Synergistic Activation of Human LDL Receptor Expression by SCAP Ligand and Cytokine Oncostatin M

Jingwen Liu, Fang Zhang, Cong Li, Meihong Lin, Michael R. Briggs

Objective—A recent study identified a new class of compounds designated as the sterol-regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) ligands that putatively bind to SCAP, leading to increased LDL receptor (LDLR) expression. In this study, we examined the effects of SCAP ligand GW707 in comparison with lovastatin and cytokine oncostatin M (OM) on the regulation of LDLR expression in cultured HepG2 cells.

Methods and Results—Our studies uncovered several new features that distinguish SCAP ligand from lovastatin, a classic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, and from OM, which utilize an SREBP-independent regulatory pathway. We show that the induction of LDLR mRNA expression by GW707 is not affected by intracellular cholesterol but is completely abolished by blocking de novo protein synthesis. Moreover, the effects of GW707 but not lovastatin on LDLR promoter activity, mRNA expression, and uptake of 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanin perchlorate–LDL are markedly enhanced by OM. We further demonstrate that the amounts of the mature form of SREBP-2 translocated to the nucleus under GW707 treatment are increased by costimulating cells with OM.

Conclusions—Our studies provide the first evidence that higher levels of LDLR expression and function can be achieved through simultaneous stimulation of the SREBP-dependent and SREBP-independent pathways, suggesting a strategy to develop an adjunct therapeutic intervention utilizing both pathways. (*Arterioscler Thromb Vasc Biol*. 2003;23:90-96.)

Key Words: LDL receptor ∎ SCAP ligand ∎ oncostatin M ∎ sterol-regulatory element ∎ sterol-independent regulatory element

Increased LDL cholesterol (LDL-c) in plasma is a widely recognized risk factor for atherosclerosis and 1 underlying cause of cardiovascular diseases.[1,2] The LDL receptor (LDLR) plays a pivotal role in the control of plasma cholesterol levels.[3,4] LDL-c binds to the LDLR expressed on the surface of hepatocytes and is removed from the circulation by LDLR-mediated endocytosis. Thus, the expression level of hepatic LDLR directly influences plasma LDL-c.

LDLR expression is predominantly regulated at the transcriptional level through a negative-feedback mechanism by the intracellular cholesterol pool. This regulation is controlled through specific interactions of the sterol-regulatory element (SRE-1) of the LDLR promoter[5,6] and a family of SRE-binding proteins, namely, SREBP-1 and SREBP-2.[7–10] SREBPs are members of the basic helix-loop-helix leucine zipper family of transcription factors. SREBPs contain 2 transmembrane domains and are localized to the endoplasmic reticulum (ER) after synthesis. In the inactive state within the ER, SREBP associates with another transmembrane protein, SREBP cleavage-activating protein (SCAP), that provides conditional chaperone activity to the SREBP.[11–13] The SCAP contains a cholesterol-sensing domain that responds to the depletion of sterol with activation of SCAP-SREBP–trans-porting activity.[14–16] Under cholesterol-depleted conditions, SCAP transports SREBP to the Golgi, where the NH₂-terminal transcription-activation domain of the SREBP is released from the precursor protein through specific proteolytic cleavages.[12] The active form of the SREBP translocates to the nucleus, binds to its cognate SRE-1 site, and activates transcription of the LDLR gene. In contrast, under cholesterol-replete conditions, the SCAP-SREBP complex remains in an inactive form in the ER through active repression by cholesterol and oxysterols, and LDLR gene transcription is maintained at a minimal constitutive level.

Clinically, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, including lovastatin and its derivatives, have been the most widely prescribed drugs for the treatment of hypercholesterolemia.[1,2] They effectively lower the plasma concentration of LDL-c and reduce mortality and morbidity from coronary artery disease. Statins specifically inhibit the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. The inhibition of cellular cholesterol biosynthesis leads to a depletion of intracellular cholesterol and activation of SCAP-SREBP–transporting activity, thereby resulting in upregulation of the LDLR and subsequent lowering of LDL-c in the blood.

However, despite the successes with statin treatment, the efficacy in lowering plasma LDL-c, especially with respect to the newer, more stringent guidelines,[17] is seen as a challenge for most statin therapies. Limitations of dose caused by side effects and intolerance in some patients provide an impetus to develop new therapeutic agents to reduce plasma LDL-c.

Very recently, another class of potential cholesterol-lowering drugs was described by investigators from Glaxo-SmithKline.[18] These agents function presumably as SCAP ligands, because they have been shown to interact with the SCAP sterol-sensing domain and activate SCAP-SREBP–transporting activity. In vitro studies showed that treating cells with SCAP ligand GW707 increased the translocation of SREBP-1 and SREBP-2 into the nucleus and elevated LDLR mRNA and protein expression. In vivo, administration of SCAP ligand GW532 to hyperlipidemic hamsters reduced LDL-c and triglycerides up to 80%, with a 3-fold increase in LDLR mRNA in the liver. Thus, these novel compounds may emerge as a new class of promising LDL-c–lowering therapeutic drugs.[19] However, the mechanism(s) whereby SCAP ligand activates SCAP-SREBP–transporting activity is currently unclear, and the effects of these compounds on the activation of LDLR transcription have not been fully examined.

Other than cholesterol and oxysterols, LDLR transcription can also be regulated through sterol-independent mechanisms, by growth factors[20–25] and cytokines.[26–32] However, among these nonsterol modulators, only for cytokine oncostatin M (OM) has a direct effect been clearly shown at the transcriptional level through a regulatory element located downstream of the SRE-1.[33] In HepG2 cells, OM induces rapid upregulation of LDLR transcription, with a 3- to 5-fold increase in the LDLR mRNA level after a 1-hour treatment, and LDLR mRNA expression remains elevated for 24 hours. The regulatory element that mediates the OM effect has been identified in our laboratory and is designated as a sterol-independent regulatory element (SIRE).[33] The SIRE is located in the LDLR promoter region (-17 to -1) that overlaps the previously described TATA-like sequences (-23 to -8).[34] The SIRE motif consists of a binding site for CCAAT/enhancer binding protein (c/EBP; -17 to -9) and a cAMP-responsive element (CRE, -8 to -1). Although mutations within the SIRE sequence have no effect on cholesterol-mediated suppression, even a single base alteration within the SIRE motif completely abolishes OM- and c-AMP–induced promoter activity and the activation synergy between OM and cAMP.

Thus, LDLR expression can be upregulated by 3 different classes of synthetic or endogenous modulators through different mechanisms and distinct cellular pathways. The aim of the present study was to evaluate the effect of SCAP ligand GW707 in comparison with OM and lovastatin on LDLR transcription, the first 2 acting directly on LDLR transcription mechanisms and the latter acting indirectly through cholesterol regulation. We further addressed the critical question of whether these agents could work together in an additive or a synergistic manner to upregulate LDLR expression with a potential for adjunct therapy.

Methods

Cells and Reagents
The human hepatoma cell line HepG2 was obtained from American Type Culture Collection (Manassas, Va) and was cultured in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS). GW707 was obtained from Ligand Pharmaceuticals. Human OM was obtained from R&D Systems. Cholesterol, 25-hydroxylcholesterol, lovastatin, and puromycin were purchased from Sigma.

LDL Uptake Assay
HepG2 cells were seeded in 6-well culture plates at a density of 0.8×10^6 cells/well in medium containing 10% FBS. On the next day, cells were washed with warm PBS, incubated in medium without serum, and treated with compounds for 18 hours. The fluorescent 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanin perchlorate (DiI)–LDL (Biomedical Technologies) at a concentration of 6 μg/mL was added to the cells at the end of treatment for 4 hours, and cells were then trypsinized. The mean red fluorescence of 2×10^4 cells was measured with a FACScan (Becton Dickinson).

Western Blots of SREBP-2
HepG2 cells seeded in 100-mm-diameter culture dishes were incubated with 10% delipidated serum (LPDS) overnight. After stimulation with inducers at the indicated concentration for 6 hours, nuclear extracts were isolated according to the vendor's protocol by using a nuclear extract isolation kit purchased from Pierce (NE-PER nuclear and cytoplasmic extraction reagents). Aliquots of each sample (50 μg protein) were separated by denaturing SDS–polyacrylamide gel electrophoresis (4% to 20%), transferred onto a nitrocellulose membrane, blotted with anti–SREBP-2 monoclonal antibody (IgG-1C6, 1:200; BD Biosciences), and detected with an enhanced chemiluminescence detection system (Amersham).

Plasmid Constructs
Native human LDLR promoter luciferase reporter construct pLDLR234Luc and the SRE-1 mutant (pLDLR234-R2 mu) have been described previously.[31] The SIRE mutants SIRE-mu4 and SIRE-mu6 were generated by site-directed mutagenesis on the template DNA (pLDLR234Luc) with a commercially available kit (QuikChange site-directed mutagenesis kit, Stratagene). The mutant vector SIRE-mu4 contains a single base substitution at the c/EBP site (TGCTGTAAA→TGCTcTAAA), and the vector SIRE-mu6 contains a single base mutation at the CRE site (TGACGT→gGACGT) of the SIRE motif.

Transient Transfections and Northern Blot Analysis
Detailed procedures for transfection and detection of LDLR mRNA expression and promoter activity were described previously.[31]

Results

Induction of LDLR Transcription by SCAP Ligand, Lovastatin, and OM With Different Kinetics
HepG2 cells were treated with effective doses of GW707 (1 μmol/L), lovastatin (1 μmol/L), and OM (50 ng/mL) for different lengths of time, and mRNA levels of LDLR and GAPDH were determined by Northern blot analysis. Figure 1A shows that the level of LDLR mRNA began to increase (1.8-fold of control) 2 hours after addition of GW707, and it reached a maximum level of 2.4-fold at 8 hours and remained elevated for 24 hours. In lovastatin-treated cells, the level of LDLR mRNA was notably increased (1.3-fold) by 15 hours, and a 1.8-fold increase was detected after 24 hours of
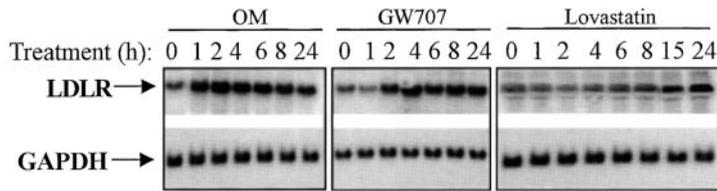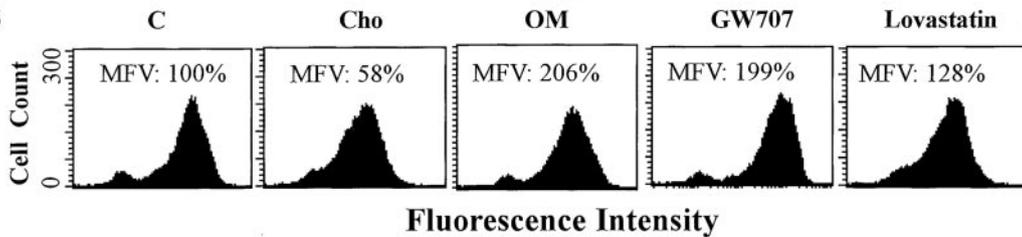
Figure 1. Effects of GW707, lovastatin, and OM on LDLR mRNA expression and LDLR function. (A) Northern blot: HepG2 cells cultured in EMEM containing 10% FBS were incubated with 1 μmol/L GW707 or 50 ng/mL OM individually for different times as indicated. For lovastatin treatment (1 μmol/L), cells were cultured in medium containing 10% LPDS. Total RNA was isolated, and 15 μg per sample was analyzed for LDLR mRNA by Northern blot. The membrane was stripped and hybridized to a human GAPDH probe. The figure shown is representative of 2 separate kinetic studies. (B) DiI-LDL uptake: HepG2 cells cultured in 10% LPDS medium were treated for 18 hours with 10 μg/mL cholesterol and 1 μg/mL 25-hydroxycholesterol, 50 ng/mL OM, 1 μmol/L GW707, or 1 μmol/L lovastatin. After treatment, DiI-LDL at a dose of 6 μg/mL was added to the medium, and cells were trypsinized 4 hours later. The uptake of DiI-LDL was measured by FACScan (Becton Dickinson) with 2×10^4 cells per sample. The mean fluorescence value (MFV) of control cells is expressed as 100%. The data shown are representative of 3 separate assays.

treatment. In contrast to GW707 and lovastatin, LDLR mRNA expression was rapidly increased by OM, with maximal induction of 2.8-fold by 1 hour, and it stayed at this elevated level for 24 hours. None of the drug treatments changed the level of GAPDH mRNA through the time course of the experiments.

To confirm the upregulatory effects of these agents on the induction of functional LDLR, LDL uptake assays with fluorescent DiI-LDL were conducted in HepG2 cells treated with GW707 (1 μmol/L), lovastatin (1 μmol/L), or OM (50 ng/mL) for 18 hours. In these experiments, cells treated with cholesterol and 25-hydroxycholesterol (Chol) were used as a positive control for LDLR regulation. Figure 1B shows that the amount of fluorescent dye that accumulated inside the cells was reduced by 42% when cells were incubated with Chol, thus demonstrating repression of LDLR expression. DiI-LDL uptake was increased approximately 2-fold by OM as well as by GW707, whereas a 28% increase was detected in lovastatin-treated cells. These results corroborate the finding on LDLR mRNA expression and confirm the inducing ability of these modulators on LDLR expression.

De Novo Protein Synthesis Is Required for GW Compound to Activate LDLR Transcription

The slower kinetics of GW707 and lovastatin versus the rapid action of OM on induction of LDLR mRNA expression suggest that the regulatory pathways of sterol-dependent and sterol-independent mechanisms may have different requirements for de novo protein synthesis. To address this question, we used the protein synthesis inhibitor puromycin. Cells were treated with puromycin for 1 hour, followed by a 4-hour incubation with OM or GW707 (Figure 2, lanes 1 through 6).

To examine the effects of sterol on the activity of GW707, the same treatment was applied to cells that were cultured in medium containing Chol (Figure 2, lanes 7 through 12). Although basal transcription of LDLR was decreased by 50% with cholesterol repression, GW707-induced and OM-induced transcriptions of LDLR were both unaffected by sterol. However, in contrast to sterol, puromycin produced differential effects. It totally abolished the activity of GW707 without an inhibitory effect on OM, although puromycin alone caused a slight decrease in the level of LDLR mRNA of controls. These results suggest that the upregulation of LDLR
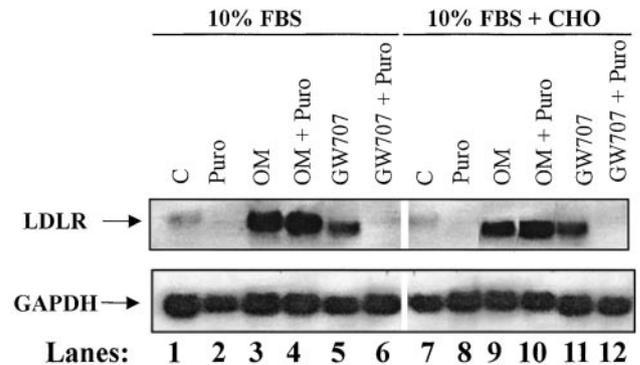

Figure 2. Inhibiting the effect of GW707 on the upregulation of LDLR transcription by puromycin. HepG2 cells were cultured overnight in 10% FBS/EMEM (lanes 1 through 6) or in 10% FBS/EMEM supplemented with 10 μg/mL cholesterol and 1 μg/mL 25-hydroxylcholesterol. Puromycin at a concentration of 150 μg/mL was added to cells 1 hour before the addition of GW707 or OM, and total RNA was isolated 4 hours later. LDLR and GAPDH mRNA expressions were analyzed by Northern blot. The figure shown is representative of 3 separate experiments.

transcription by GW707 depends on new protein synthesis, whereas the activity of OM on LDLR transcription proceeds in the absence of new protein synthesis. We could not assess the effect of puromycin on lovastatin-activated LDLR transcription, because lovastatin requires 15 to 24 hours to show a detectable inducing effect on LDLR mRNA; however, by then, a severe toxic effect caused by puromycin was already shown.

SRE-1 and SIRE Are Uniquely Involved in the Regulation of LDLR Transcription by SCAP Ligand, Lovastatin, and OM

The next set of experiments was designed to distinguish the dependency of these compounds on the promoter regulatory elements SRE-1 versus SIRE. First, the wild-type LDLR promoter luciferase reporter (pLDLR234Luc) and the SRE-1 mutant (pLDLR234-R2 mu) were transiently transfected into HepG2 cells along with a Rous sarcoma virus promoter–based β-galactosidase expression vector, BG2-β-gal, as an internal control of transfection efficiency. After transfection, cells were treated with inducers for 24 hours. Native LDLR promoter activities were increased 3.1-, 2-, and 10-fold by OM, lovastatin, and GW707, respectively. Mutation of the SRE-1 site to interrupt SREBP binding did not diminish the activity of OM at all but totally abolished the activities of lovastatin and GW707 (Figure 3A). Second, the effects of SIRE mutation on the activities of OM, GW707, and lovastatin were assessed. SIRE mutants, carrying a signal base mutation either at the c/EBP site (SIRE-mu4) or the CRE site (SIRE-mu6) of the SIRE motif, totally lost the response to OM, but they remained fully inducible for GW707 and lovastatin in a manner identical to the wild-type promoter (Figure 3B). These data clearly demonstrate that at the promoter level, there is no interplay between SRE-mediated and SIRE-mediated LDLR transcription. These 2 regulatory elements are utilized separately and independently.

GW707 Synergistically Activates LDLR Promoter Activity With OM

The results in Figure 3 suggested that an additive or even a synergistic effect of upregulation of LDLR might be achieved by simultaneous activation of the SRE-1–mediated and the SIRE-mediated regulatory pathways. To test this hypothesis, HepG2 cells were transfected with pLDLR234Luc and thereafter treated with lovastatin (3 μmol/L), OM (50 ng/mL), or GW707 (3 μmol/L), individually or combined, for 24 hours. Figure 4A shows that the effects of OM and lovastatin on the LDLR promoter were additive, whereas the effects of OM and GW707 were synergistic. To examine this synergy in detail, transfected HepG2 cells were treated with a wide concentration range of GW707 in the absence or presence of OM (Figure 4B). GW707 displayed a dose-dependent effect on LDLR promoter activity. At 0.2 μmol/L, LDLR promoter activity was increased 2.5-fold over controls and was further increased >6-fold at 2 μmol/L concentration. Strikingly, in the presence of OM, an 11-fold increase was obtained at 0.2 μmol/L concentration of GW707, although OM by itself only led to a 3-fold increase. These results demonstrate that although the SRE-1/SREBP pathway is utilized in the actions

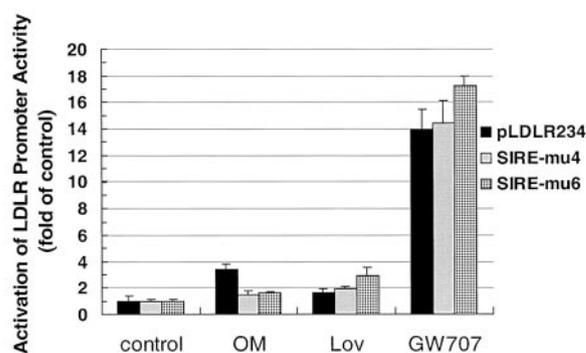**A** Effects of SRE-1 Mutation
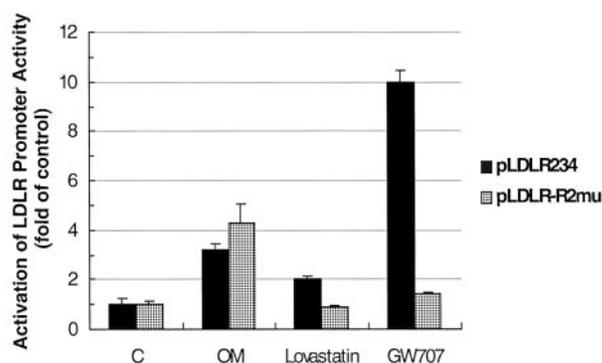
B Effects of SIRE Mutation

Figure 3. Analysis of wild-type and mutant LDLR promoter activities. HepG2 cells were either transiently transfected with pLDLR234Luc and the SRE-1 mutant reporter (A) or transfected with the SIRE mutant vectors (B). The expression vector for β-gal was cotransfected with the luciferase vectors as an internal control of transfection efficiency. After transfection, cells were treated with GW707, lovastatin, or OM for 24 hours. Cell lysates were collected, and luciferase and β-gal activities were measured. The normalized luciferase activity is expressed as the fold of luciferase activity in untreated control cells. The data (mean±SD) shown were derived from 3 independent transfection assays in which triplet wells were assayed for each condition.

of both GW707 and lovastatin, the effect of OM is synergistic with GW707 but additive with lovastatin.

Synergistic Induction of LDLR mRNA and Protein Expression by GW707 and OM

To determine whether the synergistic effect of GW707 with OM on LDLR promoter reporter activity reflected the changes in endogenous LDLR mRNA, HepG2 cells were treated with GW707 for different intervals in the absence or presence of OM. Figure 5A shows that GW707 alone at a suboptimal concentration of 0.5 μmol/L only increased the levels of LDLR mRNA to 28% over controls at 2 hours and to 95% over controls at 8 and 24 hours. However, in the presence of OM, the levels of LDLR mRNA were increased 287% over controls at 2 hours and 323% by 24 hours, whereas the maximal increase by OM itself was 179% over controls, which occurred at 2 hours. To determine whether this synergistic effect on LDLR transcription could be translated into enhanced induction of a functional LDLR, we
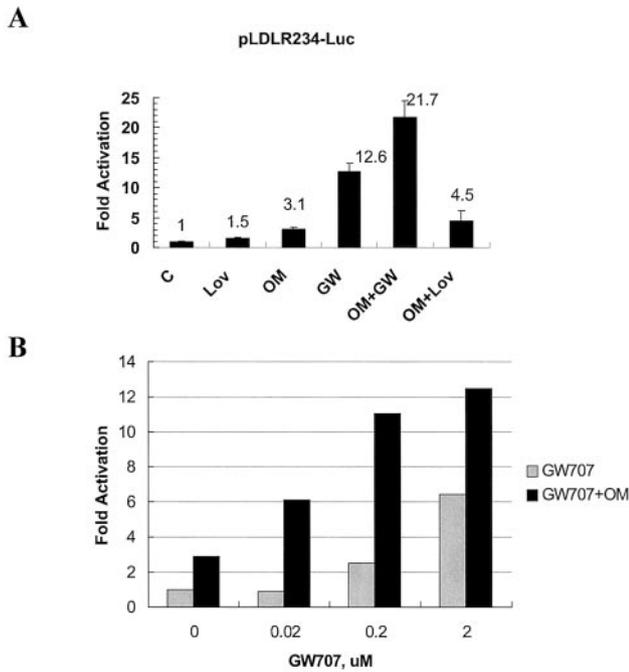
A

pLDLR234-Luc

B

Figure 4. Simultaneous activation of sterol-dependent and sterol-independent pathways leads to a synergistic stimulation of LDLR promoter activity. HepG2 cells were transfected with pLDLR234Luc and BG2-β-gal. In A, after transfection, cells were stimulated with a single dose of GW707 (1 μmol/L) or lovastatin (3 μmol/L) in the absence or presence of OM (50 ng/mL) for 24 hours. The asterisk indicates synergy. In B, the dose-dependent effects of GW707 on LDLR promoter activity were examined without or with OM. The normalized luciferase activity is expressed as the fold of luciferase activity in untreated control cells.

conducted the DiI-LDL uptake assay with control cells and with cells treated with GW707 alone, OM alone, or the combination. Because the activities of SCAP ligand and OM are not affected by intracellular sterol, to detect a maximal inducing effect Chol was present in the culture medium to suppress basal transcriptional activity. As shown in Figure 5B, the LDLR activity in taking up DiI-LDL was increased 150% by 0.5 μmol/L GW707 and 285% by OM individually. In contrast, adding GW compound and OM together increased the DiI-LDL uptake by >600%. These data together clearly demonstrate that LDLR expression and function are stimulated by GW707 and OM in a multiplicative manner.

The previous study reported by GlaxoSmithKline investigators showed that GW707 increased the nuclear fraction of SREBP-2 in Chinese hamster ovary cells that were transiently transfected with a herpes simplex virus–tagged precursor form of human SREBP-2. In an attempt to characterize the mechanisms underlying the enhancing effect of OM on the activity of GW707, we examined the GW707-induced maturation of endogenous SREBP-2 in the absence and presence of OM. As detected by Western blotting, although OM did not increase the mature form of the 68-kDa SREBP-2 by itself, it substantially increased the nuclear mature form of SREBP-2 in GW707-treated HepG2 cells (Figure 5C). These results suggest that elevated production of the transcription active form of SREBP-2 may account, at least in part, for the enhanced activity of GW707 on LDLR transcription by OM.
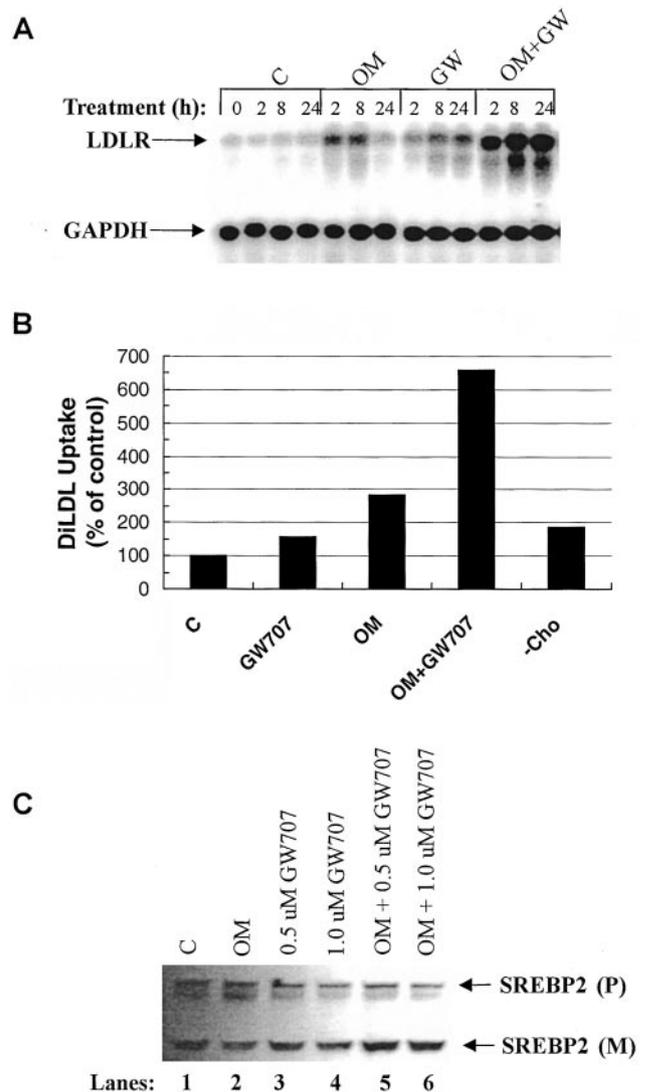
A

B

C

Figure 5. Examination of the effects of GW707 on LDLR mRNA expression, DiI-LDL uptake, and maturation of SREBP-2 in the absence and presence of OM. (A) LDLR mRNA expression: HepG2 cells were treated with 0.5 μmol/L GW707 or with 50 ng/mL OM individually or combined for the indicated lengths of time. Northern blotting was performed to analyze the levels of LDLR and GAPDH mRNAs. The LDLR mRNA signals were quantified and normalized to the signals of GAPDH. (B) DiI-LDL uptake: HepG2 cells cultured in serum-free medium containing 10 μg/mL cholesterol and 1 μg/mL 25-hydroxylcholesterol were treated with GW707 (0.5 μmol/L), OM (50 ng/mL), or OM plus GW707 for 18 hours, followed by addition of DiI-LDL for 4 hours. The mean red fluorescence intensity in untreated cells was expressed as 100%. The synergistic effect was also observed under culture conditions without cholesterol suppression. (C) Detection of the mature form of SREBP-2: HepG2 cells seeded in 100-mm-diameter culture dishes were incubated with 10% LPDS overnight. Cells were either left untreated (lane 1) or treated with 50 ng/mL OM (lane 2), 0.5 μmol/L GW707 (lane 3), 1 μmol/L GW707 (lane 4), or OM plus GW707 (lanes 5 and 6) for 6 hours. Nuclear extracts of 50 μg protein per sample were used to detect the mature form (M) and the precursor form (P) of SREBP-2 by Western blotting. as described in Methods.

Discussion

In this study, we compared the effects of 3 different classes of LDLR modulator, including the HMG-CoA reductase inhibitor lovastatin, SCAP ligand GW707, and cytokine OM. We

have shown that each of these agents regulates LDLR transcription with unique characteristics. Importantly, for the first time, we have demonstrated that upregulation of LDLR expression can be achieved through simultaneous activation of the SREBP-dependent and SREBP-independent pathways, resulting in a synergistic or an additive effect, depending on the agents used.

We showed that LDLR transcription was induced by these modulators with different kinetics. The maximal induction of LDLR mRNA expression by OM was ≈1 hour, by GW707 was 6 to 8 hours, and by lovastatin was ≈24 hours. The requirement for new protein synthesis is likely to be 1 of the factors accounting for the slower kinetics of GW707 and lovastatin. The activity of OM on LDLR transcription proceeds in the absence of de novo protein synthesis, a feature consistent with its rapid action and kinase pathway activation mechanisms. In contrast, even though a significant effect on LDLR mRNA expression by GW707 was detected by 2 hours, the SCAP ligand apparently required new protein synthesis to support its action on the activation of LDLR transcription. It has previously been reported that the regulatory mechanism of GW compound involves activation of the transporting activity of SCAP, resulting in the cleavage of SREBP precursor protein and translocation of the mature form of SREBP to the nucleus.[18] Thus, it is an unexpected finding that new protein synthesis is involved in the regulation of LDLR transcription by GW707. Our new finding raises the possibility that an unidentified protein with a rapid turnover rate might associate with SCAP and participate in the action of GW707. Alternatively, the processes of sequential proteolytic cleavages may require new protein synthesis. Additional studies will be needed to fully elaborate this observation.

By using SRE-1/SIRE mutant promoter constructs, we clearly demonstrate that the activities of GW707 and lovastatin are mediated entirely through the SRE-1 sequence, whereas the activity of OM is completely mediated through the SIRE sequence. Although the SRE-1 and SIRE sites are separated by only 39 nucleotides, these 2 regulatory elements are functionally separable. Apparently, at the promoter level, there is no cross-talk between sterol-dependent and sterol-independent pathways. On the basis of the results of promoter analysis, we thought that activation of both pathways would upregulate LDLR transcription in an additive manner. Surprisingly, we found that the LDLR mRNA and protein expressions (as measured by DiI-LDL uptake) were synergistically stimulated by treating cells with OM and GW707, whereas the effects of OM and lovastatin were additive. Because nuclear run-on assays demonstrated that GW707 and OM both regulate LDLR at the transcriptional level, it is unlikely that the observed enhanced induction of LDLR mRNA by these 2 agents was due to stabilization of the mRNA.

The previous study of SCAP ligands suggested the mechanism of action of the GW compounds as direct activation of SCAP/SREBP translocation, leading to the maturation of SREBP. One of the mechanisms responsible for the enhancing effect of OM on the activity of GW707 might be to promote this process. We have detected a moderate increase

in the amounts of mature SREBP-2 in the nuclear fraction of cells treated with GW707 plus OM compared with cells treated with GW707 alone. However, the magnitude of this effect (<2-fold) by OM was less than the extent of OM-induced LDLR transcription in GW707-treated cells. This implies that additional mechanisms are involved in this synergy, such as activation of intracellular signaling.

OM activates several signaling pathways in HepG2 cells, including the Janus kinase/signal inducers and activators of transcription pathway and the MAP kinase kinase (MEK)/extracellular signal–related kinase (ERK) kinase pathway.[32] It has been reported that the transactivating activity of SREBP could be enhanced by ERK-induced phosphorylation.[35,36] It is possible that the observed synergy between OM and GW707 partially results from an increased transactivation activity of SREBP because of the OM-induced activation of the ERK signaling cascade.

The precise mechanisms underlying the synergistic effect of OM and GW707 need to be investigated further. However, the new findings reported here may shed some light on the understanding of how SCAP ligand regulates SCAP/SREBP transporting activity. In addition, our studies provide the first illustration that higher levels of LDLR expression and function can be achieved through simultaneous stimulation of the SREBP-dependent and SREBP-independent pathways, at least, in the cell culture model. These results hold promise for developing an adjunct therapeutic strategy that drives both pathways.

Acknowledgments

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Jingwen Liu, Fang Zhang, Cong Li, Meihong Lin and Michael R. Briggs

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