Effect of liver X receptor activation on the very low density lipoprotein secretion and messenger ribonucleic acid level of related genes in goose primary hepatocytes[1]

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ABSTRACT In this study, we investigated the role of liver X receptor (LXR) activation in hepatic assembly and in the secretion of very low density lipoprotein-triglycerides in goose primary hepatocytes. Goose primary hepatocytes were isolated and treated with the LXR agonist T0901317. Total triglyceride accumulation, intracellular and extracellular triglyceride concentrations, extracellular very low density lipoprotein concentration, and gene expression levels of LXRα, microsomal triglyceride transfer protein, acyl coenzyme A:diacylglycerol acyltransferase (DGAT) 1, and DGAT2 were measured in primary hepatocytes. We found a dose-dependent upregulation of total and intracellular TG accumulation when using 0, 0.01, 0.1, 1, and 10 μ*M* T0901317, but the extracellular triglyceride and very low density lipoprotein concentrations were dose dependent only when the T0901317 concentration was below 1 μ*M*; as compared with 1 μ*M* T0901317, 10 μ*M* T0901317 had an inhibiting effect ($P < 0.05$). The mRNA levels of all the detected genes increased in the presence of T0901317. The change in LXRα and DGAT1 was dose dependent, and the mRNA levels of microsomal triglyceride transfer protein and DGAT2 increased with a T0901317 concentration up to 1 μ*M*, but decreased when treated with 10 μ*M* T0901317 ($P < 0.05$). In conclusion, the secretion of very low density lipoprotein plays a role in pharmacologically activating the LXR-induced development of hepatocellular steatosis in geese.

Key words: liver X receptor activation, assembly and secretion, very low density lipoprotein-triglyceride, goose

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INTRODUCTION

The assembly and secretion of very low density lipoprotein (**VLDL**)-triglycerides (**TG**) by the liver is a key process in hepatic steatosis. Some studies have examined the link between liver X receptor (**LXR**) activation and hepatic VLDL production. Treatment with the synthetic LXR agonist T0901317 results in hepatic steatosis and an increase in VLDL-TG secretion in mice and hamsters (Grefhorst et al., 2002; Basciano et al., 2009; Grefhorst and Parks, 2009). Several genes, in addition to LXR, are critical for the regulation of VLDL-TG assembly. Apolipoprotein B (**apoB**) is an essential component of these liver-derived VLDL and provides the integral protein component for the nascent VLDL particle. During the assembly of nascent VLDL particles with TG droplets, apoB-containing pre-VLDL particles are assembled within the lumen of the rough endoplasmic reticulum through a process partly catalyzed by the microsomal TG transfer protein (**MTTP**; Wu et al., 1996; Fisher and Ginsberg, 2002). Research has established that MTTP is required for VLDL assembly. Inhibition of MTTP impairs the assembly and probably the secretion of VLDL-TG particles and results in the intrahepatic accumulation of TG (Lettéron et al., 2003). Hepatic MTTP overproduction results in excessive VLDL-TG secretion and elevated plasma TG levels in mice (Tietge et al., 1999). Pharmacological inhibition of MTTP activity was shown to reduce VLDL production (Cuchel et al., 2007). There is emerging evidence that diminished MTTP activity is a compounding factor for advanced alcoholic liver disease (Day, 2006). Although the inverse relation between steatosis and VLDL production is self-evident in the case of MTTP blockers, in other conditions the relationship between steatosis and VLDL production is not straightforward,

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[1]The nucleotide sequence data reported in this paper have been submitted to the GenBank Submission (Los Alamos National Laboratories, Los Alamos, NM) nucleotide sequence database and have been assigned the accession numbers DGAT1: GW342946; DGAT2: GW342947; LXRα: HM138512; MTTP: GO240734; and apoB: GW342984.
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which can be illustrated by several examples. In obese *ob*/*ob* mice that have steatosis, hepatic VLDL production is not increased but instead is decreased (Li et al., 1997). This decrease in VLDL production, despite the high flux of fatty acids to the liver, contributes to the massive steatosis that is observed in these animals.

Acyl coenzyme A:diacylglycerol acyltransferase (**DGAT**) 1 and DGAT2 are found mainly in the endoplasmic reticulum (Shockey et al., 2006; Stone et al., 2006), where enriched DGAT activity is found and TG synthesis occurs (Yen et al., 2008). This suggests that one or both DGAT may play a direct role in synthesizing TG for incorporation into new VLDL-TG molecules. Overexpression of DGAT enzymes in cultured cells (Stone et al., 2004a) or in the liver (Millar et al., 2006; Monetti et al., 2007), skeletal muscle (Levin et al., 2007; Roorda et al., 2005), or adipose tissue (Chen et al., 2002, 2005) of mice leads to intracellular TG accumulation. Acyl coenzyme A:diacylglycerol acyltransferase 1 knockout mice have normal plasma TG levels, suggesting that this enzyme may not play a major role in modulating hepatic lipoprotein production (Smith et al., 2000). However, lipoprotein synthesis and secretion in DGAT1 knockout mice have not been studied extensively. Liang et al. (2004) showed that the overexpression of DGAT1 and DGAT2 in a rat hepatoma cell line results in increased secretion of TG and apoB. A study using DGAT2 knockout mice suggested that TG synthesis by DGAT2 may modulate the production of apoB-containing lipoproteins (Stone et al., 2004b). Millar et al. (2006) reported that DGAT1 or DGAT2 overexpression in the wild-type mouse liver results in increased hepatic TG content with no effect on the VLDL-TG or apoB production rate. Therefore, the role of DGAT in VLDL assembly is controversial.

During the last several years, we and other researchers have provided experimental evidence supporting the notion that the activation of LXR can result in hepatic steatosis and de novo lipogenesis, and we have found that VLDL-TG assembly and secretion play an important role in overfeeding-induced hepatic steatosis (Han et al., 2008), although there are some differences in the role of VLDL-TG assembly and secretion between geese and mammals. In mammals, hepatic steatosis is often accompanied by an increase in VLDL-TG assembly and secretion, but in geese, overfeeding-induced hepatic steatosis is followed by the inhibition of VLDL-TG assembly and secretion (Han et al., 2008). To investigate the role of LXR activation in regulating the assembly and secretion of VLDL and the development of steatosis in goose primary hepatocytes, we isolated primary hepatocytes from Sichuan white geese (*Anser cygnoides*) and investigated the effect of a synthetic LXR agonist, T0901317, on the intracellular and extracellular TG concentrations, the extracellular VLDL concentration, and the gene expression of related genes involved in the VLDL-TG assembly and secretion pathway.

MATERIALS AND METHODS

Primary Hepatocyte Isolation and Culture

Hepatocytes were isolated from three 10-d-old Sichuan White geese from the Experimental Farm for Waterfowl Breeding at Sichuan Agricultural University using a modification of the 2-step procedure described by Seglen (1976). The method differed from that of Seglen (1976) in that the liver was removed before the preperfusion step. Cellular viability was greater than 90%, as assessed by a trypan blue dye-exclusion test. Freshly isolated hepatocytes were diluted to 1×10^6 cells/mL. The culture medium was composed of Dulbecco's modified Eagle's medium (containing 4.5 g/L of glucose; Gibco, Carlsbad, CA) with 100 IU/L of insulin (Sigma, St. Louis, MO), 100 IU/mL of penicillin (Sigma), 100 g/mL of streptomycin (Sigma), 2 mmol/L of glutamine (Sigma), and 100 mL/L of fetal bovine serum (Gibco). The hepatocytes were then either plated in 60-mm culture dishes at 3×10^6 cells/dish for total RNA and nuclear protein isolation or plated in 24-well plates at 1×10^6 cells/well to measure TG levels and VLDL concentrations. Cultures were incubated at 40°C in a humidified atmosphere containing 5% CO_2, and the media were renewed after 3 h, followed by the addition of serum-free medium after 24 h. After another 24 h, cells were separately treated with serum-free medium supplemented with 0.01, 0.1, 1, or 10 μM T0901317 and incubated for 72 h, whereas the control sample cells were cultured with serum-free medium for 72 h (serum-free medium was renewed every 24 h). After the 72-h incubation, the culture media and cells were cooled on ice and collected for analysis of extracellular TG and VLDL concentrations in the media, as well as analysis of intracellular and total TG concentrations. In each case, the experiments were repeated 3 times.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay

The assay for cell viability was performed according to the method of Natali et al. (2007), with some modifications. Primary cultures of goose hepatocytes were plated at a density of 0.5×10^4 cells/well in a 96-well culture dish. After 3 h, the serum-rich medium was refreshed, followed by serum-free medium after 24 h. Cells were then separately treated with culture medium supplemented with 0.01, 0.1, 1, or 10 μM T0901317 and incubated for 24, 48, or 72 h, whereas the control sample cells were cultured with serum-free medium (serum-free medium was renewed every 24 h). Cell monolayers were then incubated for 4 h with 1 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (**MTT**). Mitochondria of living cells convert the yellow tetrazolium compound to its purple formazan derivative. After removal of the unconverted MTT,

the formazan product was dissolved in isopropanol, and the absorbance of formazan dye was measured at 490 nm.

Bromodeoxyuridine-Incorporation Assay

Primary cultures of goose hepatocytes were plated at a density of 0.5×10^4 cells/well in a 96-well culture dish. After 3 h, the serum-rich medium was refreshed, followed by serum-free medium after 24 h. Cells were then separately treated with culture medium supplemented with 0.01, 0.1, 1, or 10 μM T0901317 and incubated for 72 h, whereas the cells from the control sample were cultured with serum-free medium (serum-free medium was renewed every 24 h). Cell monolayers were then incubated for 24 h with 10 μM bromodeoxyuridine in culture medium. The cells were then washed and fixed, and the incorporated bromodeoxyuridine was detected by a specific ELISA (Roche, Indianapolis, IN) in an ELISA reader (Thermo Fisher Scientific Inc., Asheville, NC).

Isolation of Total RNA and Real-Time Reverse Transcription-PCR

Total RNA was isolated from cultured cells using Trizol (Invitrogen, Carlsbad, CA) and reverse-transcribed using the PrimerScript reverse transcription system kit for real-time PCR (TaKaRa, Dalian, China) according to the manufacturer's instructions (http://www.takara.com.cn/?action=Page&Plat=pdetail&newsid=536&subclass=1). The quantitative real-time PCR reaction contained the newly generated cDNA template, SYBR Premix Ex Taq (TaKaRa), sterile water, and primers of target genes. Real-time PCR was performed on an iCycler iQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA; 1 cycle of 95°C for 10 s, followed by 40 cycles of 95°C for 5 s, and 60°C for 40 s). An 80-cycle melt curve was performed, beginning at 55°C and increasing by 0.5°C every 10 s, to determine primer specificity. Specific primers are listed in Table 1 and were designed according to the goose gene sequences, DGAT1: GenBank No. GW342946; DGAT2: GenBank No. GW342947; LXRα: GenBank No. HM138512; MTTP: GenBank No. GO240734; apoB: GenBank No. GW342984; 18S: GenBank No. L21170; and β-actin: GenBank No. M26111.

Amplicons corresponding to each target were examined by agarose gel to confirm the presence of a unique band of the expected size. Negative controls, which consisted of PCR amplifications with non-reverse-transcribed RNA, did not generate any signal. All samples were amplified in duplicate, with the same PCR mixture and in the same 96-well plate. The cycle threshold (**Ct**) variation observed between duplicates was on average 0.12 ± 0.1, thus demonstrating a high level of intraassay reproducibility. Each sample was also repeated in another 96-well plate. The variation in Ct between the 2 independent plates was 0.28 ± 0.22, showing a fair level of interassay reproducibility as well. The PCR products were then diluted 16-fold and were used to generate the calibration curve and the amplification rate (R) for each gene (LXRα, MTTP, DGAT1, DGAT2, β-actin, and 18S). For each experimental sample, a normalized target gene level (Exp), corresponding to the target gene expression level relative to the β-actin and 18S (housekeeping genes) expression levels, was determined by the $2^{-\Delta\Delta Ct}$ method, as described previously (Livak and Schmittgen, 2001):

$$\mathrm{Exp}_{\text{target gene in sample}} = (1 + \mathrm{R}_{\text{target gene}})^{\mathrm{Ct(target\ gene\ in\ sample)}} / (1 + \mathrm{R}_{\beta\text{-actin and 18S}})^{\mathrm{Ct}(\beta\text{-actin and 18S in sample})}.$$

For the analyses of target gene expression, the normalized target gene expression level for each sample was compared with the positive control sample. Therefore, the final results were expressed as n-fold differences in the normalized target gene expression level between each treated and control sample.

Measurement of Total TG Accumulation, Intracellular and Extracellular TG Concentrations, and Extracellular VLDL Concentrations

The cell samples used for detecting intracellular TG concentrations were collected and shaken for 1 h using an ultrasonic processor, and then washed 3 times with

Table 1. Primer sequences for real-time PCR

Gene name[1]	Upstream (5′–3′)	Downstream (5′–3′)	Product size (bp)
DGAT2	CGCCATCATCATCGTGGT	CGTGCCGTAGAGCCAGTTT	113
MTTP	CCCGATGAAGGAGAGGAA	AAAATGTAACTGGCCTGAGT	85
LXRα	CCCAGCCCTTCCCACAAACT	CTGCCTCGCTTCACGGTTATTAG	156
DGAT1	CCTGAGGAACTTGGACACG	CAGGGACTGGTGGAACTCG	265
apoB	CTCAAGCCAACGAAGAAG	AAGCAAGTCAAGGCAAAA	153
β-Actin	CAACGAGCGGTTCAGGTGT	TGGAGTTGAAGGTGGTCTCG	92
18S	TTGGTGGAGCGATTTGTC	ATCTCGGGTGGCTGAACG	129

[1]DGAT2 = acyl coenzyme A:diacylglycerol acyltransferase 2; MTTP = microsomal triglyceride transfer protein; LXRα = liver X receptor α; DGAT1 = acyl coenzyme A:diacylglycerol acyltransferase 1; apoB = apolipoprotein B.

ice-cold PBS and added to an isovolumic mixture of chloroform and methanol (2:1, vol/vol). Samples used to measure total and extracellular TG concentrations were collected and shaken for 1 h using an ultrasonic processor and were then added directly to an isovolumic mixture of chloroform and methanol (2:1, vol/vol). The TG level was quantified by a colorimetric method (Fossati and Prencipe, 1982) using a TG glycerol-3-phosphate oxidase-peroxidase assay kit (Biosinc, Shanghai, China).

Measurement of Extracellular VLDL Concentration

Culture media samples used to measure extracellular VLDL concentrations were collected and centrifuged for 20 min at 1,000 × *g* at 4°C, and the supernatant was used to measure the extracellular VLDL concentration using a Chicken Very Low Density Lipoprotein ELISA kit (GBD, San Diego, CA). As described in the manufacturer's instructions, the microtiter plate provided in this kit had been precoated with an antibody specific to VLDL. Standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for VLDL, and avidin conjugated to horseradish peroxidase was added to each microplate well and incubated. A 3,3′5,5′ tetramethyl-benzidine substrate solution was then added to each well. Only those wells that contained VLDL, biotin-conjugated antibody, and enzyme-conjugated avidin exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution, and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of VLDL in the samples was then determined by comparing the optical density value of the samples to the standard curve.

Statistical Analysis

The data were subjected to ANOVA and the means were compared for significance by Tukey's test. Analysis of variance and a *t*-test were performed using the SAS 6.12 software package (SAS Institute Inc., Cary, NC). The results are presented as the mean ± SD.

RESULTS

Effect of T0901317 on the Viability of Goose Hepatocytes

Cell viability was determined by the optical density value at 490 nm by MTT assay. Treatment with T0901317 at all concentrations had a significant effect ($P < 0.05$) on cell viability as compared with the control group, and cell viability showed an upward trend with increasing T0901317 concentrations (Figure 1). The culture time (24 h, 48 h and 72 h) with 0.01, 0.1, or 10 μ*M* T0901317 had no evident effect on cell viability; in the group treated with 1 μ*M* T0901317, the 48-h culture time had an evident effect on cell viability as compared with the effects of culturing for 24 and 72 h.

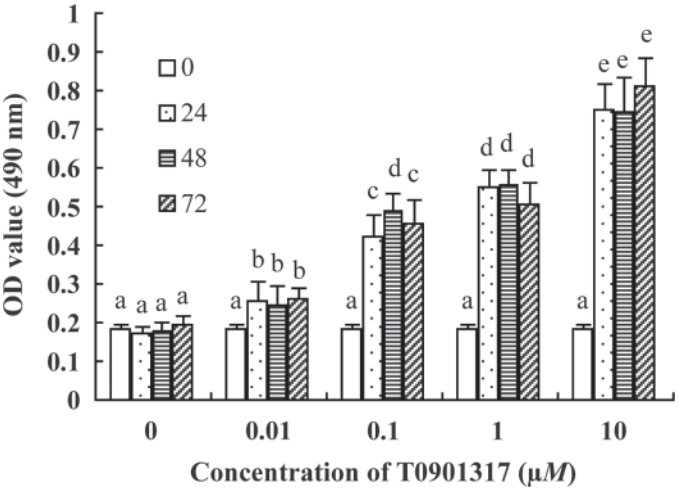

Figure 1. Viability of goose hepatocytes treated with T0901317 for 0 to 72 h by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Different lowercase letters (a–e) indicate differences among treatments ($P < 0.05$). After 24 h in serum-free medium, hepatocytes were incubated for 0 to 72 h in medium with nothing added as a control or with T0901317 added to a concentration of 0.01, 0.1, 1, or 10 μ*M*. OD = optical density.

A bromodeoxyuridine incorporation assay was performed to measure the changed DNA synthesis of treated cells as for the [^{3}H]thymidine incorporation assay (Figure 2). Data from Figure 2 in goose primary hepatocytes showed the DNA synthesis rate had no evident change ($P > 0.05$) after treatment with 0, 0.01, 0.1, and 1 μ*M* T0901317.

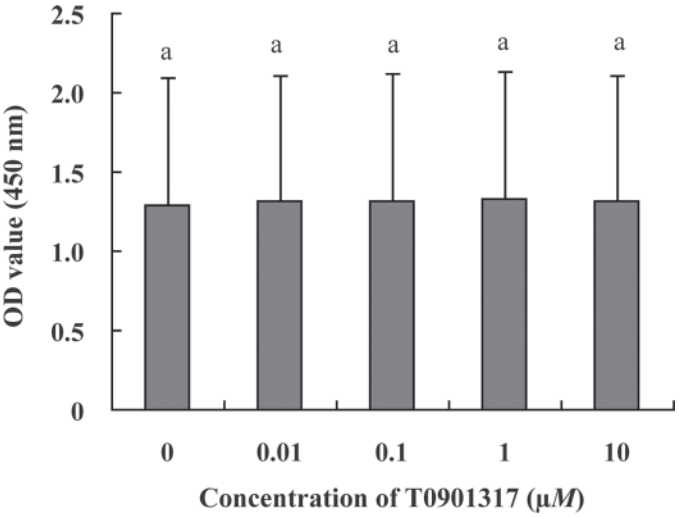

Figure 2. Viability of goose hepatocytes treated with T0901317 for 48 h by a bromodeoxyuridine-incorporation assay. The same lowercase letters indicate there was no difference among treatments with $P < 0.05$. After 24 h in serum-free medium, hepatocytes were incubated for 48 h in medium with nothing added as a control or with 0.01, 0.1, 1 or 10 μ*M* T0901317. OD = optical density.

Table 2. Intracellular and extracellular triglyceride (TG) concentrations of goose primary hepatocytes treated with T0901317[1]

T0901317 (μM)	Intracellular TG concentration (mM)	Extracellular TG concentration (mM)	Total TG accumulation (mM)
0	0.203 ± 0.012[a]	0.210 ± 0.026[a]	0.703 ± 0.056[a]
0.01	0.237 ± 0.023[a]	0.217 ± 0.031[a]	0.891 ± 0.092[b]
0.1	0.344 ± 0.024[b]	0.228 ± 0.024[a]	0.972 ± 0.126[b]
1	0.649 ± 0.025[c]	0.385 ± 0.047[c]	1.145 ± 0.154[c]
10	0.961 ± 0.082[d]	0.270 ± 0.031[b]	1.228 ± 0.213[d]

[a–d]Different lowercase letters in the same array indicate statistically significant differences between treatments ($P < 0.05$).

[1]Data are shown as the mean ± SD. After 24 h in serum-free medium, hepatocytes were incubated for 72 h with nothing added as a control or with the addition of 0.01, 0.1, 1, or 10 μM T0901317.

Effect of T0901317 on Total TG Accumulation and on Intracellular and Extracellular TG Concentrations

As shown in Table 2, T0901317 induced an increase in total TG accumulation in a dose-dependent manner from 0 to 10 μM. Doses of 0.01, 0.1, 1, or 10 μM T0901317 all had a significant effect ($P < 0.05$) compared with the control group, and the increase in TG accumulation induced by 10 μM T0901317 was the most evident ($P < 0.05$).

The regulation of intracellular TG mass by T0901317 was similar to that of total TG accumulation, and also occurred in a dose-dependent manner. The amount of extracellular TG in the medium after incubation with T0901317 was compared with that in the control medium. Incubations with 0.01 and 0.1 μM T0901317 had no significant effect ($P > 0.05$), but incubations with 1 and 10 μM T0901317 clearly had an influence. Compared with the effect of 1 μM T0901317 on extracellular TG accumulation, 10 μM T0901317 showed an inhibiting effect.

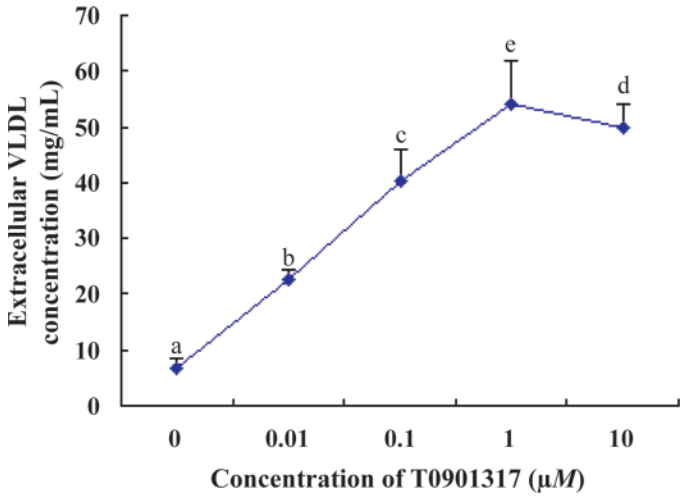

Figure 3. Extracellular very low density lipoprotein (VLDL) concentrations of goose primary hepatocytes treated with T0901317. Data are shown as the mean ± SD. Different lowercase letters (a–e) indicate statistically significant differences between treatments ($P < 0.05$). After 24 h in serum-free medium, hepatocytes were incubated for 72 h without the addition of T0901317 as a control or with 0.01, 0.1, 1, or 10 μM of T0901317. Color version available in the online PDF.

Effect of T0901317 on Extracellular VLDL Concentration

Figure 3 shows the regulation of the extracellular VLDL concentration in response to doses of T0901317 ranging from 0 to 10 μM. Incubating hepatocytes with 0.01, 0.1, 1, and 10 μM T0901317 increased the extracellular VLDL concentration ($P < 0.05$). Compared with the effect of 1 μM T0901317, 10 μM T0901317 had an inhibiting effect on extracellular VLDL concentration ($P < 0.05$).

Effect of T0901317 on Relative mRNA Levels

The genes involved in the assembly and secretion of VLDL-TG may respond to the activation of T0901317. Table 3 presents the effect of T0901317 on the expression of LXRα, DGAT-1, DGAT-2, and MTTP by quantitative real-time PCR analysis. The gene expression levels of LXRα and DGAT1 were similarly regulated by T0901317 in a dose-dependent manner. The effect of T0901317 on MTTP and DGAT2 gene expression levels was similar, and for the most part, this effect increased along with T0901317 concentration, but when T0901317 concentration reached 10 μM, the effect decreased. Compared with the effect of T0901317 on the mRNA levels of other genes, DGAT1 gene expression levels were affected the most (up to 90-fold).

DISCUSSION

To investigate whether LXR activation would directly stimulate VLDL-TG secretion in goose liver cells, we performed experiments with goose primary hepatocytes treated with LXR agonist T0901317. In these in vitro studies, when the T0901713 concentration was between 0 and 1 μM, we found gene expression profiles of MTTP and DGAT consistent with stimulated cellular TG synthesis and VLDL secretion. Actions of the LXR agonist in hepatocytes may be responsible for the observed increase in VLDL-TG production rate in

vivo because these cells are primarily involved in this process. It should be stressed that increased hepatic de novo lipogenesis is not always associated with increased VLDL-TG secretion. For instance, the strong induction of de novo lipogenesis on inhibition of glucose-6-phosphatase activity in rats is not associated with an increase in VLDL production (Bandsma et al., 2001). Likewise, genes encoding proteins involved in the assembly and secretion of VLDL, such as *MTTP*, were not affected upon in vivo treatment with the LXR agonist (Grefhorst et al., 2002). We found that the effects of T0901317 on the gene expression levels of MTTP, apoB, and DGAT2 were similar to the regulation of extracellular TG and VLDL concentrations. The activation of LXR could activate the gene expression of MTTP and apoB and upregulate extracellular TG and VLDL concentrations. Compared with the effect of 1 μM T0901317, extracellular TG and VLDL concentrations and expression levels of MTTP, apoB, and DGAT2 were decreased in goose hepatocytes cultured with 10 μM T0901317, although the total and intracellular TG concentrations increased. These results indicate that a high concentration of T0901317 could disturb the pathway of VLDL-TG assembly and secretion, which could contribute to the formation of hepatocellular steatosis. Thus, we predicted that in hepatocellular steatosis induced by high concentrations of the LXR agonist, MTTP, apoB, and DGAT2 might inhibit VLDL-TG assembly.

Increased TG synthesis is frequently associated with VLDL overproduction. This has led to the common belief that hepatic TG synthesis can directly modulate VLDL production. Triglyerides in hepatocytes are not an inert storage unit but are continuously recycled (Gibbons et al., 2000). Intracellular TG undergo lipolysis in larger quantities than necessary to form VLDL-TG. The fatty acids that are not built into VLDL are reesterified into TG and transported back into the cytoplasmic pool. When this equilibrium is disturbed, TG accumulation can occur. In this study, when the T0901317 concentration was greater than 1 μM, TG metabolism was disturbed and TG accumulation and VLDL secretion were increased. When the T0901317 concentration reached 10 μM, the VLDL secretion pathway was disturbed, most of the intracellular TG could not be transported out of the cells, and hepatocellular steatosis occurred.

In the liver, the complex effects of DGAT1 and DGAT2 on TG homeostasis and on the assembly and secretion of VLDL have been studied in cell cultures and animal models. The activities of DGAT1 and DGAT2 are compartmentalized into an overt fraction and a latent fraction (Owen et al., 1997; Abo-Hashema et al., 1999; Waterman et al., 2002), which may catalyze the synthesis of TG designated for storage in lipid droplets or for secretion in nascent lipoproteins, respectively. Based on the topology of DGAT1 and DGAT2, DGAT1, but not DGAT2, could contribute to the luminal (latent) activity. When tested in vivo with adenovirus, short-term overexpression of DGAT1, but not DGAT2, in the livers of mice resulted in an increase in endoplasmic reticulum luminal DGAT activity and hepatic TG secretion (Yamazaki et al., 2005). In a different study using mice 4 d after adenoviral infection, overexpression of both DGAT1 and DGAT2 increased cytosolic TG levels in hepatocytes but did not alter microsomal or VLDL-TG levels, suggesting that neither enzyme is involved in determining the rate of TG secretion in VLDL and that factors other than DGAT1 or DGAT2 activity are limiting (Millar et al., 2006). Thus, the existing data are mixed concerning whether the overexpression of DGAT1 increases the capacity for TG secretion from the liver. Studies in transgenic mice confirm that the overexpression of either DGAT enzyme can cause TG accumulation in cytosolic lipid droplets in the liver (Levin et al., 2007). Interestingly, small increases in DGAT2 mRNA in murine liver were sufficient to promote marked TG accumulation and hepatic steatosis in vivo, whereas relatively larger increases in DGAT1 mRNA levels produced a smaller increase in hepatic TG storage (Levin et al., 2007). Using goose primary hepatocytes, we found the regulation of DGAT1 mRNA levels by 0, 0.01, 0.1, 1, and 10 μM T0901317 to be consistent with the change in concentrations of total and intracellular TG. However, only when the T0901317 concentration was less than 1 μM was the DGAT2 mRNA level similar to the changes in DGAT1 mRNA levels, whereas 10 μM T0901317 inhibited the DGAT2 gene expression level when compared with inhibition of the DGAT2 mRNA levels after treatment with 1 μM T0901317, indicating that DGAT2 may play a more important role in VLDL-TG assembly and secretion than DGAT1.

Table 3. Relative mRNA levels of liver X receptor α (LXRα), microsomal triglyceride transfer protein (MTTP), acyl coenzyme A:diacylglycerol acyltransferase (DGAT) 1, DGAT2, and apolipoprotein B (apoB) in goose primary hepatocytes treated with different concentrations of T0901317[1]

Gene name	0 μM	0.01 μM	0.1 μM	1 μM	10 μM
LXRα	1.00 ± 0.31^{a}	1.58 ± 0.32^{a}	2.73 ± 0.64^{b}	4.12 ± 1.63^{c}	6.42 ± 1.89^{d}
MTTP	1.00 ± 0.18^{a}	1.07 ± 0.22^{a}	1.35 ± 0.41^{b}	6.02 ± 1.19^{c}	1.95 ± 0.43^{b}
DGAT1	1.00 ± 0.26^{a}	2.15 ± 0.45^{b}	21.69 ± 4.51^{c}	55.24 ± 17.6^{d}	93.67 ± 18.50^{e}
DGAT2	1.00 ± 0.50^{a}	3.07 ± 0.98^{b}	22.43 ± 3.48^{c}	62.16 ± 14.17^{d}	11.21 ± 4.03^{e}
apoB	1.00 ± 0.29^{a}	2.24 ± 0.52^{b}	4.13 ± 0.89^{c}	8.26 ± 1.98^{d}	4.26 ± 1.11^{c}

[a–e]Different lowercase letters in the same row indicate statistically significant differences among the treatments with $P < 0.05$.

[1]Data are shown as the mean ± SD. After 24 h in serum-free medium, hepatocytes were incubated for 72 h without the addition of T0901317 as a control or with 0.01, 0.1, 1, or 10 μM T0901317.

Moreover, LXR activation has been shown to regulate its targets differentially in different species (Menke et al., 2002). Liver X receptor activation induces yp7a1 in rats and mice, but not in humans or rabbits (Menke et al., 2002). Only certain genes are differentially regulated between species. In vivo activation of LXR using T0901317 in hamsters led to increased plasma TG, apoB, and VLDL-TG, and the activation of LXR in primary hamster hepatocytes increased the secretion of newly synthesized apoB, although the levels of MTTP mRNA and protein were unchanged and apoB mRNA were slightly decreased (Basciano et al., 2009). Jamil et al. (1998) used an MTTP inhibitor to inactivate MTTP in HepG2 cells and found that the resulting decrease in MTTP activity produced a proportional decrease in apoB secretion. Grefhorst et al. (2002) found no appreciable change in apoB100 or apoB48 in C57BL/6J mice treated with T0901317. In goose primary hepatocytes, LXR activation could upregulate the mRNA level of MTTP. This may have been due to species-specific differences in hepatic lipid mobilization. In conclusion, the impairment of VLDL secretion plays a role in the pharmacologically activated development of hepatic steatosis induced by LXR activation.

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