Raising HDL cholesterol without inducing hepatic steatosis and hypertriglyceridemia by a selective LXR modulator

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Abstract Liver X receptors (LXRs) are ligand-activated transcription factors that belong to the nuclear receptor superfamily. LXRs activate transcription of a spectrum of genes that regulate reverse cholesterol transport, including the ATP binding cassette transporter A1 (ABCA1), and raise HDL cholesterol (HDL-C) levels. However, LXR agonists also induce genes that stimulate lipogenesis, including the sterol response element binding protein (SREBP1-c) and fatty acid synthetase (FAS). The induction of these genes in the liver cause increased hepatic triglyceride synthesis, hypertriglyceridemia, and hepatic steatosis. As LXR response elements have been identified in these promoters, it is not clear if these two processes can be separated. Herein, we demonstrate that plasma HDL-C elevation and intestinal ABCA1 induction can occur with relatively little induction of FAS and SREBP1-c in mouse liver via a selective LXR modulator GW3965. This is in contrast to the strong induction of hepatic lipogenic genes by the well-characterized LXR agonist T0901317 (T317). Consistent with the in vivo results, GW3965 is a very weak LXR activator compared with T317 in human hepatoma cells. GW3965-liganded LXR recruits selected coactivators less effectively than T317 and may explain in part the tissue selective gene induction. This demonstration that tissue and gene selective modulation is possible with selective LXR modulators has positive implications for the development of this class of antiatherosclerotic agents.—Miao, B., S. Zondlo, S. Gibbs, D. Cromley, V. P. Hosagrahara, T. G. Kirchgessner, J. Billheimer, and R. Mukherjee. **Raising HDL cholesterol without inducing hepatic steatosis and hypertriglyceridemia by a selective LXR modulator. *J. Lipid Res.* 2004.** 45: **1410–1417.**

Supplementary key words high density lipoprotein • liver X receptor • fatty acid synthetase • sterol response element binding protein 1-c • triglyceride

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The liver X receptors (LXRs) belong to the intracellular receptor superfamily of ligand-activated transcription factors (1). Two subtypes, LXRα (NR1H3) and LXRβ (NR1H2) (1, 2), have been identified. LXRα is primarily expressed in liver, kidney, macrophages, and intestine, while LXRβ is ubiquitously expressed. Oxysterols are natural ligands for LXRs, and synthetic, high affinity ligands have also been identified, e.g., T0901317 (T317) and GW3965 (3, 4). LXR agonists induce a number of genes modulating reverse cholesterol transport, cholesterol homeostasis and lipogenesis. These genes include ATP binding cassette transporter A1 (ABCA1), ABCG1, ABCG5, ABCG8, apolipoprotein E (apoE), cholesteryl ester transfer protein (CETP), phospholipid transfer protein, fatty acid synthetase (FAS), and sterol response element binding protein (SREBP1-c) (5–10) and references therein. ABCA1 mediates the efflux of excess cholesterol and phospholipids from peripheral cells, such as macrophages, to nascent HDL particles. The HDL cholesterol (HDL-C) is further modified in the circulation through the esterification of cholesterol by lecithin:cholesterol

Abbreviations: ABCA1, ATP binding cassette transporter A1; apoE, apolipoprotein E; CBP, cAMP response element binding protein; CETP, cholesteryl ester transfer protein; DRIP, vitamin D receptor-interacting proteins; FAS, fatty acid synthetase; FRET, fluorescence resonance energy transfer; GST, glutathione-S-transferase; HDL-C, HDL cholesterol; hLXRα, human LXRα; LDLR, LDL receptor; LXR, liver X receptor; SLRM, selective LXR modulator; SR-BI, scavenger receptor class B type I; SRC-1, steroid receptor coactivator-1; SREBP1-c, sterol response element binding protein 1-c; T317, T0901317; TAMRA, 6-carboxytetramethylrhodamine; TG, triglyceride.

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acyltransferase and by interactions with apoB-containing lipoproteins mediated by CETP and is finally taken up into the liver. These and other reactions together constitute the process termed reverse cholesterol transport, whereby excess cholesterol from the periphery is transported back to the liver for excretion. LXR agonists induce ABCA1, ABCG5, and ABCG8 in the liver and small intestine. Inducing ABCG5 and ABCG8 results in increased transport of hepatic cholesterol into the bile as well as increased efflux of dietary and biliary cholesterol from the enterocytes back into the gut, thus decreasing cholesterol absorption (7, 8).

Together, these processes are thought to contribute to inhibiting the progression of atherosclerotic lesions in animal models as was demonstrated by GW3965 in the apoE$^{-/-}$ mice and LDL receptor (LDLR)$^{-/-}$ mice (11), as well as T317 treatment of LDLR$^{-/-}$ mice (12). Recently described antiinflammatory properties of LXR agonists may also play an antiatherosclerotic role (13). However, in addition to the potentially beneficial effects described above, LXR agonists also induce hypertriglyceridemia (3, 14) and hepatic steatosis (15) by inducing genes for the major lipogenic transcription factors, the SREBP1-c and FAS in the liver.

Because hypertriglyceridemia is a known risk factor for cardiovascular diseases, and hepatic triglycerides (TGs) are associated with insulin resistance and hepatotoxicity, elevating hepatic or plasma TGs is unlikely to be a tolerated side effect of LXR agonists in a clinical setting (16–18). Thus, the enthusiasm for the discovery of LXR agonists for the treatment of atherosclerosis has been tempered by this unfavorable effect on plasma and liver TG levels. However, an LXR agonist that induces ABCA1 mRNA in peripheral tissues without inducing lipogenic genes in the liver may raise HDL and increase reverse cholesterol transport without the detrimental hepatic effects.

We investigated the possibility of separating these two pathways by a selective modulator of LXR activity. We report such a compound that raises plasma HDL-C without inducing TG accumulation in the liver and that has minimal hepatic induction of SREBP1-c and FAS mRNA even at very high doses. This selective LXR modulator (SLRM), GW3965, is a weak activator of LXRα compared with T317 in HepG2 cells. Differential coactivator recruitment may explain in part the tissue selective gene induction observed with GW3965.

MATERIALS AND METHODS

Reagents

T317 (3) and GW3965 (4) were synthesized at Bristol-Myers Squibb Co.

Animals and treatments

C57Bl/6 male mice around 20 g (four animals per group) were housed individually and maintained on standard mouse chow and water ad libitum. Blood was always drawn after an overnight fast from the retroorbital sinus. Animals were prebled and grouped so that the average TG levels of each group were similar. After 7 days they were dosed by oral gavage (0.2 mls per mouse) with compounds at the doses indicated or vehicle (0.5% methylcellulose) once a day. The initial doses were selected from published data (4, 10, 19). Animals were sacrificed 24 h after the third treatment after an overnight fast. Plasma lipids were determined. Statistically significant differences ($P < 0.05$) from the vehicle-treated group were determined by the Student's *t*-test. Livers and a portion of the small intestine (jejunum) were frozen in liquid nitrogen for RNA extraction.

RNA measurements

RNA from liver and small intestine (jejunum) was extracted, and FAS, SREBP1-c, and ABCA1 mRNA levels were measured by real-time quantitative PCR analysis. Expression levels were normalized to that of 18S and expressed relative to vehicle-treated animals set at 1. Values represent the average measurements of four tissue samples carried out in duplicate ± SEM. Probes were modified at the 5′ end with 6-carboxyfluorescein and at the 3′ end with 6-carboxytetramethylrhodamine (TAMRA) (Biosearch Technologies, Novato, CA); the 18S rRNA probe was modified at the 5′ end with VIC and the 3′ end with TAMRA. The sequences of the forward and reverse primers and probes are:

FAS: Forward CTCACGCTGCGGAAACTTCAGGAAATG
FAS: Reverse GAGACGTGTCACTCCTGGACTTG
FAS: Probe CTCACGCTGCGGAAACTTCAGGAAATG
ABCA1: Forward CCGCTTCGTCTCTCCGC
ABCA1: Reverse TTCGCCTTTACAGGTCTGGG
ABCA1: Probe CTCTTGGGACTTGGTAGGACGGAACCTTTT
SREBP-1c: Forward CATCGACTACATCCGCTTCTTG
SREBP-1c: Reverse TTGCTTTTGTGTGCACTTCGT
SREBP-1c: Probe CACAGCAACCAGAAGCTCAAGCAGGA

Liver TG measurements

Livers were homogenized in saline using 3 ml saline/g liver (~250 mg/ml protein). The homogenate was quick frozen on dry ice and kept at −80°C till assayed.

Homogenates were quickly thawed at 37°C and then diluted 5× with saline, resulting in a homogenate at ~50 mg/ml. Solubilization was performed in a 96-well plate with 20 μl of homogenate and 20 μl of 1% deoxycholate and incubated at 37°C for 5 min. For TG measurements, 200 μl of reagent (Infinity TG Reagent, Sigma, St. Louis) was added and incubated for 15 min at 37°C. For cholesterol measurements, 200 μl of reagent (Infinity Cholesterol Reagent, Sigma) was added and incubated for 5 min at 37°C. Samples were agitated during incubations and the optical density read at 500 nm. The TG content was determined using a standard curve generated with Lipid Lin-Trol prediluted standard set (Sigma). Protein was measured using the BioRad Protein Assay (BioRad, Hercules, CA) with serum albumin as standard.

Plasmids and transfection assays

The GAL4 DNA binding domain (amino acids 1–147), human LXRα (hLXRα) (amino acids 166–447), and hLXRβ (amino acids 218–461) sequences were amplified by PCR and cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA) to create GAL4-LXR fusion proteins. The reporter had five copies of the GAL4 upstream activating sequence cloned upstream of TATA-luciferase. All constructs were verified by sequencing. HEK-293 cells were stably transformed with this reporter and used in the assays. For HepG2 cell assays, the GAL4-reporter plasmid was cotransfected with the GAL4-LXR plasmid and a pSVβ-galactosidase (Promega, Madison, WI) plasmid for normalization using Fugene 6 transfection reagent (Roche Diagnosis Corporation, Indianapolis, IN). Twenty-four hours after transfection, the ligands or vehicle (DMSO, 0.1%) were added to the cells. Cells were analyzed for

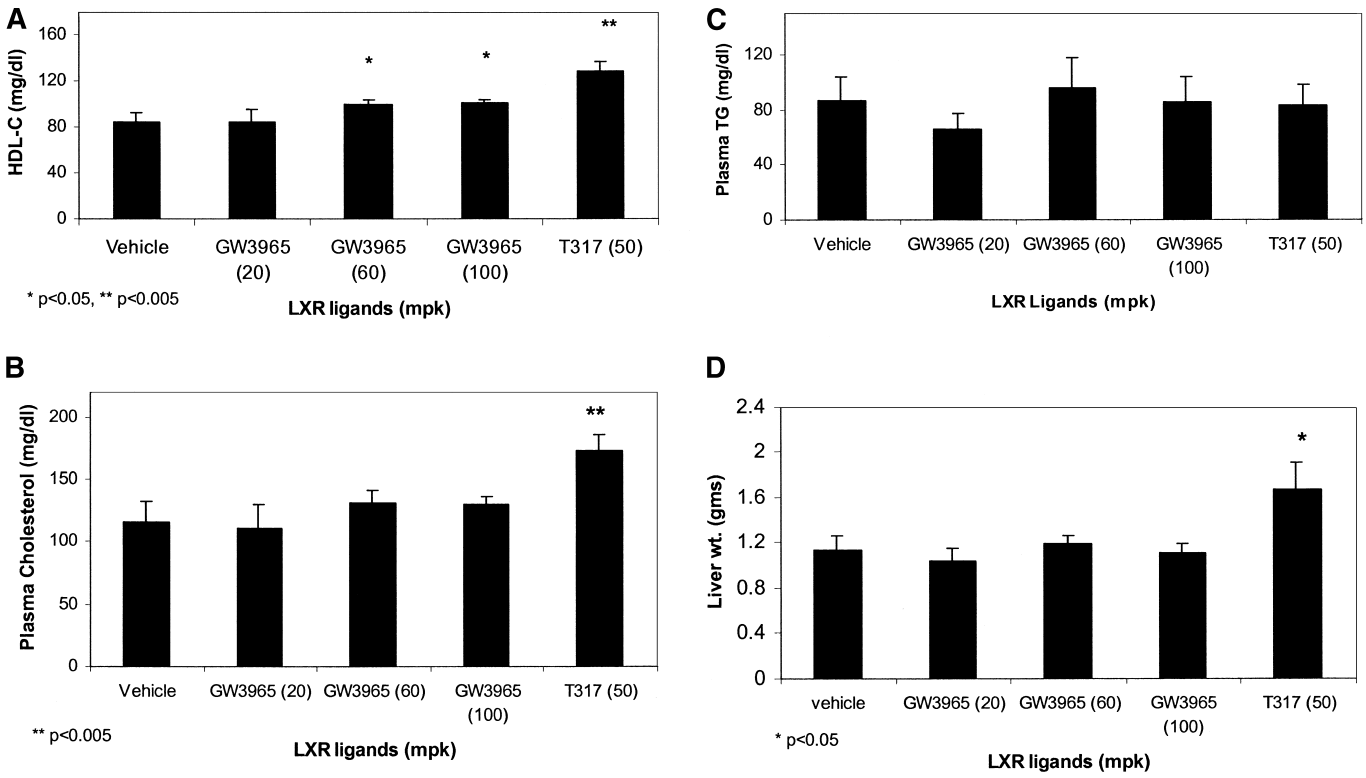

Fig. 1. Plasma lipid levels and liver weights in mice treated with liver X receptor (LXR) agonists. C57Bl/6 mice were treated for 3 days with T0901317 (T317) at 50 mg/kg (mpk) and with GW3965 at 20, 60, and 100 mpk once a day as indicated. After an overnight fast, blood was drawn and plasma HDL cholesterol (HDL-C) (A), total cholesterol (B), and triglycerides (TG) (C) were determined. Liver weights are shown (D). The data shows the mean ± SD (n = 4 mice per group). Statistically significant differences with respect to the vehicle-treated group, * $P < 0.05$ and ** $P < 0.005$, are shown.

luciferase activities and β-galactosidase according to the manufacturers (Promega and Stratagene). The data show the mean ± SEM (n = 3) of a representative experiment, which was repeated with similar results.

Fluorescence resonance energy transfer assays

hLXRα (amino acids 77–448) with an N-terminal glutathione-S-transferase (GST) tag was expressed and purified from *Escherichia coli*. Fluorescence resonance energy transfer (FRET) assay (20) was performed by incubating 5 nM GST-hLXRα protein, 100 nM biotinylated peptide from the coactivators steroid receptor coactivator-1 (SRC-1), cAMP response element binding protein (CBP) or vitamin D receptor-interacting protein (DRIP) (21–23), 1 nM Eu-labeled anti-GST antibody, 70 nM streptavidin-conjugated allophycocyanin, and 1 μM ligand for 2 h at room temperature. The assay buffer consists of 50 mM Tris, pH 8.0, 50 mM KCl, 1 mM DTT, 1 mg/ml BSA, and 1 mM EDTA. Data was expressed as the ratio of emission intensity at 665 nm to that at 620 nm. The data show the mean ± SD (n = 3), and the value for the vehicle control in each case is set at 1. The sequences of SRC-1 and CBP peptides used are published (24) and that of DRIP (GenBank accession number AF283812) is AGNTKNHPMLMNLLKDNPAQ.

RESULTS

We utilized two synthetic high affinity ligands, T317 and GW3965, which are selective LXR agonists with an EC_{50} around 20 nM and 200 nM, respectively (4, 7). C57Bl/6 mice were treated for 3 days with these compounds at the doses indicated, and plasma lipids and gene induction in the liver and small intestine were measured.

We observed a 53% rise in plasma HDL-C levels with T317 at 50 mg/kg (mpk) (**Fig. 1A**). There was no increase in HDL levels with GW3965 at 20 mpk but a significant rise (18% and 20%) with GW3965 at 60 mpk and 100 mpk, respectively (Fig. 1A). Similarly, there was a 73% rise

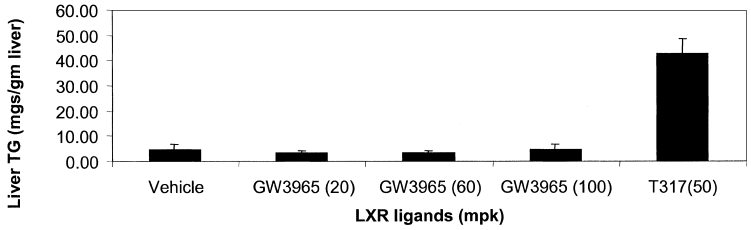

Fig. 2. T317, but not GW3965, induces TG accumulation in the liver. Animals were treated as in the legend to Fig. 1. Liver TG levels were measured as described in Materials and Methods. The data show the mean ± SD (n = 4 mice per group).

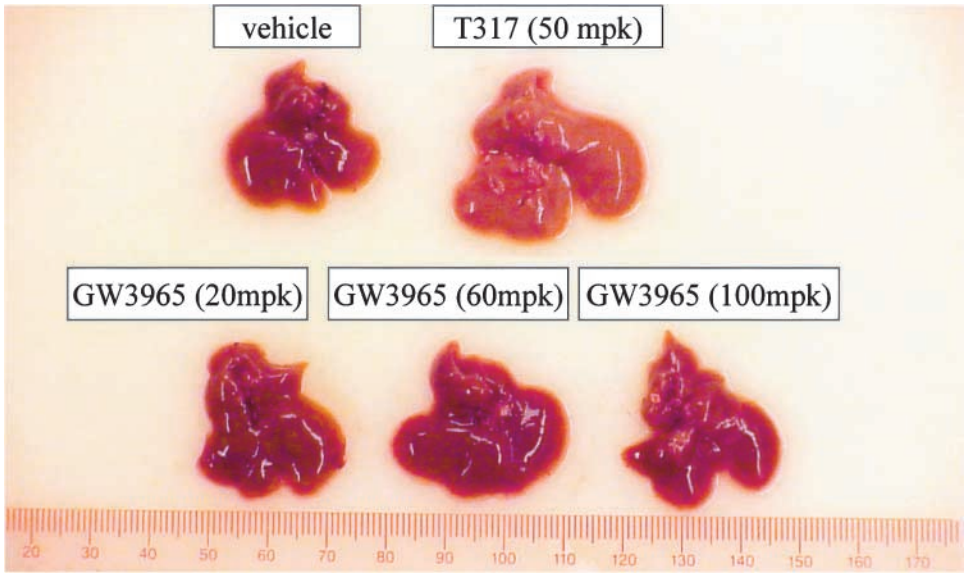

Fig. 3. GW3965 does not induce liver steatosis. Gross morphology of livers from GW3965- and T317-treated animals are shown. Animals were treated as in the legend to Fig. 1.

in total cholesterol with T317 and around a 30% rise with the higher doses of GW3965 (Fig. 1B). Interestingly, there was no significant rise in plasma TG levels by any compound at any dose (Fig. 1C). There was an increase in liver weight with T317 but no increase in liver weight with GW3965, even at 100 mpk (Fig. 1D).

We next measured TG and cholesterol levels in the liver. There was a 10-fold increase in liver TGs with T317

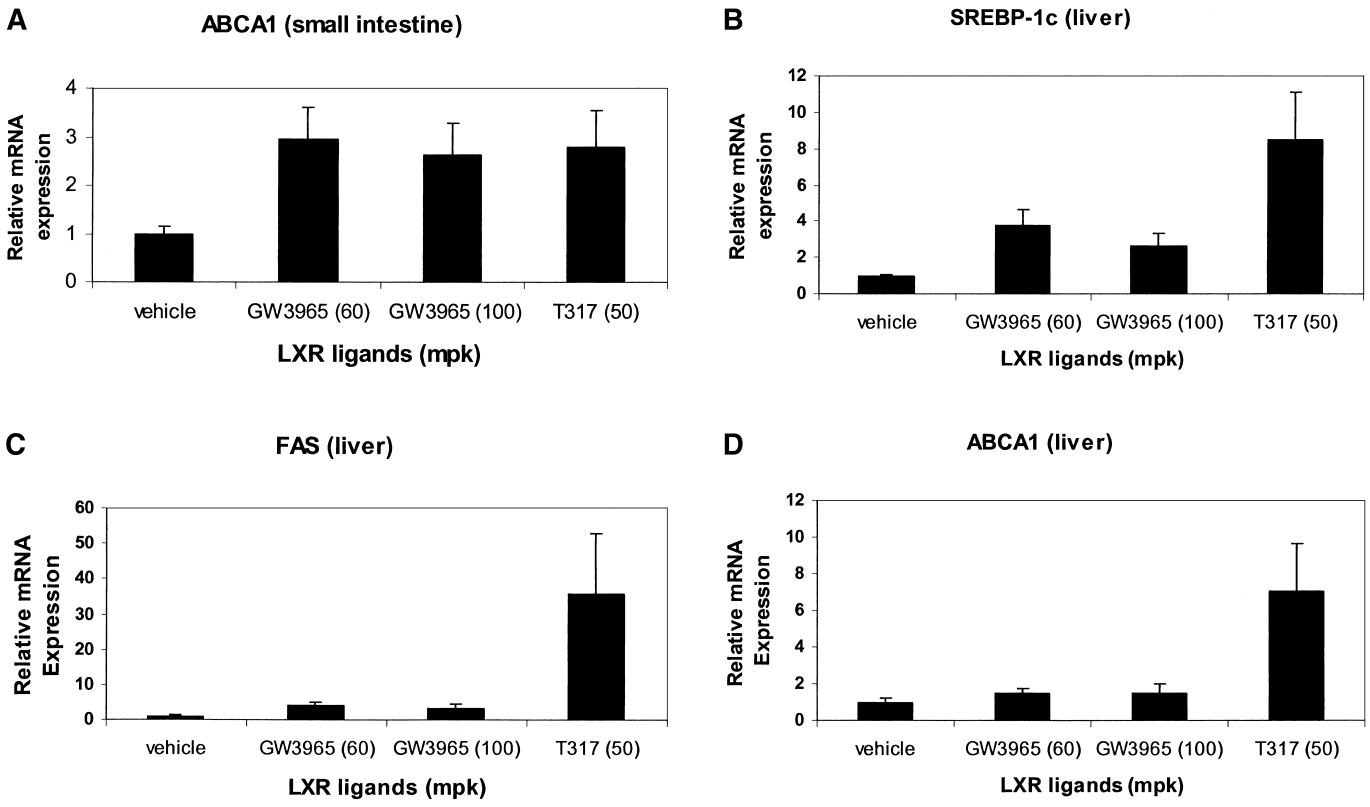

Fig. 4. GW3965 is a tissue-selective modulator of LXR. ATP binding cassette transporter A1 (ABCA1) mRNA is induced in the small intestine by GW3965 and T317 (A). In liver, compared with T317, GW3965 is a relatively weak inducer of sterol response element binding protein (SREBP1-c) (B), fatty acid synthetase (FAS) (C), and ABCA1 (D). Animals were treated as in the legend to Fig. 1. RNA was extracted from jejunum and liver, and the expression of specific mRNAs was determined by quantitative PCR analysis. Expression levels were normalized to that of 18S and expressed relative to vehicle-treated animals set as 1. Values represent the average of four tissue samples per group carried out in duplicate ± SEM.

at 50 mpk ($P < 0.005$), but no increase was observed with GW3965 at any dose (**Fig. 2**). Liver cholesterol content was unchanged with either compound (data not shown). Consistent with the large increase in liver TG content, T317-treated livers were larger and paler in color (**Fig. 3**), while the sizes and colors of GW3965-treated livers were similar to that of vehicle controls.

To account for the differences in hepatic TG accumulation in mice treated with the two LXR ligands, we measured changes in gene expression in the liver and small intestine. ABCA1 is expressed in the small intestine and is induced by LXR agonists (7). In the small intestine, both T317 and GW3965 induced ABCA1 expression to similar extents (~3-fold, $P < 0.05$; **Fig. 4A**). However, the two compounds behaved very differently in the liver (Fig. 4B–C). T317 induced SREBP1-c (8-fold, $P < 0.05$) and robustly induced FAS (38-fold). T317 also induced ABCA1 (8-fold) in the liver. In contrast, GW3965 was a very weak inducer of these genes (2- to 4-fold). We conclude that GW3965 at 60 mpk raises plasma HDL-C levels and induces ABCA1 in small intestine. Unlike T317, GW3965 does not induce TG accumulation and minimal SREBP1-c and FAS expression in the liver. The induction saturates at 60 mpk and does not increase at 100 mpk.

In trying to decipher the molecular mechanism for this difference in tissue selectivity of T317 and GW3965, we hypothesized that these ligands transactivate LXR to very different extents in liver. The transcriptional activity of the ligands was compared in cotransfection assays using GAL4LXRα and GAL4LXRβ in HEK-293 and HepG2 cells. In HEK-293 cells, T317 activated LXRα (10-fold) with an EC_{50} of 0.04 μM (**Fig. 5A**), while GW3965 activated LXRα 7-fold with an EC_{50} of 0.29 μM. These values are comparable with those observed by other researchers

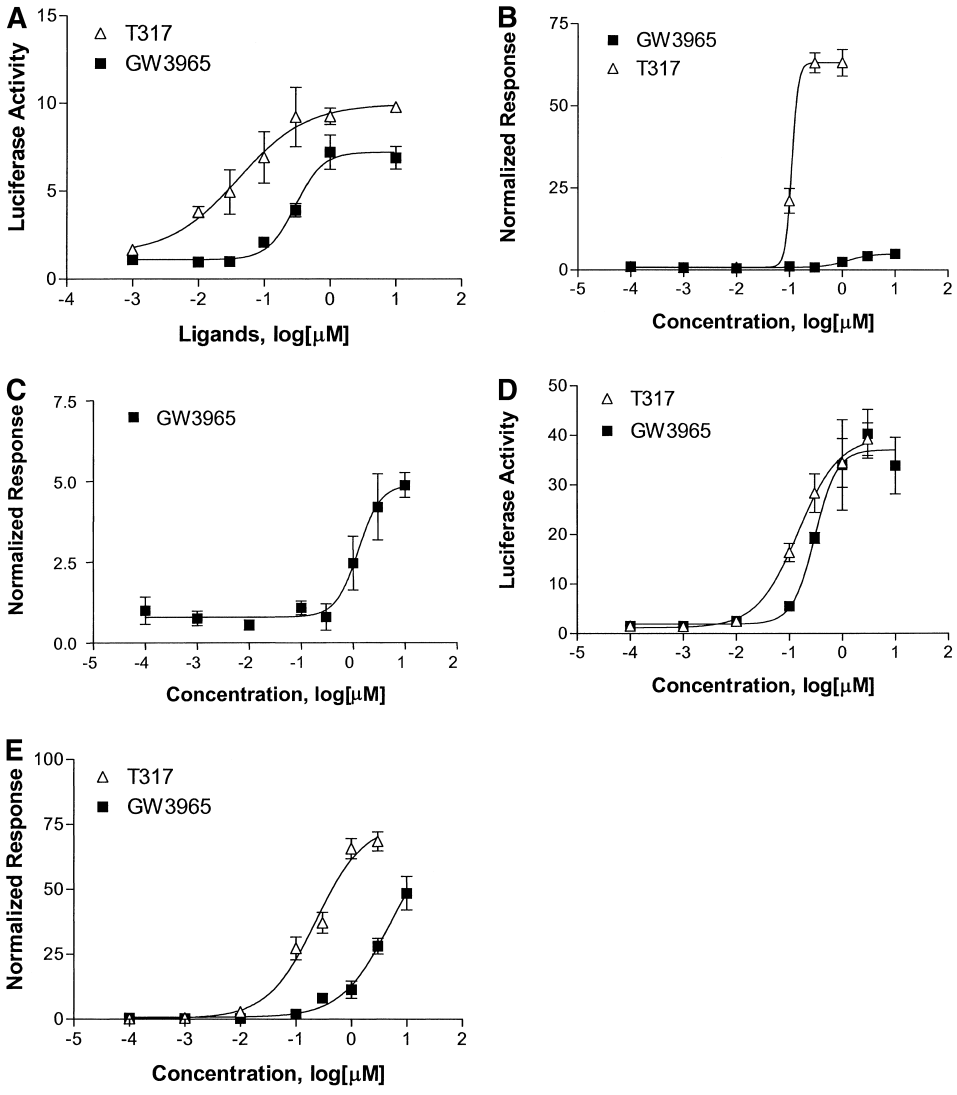

Fig. 5. GW3965 is a weak activator of LXR compared with T317 in HepG2 cells but not in HEK-293 cells. Cotransfection assays were performed with GAL4LXRα in HEK-293 cells (A) and HepG2 cells (B, C) and GAL4LXRβ in HEK-293 cells (D) and HepG2 cells (E) as described in Materials and Methods. The data represent the mean ± SEM (n = 3) of a representative experiment. This experiment was repeated with similar results.

(4, 7). In HepG2 cells, T317 is an extremely strong activator of LXRα (65-fold, EC_{50} 0.11 μM; Fig. 5B). GW3965 is a much weaker activator (5-fold) with an EC_{50} of 1.2 μM (Fig. 5B, and shown more clearly in Fig. 5C). Therefore, compared with T317, GW3965 is a much weaker activator of LXRα (<10%) in HepG2 cells. Similarly, in HEK293 cells, both T317 and GW3965 were full agonists of LXRβ, with EC_{50}s corresponding to 0.14 μM and 0.3 μM, respectively (Fig. 5D). In HepG2 cells, the EC_{50} value for T317 is 0.2 μM, and the EC_{50} value for GW3965 is greater than 4 μM, as the curve did not reach saturation even at 10 μM (Fig. 5E). We conclude that GW3965 is a selective modulator of LXRα activity.

We hypothesized that a possible mechanism for tissue selectivity is differential coactivator recruitment induced by the two ligands of LXR. We performed coactivator recruitment assays utilizing the FRET method using GST-tagged LXRα and peptides with the signature LXXLL motifs from three different coactivators: SRC-1, CBP, and DRIP (**Fig. 6A**) (21–23). Both ligands induced recruitment of SRC-1 and DRIP to similar extents. However, recruitment of CBP was significantly less with GW3965 than with T317, even when the assay was performed as a dose response to both ligands (Fig. 6B). T317 and GW3965 liganded LXRα recruits CBP with EC_{50} values of 0.03 μM and 0.36 μM, respectively (Fig. 6B). These values are similar to their EC_{50}s in cotransfection assays (Fig. 5A). Hence, tissue-specific differences in coactivator recruitment may explain in part the selective pattern of gene induction observed in vivo.

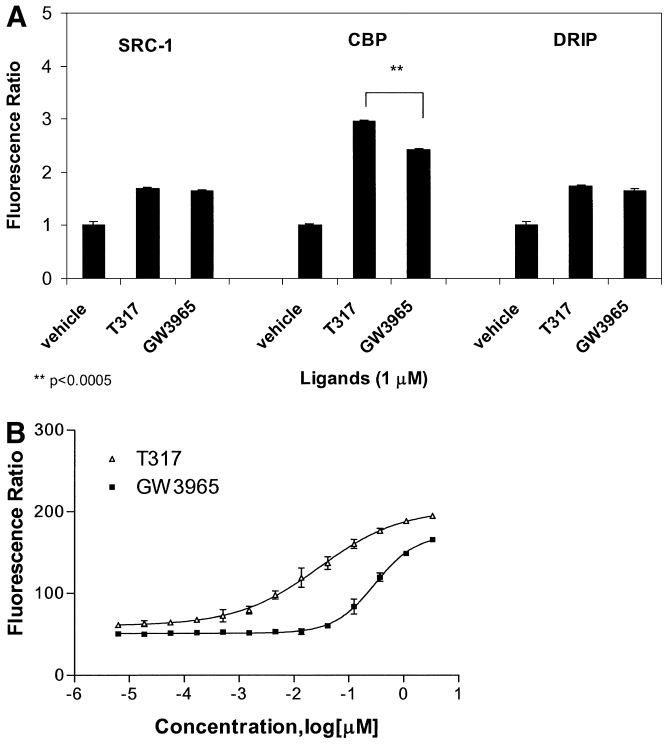

Fig. 6. Differences in coactivator recruitment by T317- or GW3965-liganded LXR. Fluorescence resonance energy transfer assay was performed with glutathione-S-transferase-tagged LXRα and biotinylated peptides from the different coactivators indicated (A) and with cAMP response element binding protein (CBP) (B). T317 and GW3965 were added at the concentrations indicated. The data show the mean and SD (n = 3). The experiment was repeated with similar results. Statistically significant differences were determined by Student's *t*-test. DRIP, vitamin D receptor-interacting protein; SRC-1, steroid receptor coactivator-1.

DISCUSSION

LXRs are attractive targets for treating atherosclerosis because they are direct transcriptional regulators of a variety of genes, including ABCA1, a transporter that mediates cholesterol efflux from lipid laden macrophages onto HDL particles for transport back to the liver. T317, the first high affinity synthetic LXR ligand identified, clearly increases ABCA1 induction and cholesterol efflux but also induces hypertriglyceridemia and fatty liver. It is counterintuitive to treat a patient at risk for cardiovascular diseases with a drug that raises TG levels. Inasmuch as LXR response elements have been identified in the FAS and SREBP1-c gene, it is not apparent if induction of ABCA1 and other beneficial reverse cholesterol transport genes can be separated from the concomitant induction of fatty acid synthesis and its downstream consequences, hypertriglyceridemia and fatty liver.

Recently, GW3965 was also identified as a high affinity ligand for LXRs, which slows progression of atherosclerotic lesions in apoE and $LDLR^{-/-}$ mice (11). GW3965 induces hypertriglyceridemia in $apoE^{-/-}$ mice but not in $LDLR^{-/-}$ mice (11). It is not known if this compound induces hypertriglyceridemia and liver steatosis in wild-type mice.

Induction of hypertriglyceridemia by LXR agonists is controversial. Increases in plasma TGs have been reported (14). However, others report no change in plasma TGs (15) or only a transient increase (19). The reason for this inconsistency is unclear at present. LXR agonists also induce TG accumulation in the liver (15). Therefore, the time-dependent normalization of TGs in the blood could partially be explained by the sequestration of fatty acids in the liver.

Is the hepatic induction of lipogenic genes a common property of all LXR agonists? We tested this hypothesis using ligands of two different chemotypes in two cell lines. While GW3965 is about 70% as active as T317 on LXRα in HEK293 cells (representing a nonhepatic cell line), it is only 8% as active as T317 in HepG2 cells (a hepatic cell line). This is consistent with the very poor induction of ABCA1, FAS, and SREBP1-c in liver even at the highest dose. The doses of GW3965 were so chosen that at the highest dose (100 mpk), the exposure was at least 5× that required to see a decrease in atherosclerosis progression in mice (11) and an induction of ABCA1 mRNA and is well above its EC_{50} in cells (4). T317 is a very strong activator of LXRα in HepG2 cells, much more than in HEK293 cells (65-fold vs. 10-fold). This is consistent with a more pronounced induction of liver FAS (35-fold vs. 4-fold), SREBP1-c (8-fold vs. 4-fold), and ABCA1 (7-fold vs. 1.4-

fold) by T317 compared with GW3965. Hence, in liver, GW3965 is a gene selective modulator. In small intestine, both compounds induce ABCA1 to a similar extent. Furthermore, administration of GW3965 at 10 mpk (bid) to the same mouse model used in this study induces ABCA1 robustly in peripheral macrophages (4). Hence, GW3965 is also a tissue selective modulator of LXRα activity. We note that GW3965 is also a less potent activator of LXRβ compared with T317 in HepG2 cells and does not reach saturation even at 10 μM (Fig. 5E). Our results suggest that LXRα is the predominant LXR subtype modulating genes that regulate TG synthesis in mouse liver, and our results are consistent with similar conclusions based on an analysis of LXRα and LXRβ null mice (25, 26).

We note that HDL-C elevation by GW3965 is 77% of that by T317, even at the highest dose (Fig. 1). This is consistent with the activity of GW3965 being less than that of T317, even at saturating concentrations of GW3965. Increasing the transactivating potential of LXR ligands may increase ABCA1 induction and HDL elevation. However, this may be accompanied by greater induction of FAS and SREBP1-c in the liver, an undesirable outcome. There may be a tradeoff between inducing peripheral ABCA1 (reverse cholesterol transport) and hepatic SREBP1-c and FAS. Alternatively, the lower induction of HDL-C by GW3965 could be due to a more efficient induction of genes that cause HDL-C clearance in the liver, e.g., SR-B1. The dose of T317 utilized (50 mpk) was chosen from several reports, indicating that this dose is an efficacious dose (15, 19), with robust gene induction and HDL-C elevation.

Differential coactivator and corepressor recruitment have been shown to be determinants of tissue specificity of nuclear receptors, e.g., the selective estrogen receptor modulators, tamoxifen and raloxifen (27). Similarly, the relative expression levels of coregulators are also important determinants for tissue selectivity (27). For LXRα, SRC-1 and DRIP are recruited to similar levels with T317 and GW3965 (Fig. 6). In contrast, GW3965 recruits CBP to a lesser extent when compared with T317. It is tempting to speculate that coactivators like SRC-1 and DRIP mediate the intestinal effects on gene regulation, while CBP mediates liver effects of these two ligands through LXRα. We note that the difference in the extent of recruitment of CBP by the two ligands is less than the difference in induction of SREBP1-c or FAS in liver. Hence, other coactivators besides the ones tested may mediate the tissue selective differences observed with T317 and GW3965. We also note that the FRET assay is an in vitro assay, and chromatin immunoprecipitation studies need to be performed to determine the extent of cofactor recruitment in vivo.

Differences in the pharmacokinetics could also explain the differences in the activity of the two LXR ligands. GW3965 has 70% bioavailability, a serum half-life of 2 h, and a C_{max} of 12.7 μM after dosing at 10 mpk (4). Similar parameters for T317 were determined in C57Bl/6 mice after a 50 mpk oral dose of T317 administered as a suspension in 0.5% carboxymethylcellulose plus 2% Tween 80. The peak plasma concentration (C_{max}) was 5 μM, and the absolute oral bioavailability was approximately 15% (10 mpk IV dose). The apparent plasma half-life, after administration of the IV dose, was approximately 5 h. Further, following a single dose of GW3965 or T317 at 50 mpk, the liver concentration of each compound was greater than 50 μM for at least 8 h, and the integrated hepatic exposure values for 8 h were similar (413 μM·hr and 499 μM·hr for T317 and GW3965, respectively). Thus, the liver concentrations of both compounds were well above the EC_{50} for LXRα activation, and total hepatic exposure was comparable, making significant differences in pharmacokinetics an unlikely explanation for the differences in gene selectivity and TG accumulation seen in liver with the two LXR ligands.

We have compared two LXR ligands of different chemotypes in the same in vivo model and conclude that GW3965 is a tissue and gene selective modulator of LXRα activity. It induces ABCA1 in the small intestine (but not liver) with minimal effects on SREBP1-c and FAS in the liver of mice treated with high doses of GW3965. Unlike T317, it does not induce TG accumulation in the liver. Compared with T317, GW3965 is a very weak activator of LXRα in HepG2 cells. The reason for this difference is not clear at present but may involve selective coactivator recruitment. Further experiments need to be done to address this issue.

Our data demonstrate the feasibility of identifying SLRMs that inhibit the progression of atherosclerosis by increasing reverse cholesterol transport without the detrimental lipogenic effects in liver. Identification of these modulators will be an important challenge for the pharmaceutical industry.

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