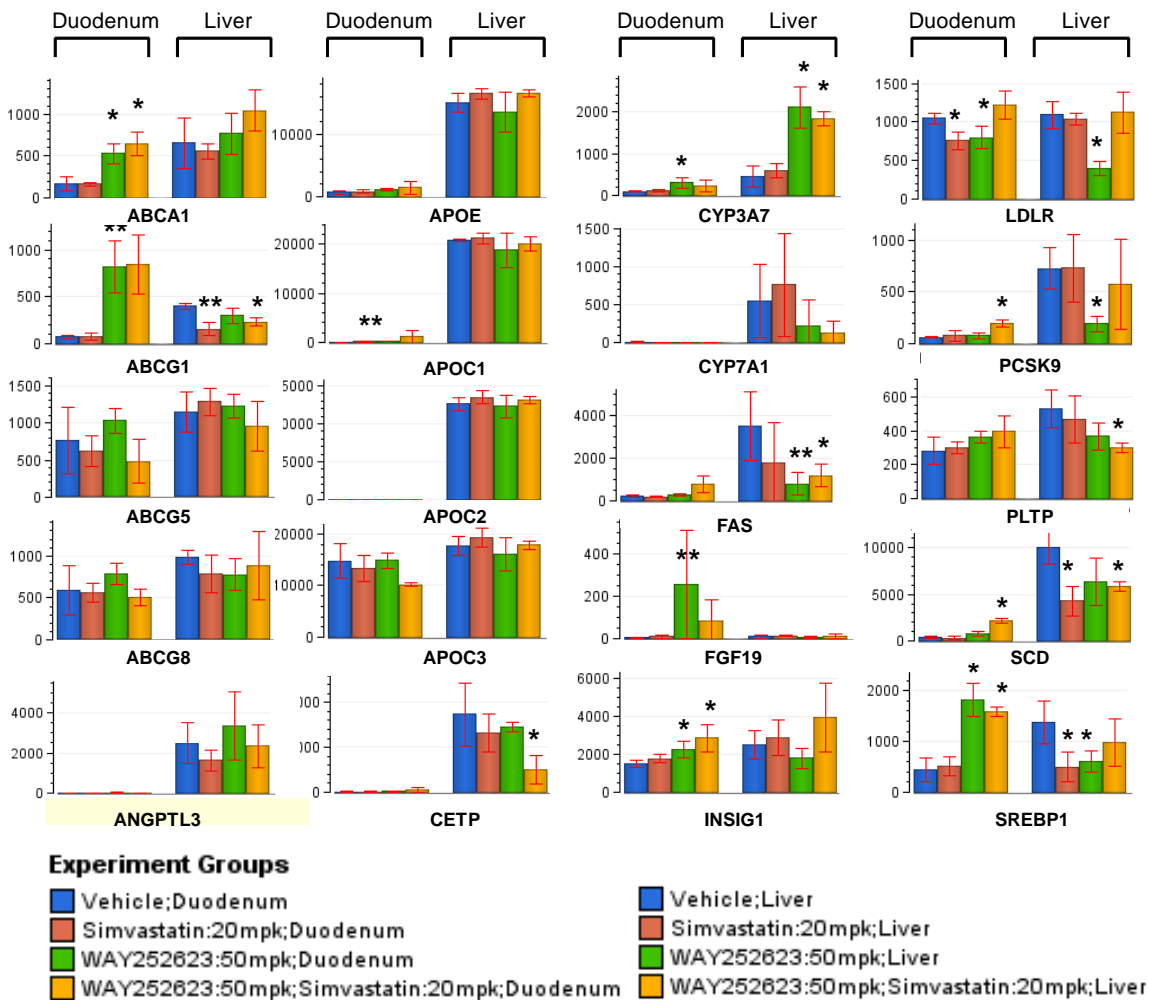


Figure 6

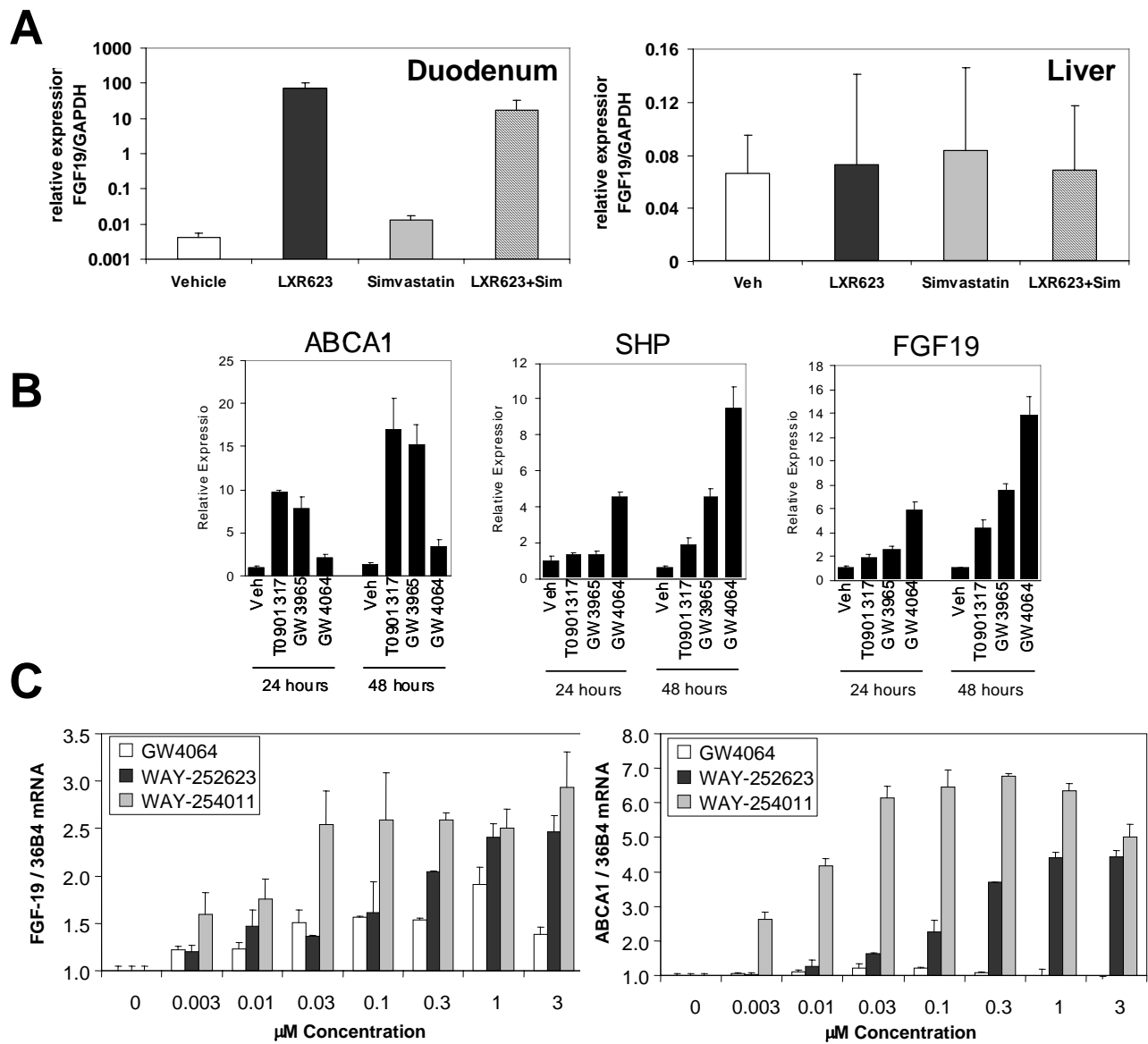


Figure 7