In vivo activities of cytokine oncostatin M in the regulation of plasma lipid levels

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Abstract Our previous studies have demonstrated the activity of oncostatin M (OM) in stimulating the transcription of the human LDL receptor (LDLR) gene in HepG2 cells through a sterol-independent regulatory mechanism. The current studies were designed to determine whether this in vitro property of OM could be recapitulated in vivo to increase LDLR expression in cholesterol-loaded livers and consequently decrease plasma levels of LDL-cholesterol (LDL-C) and total cholesterol (TC) using hypercholesterolemic hamsters as an experimental model. We show that administration of human recombinant OM for 7 days in hamsters fed a high-fat diet significantly reduced plasma levels of TC, LDL-C, and triglyceride in dose- and time-dependent manners. This lipid-lowering effect was associated with increased hepatic LDLR mRNA expression, as determined by quantitative real-time RT-PCR. Additionally, hepatic fat storage and cholesterol content in the hypercholesterolemic animals were substantially reduced by OM treatment. As a consequence, the increased aminotransferase levels in the high-fat diet-fed hamsters were normalized nearly to baseline values. These results not only corroborate the in vitro finding of OM in the regulation of LDLR but also, for the first time, demonstrate that OM has a strong lipid-lowering effect under in vivo conditions in which the levels of circulating LDL-C are high and liver LDLR transcription is repressed.—Kong, W., P. Abidi, F. B. Kraemer, J-D. Jiang, and J. Liu. **In vivo activities of cytokine oncostatin M in the regulation of plasma lipid levels. *J. Lipid Res.* 2005.** 46: **1163–1171.**

Supplementary key words low density lipoprotein receptor • dyslipidemia • hepatosteatosis • sterol-independent regulatory element

Coronary heart disease is the major cause of morbidity and mortality in the United States and other Western countries (1). Increased plasma LDL-cholesterol (LDL-C) levels contribute directly to the development of atherosclerosis and coronary heart disease (2, 3). Because in humans more than 70% of LDL is removed from the circulation by LDL receptor (LDLR)-mediated uptake in the liver, expression levels of the hepatic LDLR have a profound effect on plasma cholesterol levels (4, 5); therefore, the regulation of liver LDLR expression has been considered a key mechanism by which therapeutic agents could interfere with the development of atherosclerosis.

Over the past several decades, statins, functioning as inhibitors of HMG-CoA reductase, have been extensively developed and applied in the clinical setting (6, 7). These small molecules are very effective at decreasing cholesterol and LDL-C levels, representing an extremely important development in the pharmacotherapy of dyslipidemia and atherothrombotic vascular disease. Compared with statins, the use of bioagents such as growth factors or cytokines in the treatment of lipid disorders has been little explored, despite accumulating evidence for the regulation of liver LDLR expression by a variety of endogenous factors (8–11).

Our laboratory has shown that oncostatin M (OM), a member of the interleukin-6 family of cytokines, is a strong inducer of LDLR expression in human HepG2 cells (12–16). The level of LDLR mRNA is increased markedly by 1 h of OM treatment and is sustained for more than 24 h in HepG2 cells cultured in the absence or presence of sterols. The OM-responsive element on the LDLR promoter has been identified and designated the sterol-independent regulatory element (SIRE) (17). The SIRE motif is located in the proximal LDLR promoter region (−17 to −1), downstream of the sterol-regulatory element-1 (SRE-1) site and Sp1 binding sites. It consists of a binding site for c/EBP (−17 to −9) and a cAMP-responsive element (−8 to −1). Treatment of HepG2 cells with OM immediately activates the transcription factors Egr1 and c/EBPβ, which form an active protein complex at the SIRE site and stimulate the transcription of the LDLR gene (18, 19). These studies to characterize the LDLR promoter pro-

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Abbreviations: C_T, threshold cycle; FC, free cholesterol; HFHC, high-fat and high-cholesterol; LDL-C, low density lipoprotein-cholesterol; LDLR, low density lipoprotein receptor, OM, oncostatin M; SIRE, sterol-independent regulatory element; SRE-1, sterol-regulatory element-1; SREBP, sterol-regulatory element binding protein; TC, total cholesterol; TG, triglyceride.

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vided us with a mechanistic understanding of the cholesterol-independent actions of OM on LDLR regulation, which was distinguished from the SRE-1/sterol-regulatory element binding protein (SREBP)-dependent action of statins.

In addition to HepG2 cells, OM has been shown to increase hepatic LDLR mRNA expression in apolipoprotein B (apoB)/cholesteryl ester transfer protein transgenic mice (20). However, the effects of OM on plasma cholesterol levels were not examined in that study. To corroborate our in vitro findings, in this study we examined the in vivo activities of OM in the regulation of hepatic LDLR expression and the modulation of plasma LDL-C levels in hypercholesterolemic hamsters, an experimental model system in which the effects of added cholesterol and fat on the kinetics of hepatic LDLR-mediated LDL clearance are well characterized (21–24).

MATERIALS AND METHODS

Animals and diets

Female Golden Syrian hamsters weighing 110–120 g, purchased from the National Vaccine and Serum Institute (Beijing, China), were housed in individual cages in an air-conditioned room with a 12 h light cycle. These animals had free access to a regular rodent chow diet containing 0.05% cholesterol for 2 weeks. With the exception of three hamsters that were continuously fed the normal diet as a diet control group, all hamsters were switched to a high-fat and high-cholesterol (HFHC) diet. The HFHC diet consists of 10% lard (0.25 g of cholesterol per 100 g of lard), 10% egg yolk powder (2 g of cholesterol per 100 g of yolk power), and 1% cholesterol. The total cholesterol (TC) content of the high-fat diet is 1.3%. After a period of 16 days of HFHC feeding, hamsters were randomly assigned to three groups (n = 8 per group): HFHC control group, HFHC OM 75 μg/kg/day group (~10 μg total dose/day), and OM 150 μg/kg/day group (~20 μg total dose/day). Human recombinant OM purified from CHO cells (20, 25) in PBS containing 1 mg/ml BSA at a volume of 100 μl was administered intraperitoneally to the treatment group twice a day in the morning (8 AM) and the afternoon (4 PM) for 7 days. In the second set of experiments to test the acute effects of OM, HFHC-fed hamsters (n = 4) were injected intraperitoneally with OM at a single dose of 300 μg/kg (40 μg/animal) and the animals were killed 36 h later for collection of blood and liver tissues.

Serum isolation and cholesterol determination

Blood samples (0.1 ml) were collected using the retro-orbital plexus under light ether-induced anesthesia after a 4 h fast before (day 0) and at the noted times during the treatment course. Four hours after the last treatment, the animals were killed and blood samples were collected by cardiac puncture into tubes containing EDTA. Livers were removed and frozen in liquid nitrogen and stored in small portions at −80°C for RNA isolation, cholesterol content measurement, and histological examination. Standard enzymatic methods were used to determine TC, triglyceride (TG), LDL-C, and HDL-C levels with commercially available kits purchased from G. Cell Co., Ltd. Each sample was assayed in triplicate. For measurement of hepatic cholesterol, lipids were extracted from frozen liver tissues by thawing and homogenizing in isopropanol. TC and free cholesterol (FC) were measured using commercially available kits. Cholesteryl ester was calculated by subtraction of FC from TC. The enzymatic activities of blood samples representing liver or kidney functions were assayed using a Hitachi 7170 instrument.

RNA isolation and quantitative real-time RT-PCR analysis of hepatic LDLR mRNA levels

Approximately 200 mg of liver tissue was homogenized in 3 ml of Ultraspec RNA lysis solution (Biotecxs Laboratory, Houston, TX), and total RNA was isolated according to the supplier's protocol. For quantitative real-time RT-PCR assays, reverse transcription was conducted with random primers using Superscript II (Invitrogen) at 42°C for 50 min in a volume of 20 μl containing 1 μg of total RNA. Real-time PCR was performed with the cDNA using the ABI Prism 7900-HT Sequence Detection System and Universal MasterMix (Applied Biosystems). LDLR, apoB-100, HMG-CoA reductase, and GAPDH mRNA expression levels in hamster livers were assessed using the hamster PreDeveloped TaqMan Assay Reagents (Applied Biosystems). For detection of LDLR or HMG-CoA reductase, PCR was performed in a 10 μl reaction containing 1 μl of RT product, 5 μl of 2× universal PCR Master Mix, 0.5 μl of the premixed primers, and TagMan probe. For detection of GAPDH or apoB-100, 1 μl of a 1:20 dilution of the RT product was used. After initial incubations at 50°C for 2 min and 95°C for 10 min, the samples were amplified for 45 cycles of 95°C for 30 s, followed by 58°C for 30 s, and a final extension at 72°C for 30 s.

For data analysis, threshold cycle (C_T) values for each reaction were determined using TaqMan SDS analysis software. The C_T value for LDLR was subtracted from the GAPDH C_T value to yield the ΔC_T value for each sample, which was assayed in triplicate and the ΔC_T values were averaged. The average ΔC_T value for the positive control was then subtracted from the ΔC_T value for each sample to give $\Delta\Delta C_T$. Finally, the relative difference between tested samples and the control sample was calculated by the formula $2^{-\Delta\Delta CT}$.

Statistical analysis

Significant differences between treatment groups were assessed by Student's *t*-test. $P < 0.05$ was considered statistically significant. A paired *t*-test was used to compare differences before and after treatment, and an unpaired *t*-test was used to compare differences between two groups.

RESULTS

Reduction of TC, LDL-C, and TG in plasma and upregulation of hepatic LDLR mRNA expression by OM in hyperlipidemic hamsters

The plasma levels of TC, TG, LDL-C, and HDL-C in female hamsters on normal diet (n = 3) and HFHC diet (n = 8) feeding for 16 and 23 days are compared in **Fig. 1A**. Feeding hamsters a high-fat diet for 16 days induced pronounced increases in plasma lipid levels, with approximately a 2-fold increase in TC, a 3-fold increase in LDL-C, a 2.2-fold increase in TG, and a slight increase of HDL-C to 1.5-fold compared with hamsters fed a normal diet. The lipid levels in the HFHC group appeared to reach a plateau after 16 days of HFHC feeding, as continuous feeding with the high-fat diet for another 7 days did not further increase the plasma cholesterol levels in these animals.

To determine whether the high circulating level of LDL-C would suppress hepatic LDLR expression, hamsters of the HFHC group and the normal diet group were killed after

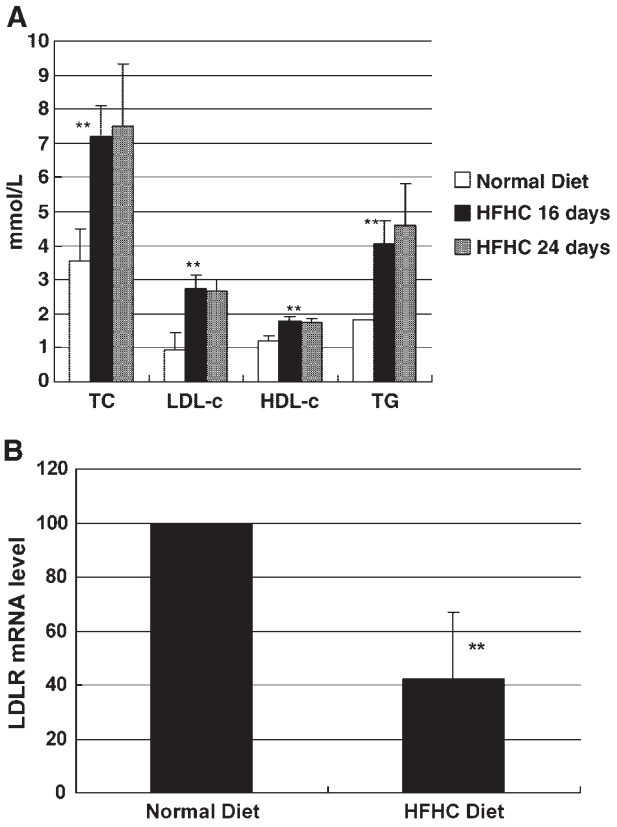

Fig. 1. Comparison of the effects of a normal diet and a high-fat and high-cholesterol (HFHC) diet on serum lipids and on hepatic low density lipoprotein receptor (LDLR) mRNA expression. Hamsters were fed a normal diet (n = 3) or a HFHC diet (n = 8) for the indicated days. A: Serum was prepared and cholesterol was assayed as described in Materials and Methods. Results shown are means ± SEM. Factors for the conversion of the lipid values to System International units are 0.02586 for total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and HDL-C and 0.01129 for triglyceride (TG). B: Liver tissues were isolated after killing the animals, and total RNA was prepared. The relative amounts of LDLR mRNA in livers were measured by a quantitative real-time RT-PCR assay using a hamster LDLR-specific fluorogenic probe from Applied Biosystems. The amount of LDLR mRNA in normal diet-fed animals (n = 3) was defined as 100, and the amount of LDLR mRNA in the HFHC group (n = 4) was plotted relative to that value. The data shown are means ± SEM from three separate experiments. ** $P < 0.01$ compared with the values for the normal diet.

23 days, livers were removed, and total RNAs were isolated. The levels of LDLR and GAPDH mRNA in these liver samples were assessed by conducting quantitative real-time RT-PCR analysis using specific probes derived from the hamster mRNA sequences. Figure 1B shows that the level of LDLR mRNA in animal livers was repressed by ~60% with the HFHC feeding. Together with the previous findings in this model, these results clearly demonstrate that lipid levels and liver LDLR expression are subject to coordinated regulation by diets, validating the suitability of this animal model for subsequent studies to examine the in vivo regulation of LDLR by OM.

Our previous studies conducted in HepG2 cells have shown that OM increases LDLR mRNA expression even under conditions of high intracellular sterol levels (13, 14). The current studies were designed to determine whether this in vitro property of OM could be recapitulated in vivo to increase LDLR expression in cholesterol-loaded livers and consequently decrease plasma levels of LDL-C and TC in hypercholesterolemic hamsters. Hamsters were fed the HFHC diet for 16 days. Human recombinant OM was then administered intraperitoneally at daily doses of 75 μg/kg (n = 8) and 150 μg/kg (n = 8) for 7 days while the hamsters were maintained on the HFHC diet. The control group (n = 8) received the same daily treatment with OM dilution buffer (100 μl of 1 mg/ml BSA in PBS) and continued with the HFHC diet. **Figure 2A** shows that OM treatment resulted in dose-dependent decreases in both serum TC and LDL-C. After the 7 day treatment, OM at 150 μg/kg/day reduced TC by 30% and LDL-C by 20% compared with pretreatment levels and reduced TC by 28% and LDL-C by 23% compared with the untreated control. The OM effect was also time-dependent. The decreases in serum cholesterol and LDL-C were clearly observed by day 3 and became highly significant by day 5. Interestingly, in addition to LDL-C, plasma levels of TG were robustly decreased in OM-treated animals. The magnitude of the effect of OM on TG was greater than the LDL-C reduction, as a significant reduction of TG was observed at day 3 by OM at the lower dose of 75 μg/kg/day. Levels of HDL-C were not significantly affected by OM administration at either dose. At the end of treatment, animals from the untreated control group and from the OM 150 μg/kg/day group were killed and the mRNA levels of LDLR, HMG-CoA reductase, apoB-100, and GAPDH of liver samples were measured by quantitative real-time RT-PCR. Figure 2B shows that OM treatment did not alter the mRNA expressions of apoB or HMG-CoA reductase but specifically increased the expression of LDLR mRNA by 2.4-fold. These results corroborated the previous in vitro findings with OM in the regulation of LDLR mRNA expression and further demonstrated that OM has a strong lipid-lowering effect under in vivo conditions in which the levels of circulating LDL-C are high and liver LDLR transcription is repressed.

OM treatment reduces hepatic fat storage and improves liver function in hyperlipidemic hamsters

HFHC feeding is known to lead to increased hepatic cholesterol content and prominent increases in hepatic fat storage (21, 26). To determine whether OM treatment could affect the hepatic fat content in animals fed a HFHC diet, liver tissue sections from animals under different diets and treatment were examined by hematoxylin and eosin staining and Sudan IV staining to reveal the fat droplets. Histological examination showed that liver tissue from hamsters fed a normal diet displayed a clear hepatic cord-sinusoid structure; a small amount of microvesicular lipid droplets was lightly stained intracellularly with Sudan IV (**Fig. 3A**, top panel). In liver tissues taken from untreated HFHC-fed hamsters, lipids accumulated to the point of creating large macrovesicular spaces, compressing and displacing the nucleus to the periphery of the hepatocytes (Fig. 3A, middle panel). Interestingly, OM treatment at

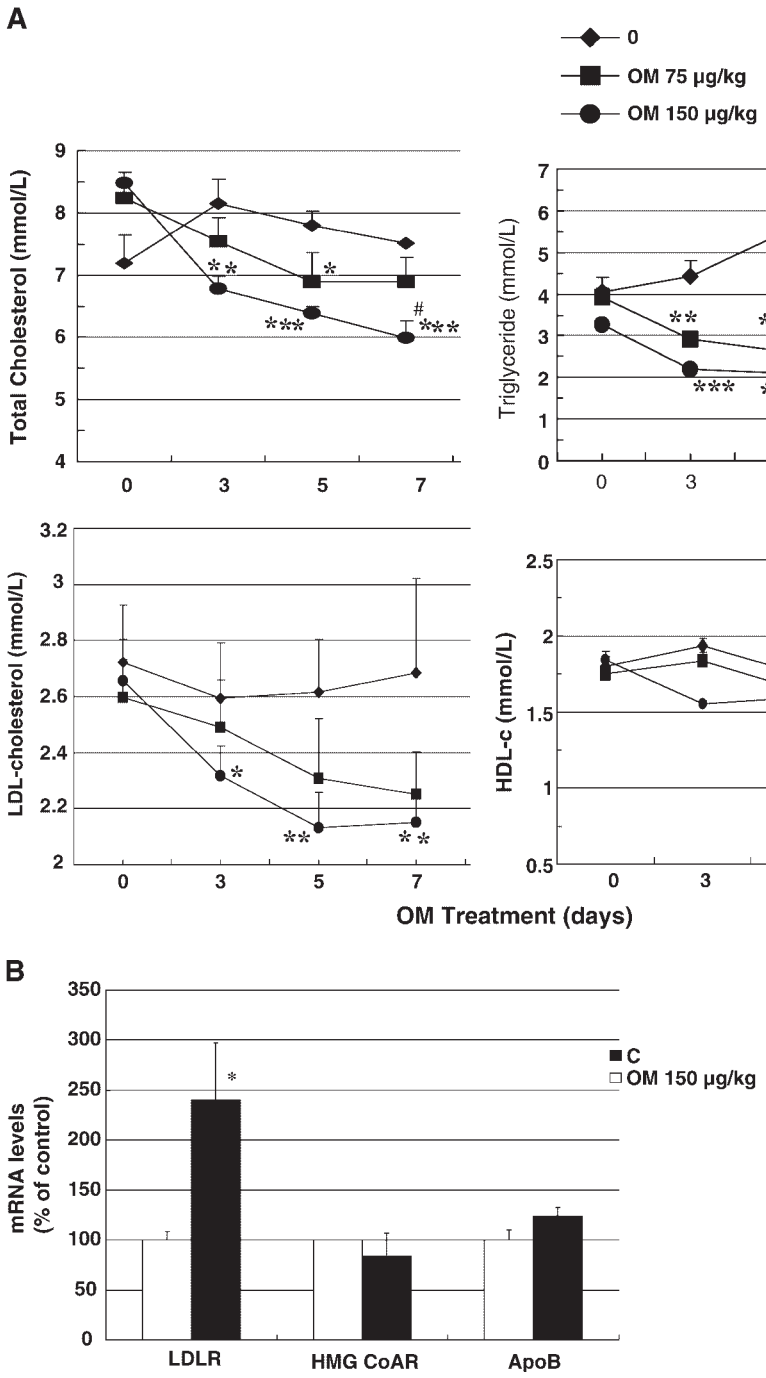

Fig. 2. Oncostatin M (OM) reduces serum TC, TG, and LDL-C and increases liver LDLR expression in hyperlipidemic hamsters. A: Serum was taken before, during, and after a 7 day OM treatment at the indicated doses from hamsters fed a HFHC diet. Results represent means ± SEM of six to eight animals. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with the values at day 0; # $P < 0.05$ and ## $P < 0.01$ compared with the untreated control group. B: OM increases liver LDLR expression in hypercholesterolemic hamsters. Four hours after the last drug treatment, four animals from the untreated group and four animals from the OM 150 μg/kg group were killed, and liver total RNAs were immediately prepared and analyzed for mRNAs of LDLR, HMG-CoA reductase (HMG-CoA R), and apolipoprotein B-100 (ApoB) by quantitative real-time RT-PCR. Error bars represent means ± SEM of all four animals.* $P < 0.05$ compared with the control (C) group.

150 μg/kg/day for only 7 days substantially diminished the fat staining (Fig. 3A, bottom panel). The decrease in lipid droplets was initially perilobular with some centrilobular cells still loaded with lipid, indicating the process of reduction of hepatic steatosis by OM.

To quantitatively assess the effect of OM in reducing lipid storage, hepatic cholesterol contents in normal chow-fed, HFHC-fed untreated, and HFHC-fed OM-treated hamsters were measured (Fig. 3B). Compared with animals fed the normal chow diet, the level of hepatic TC was in-

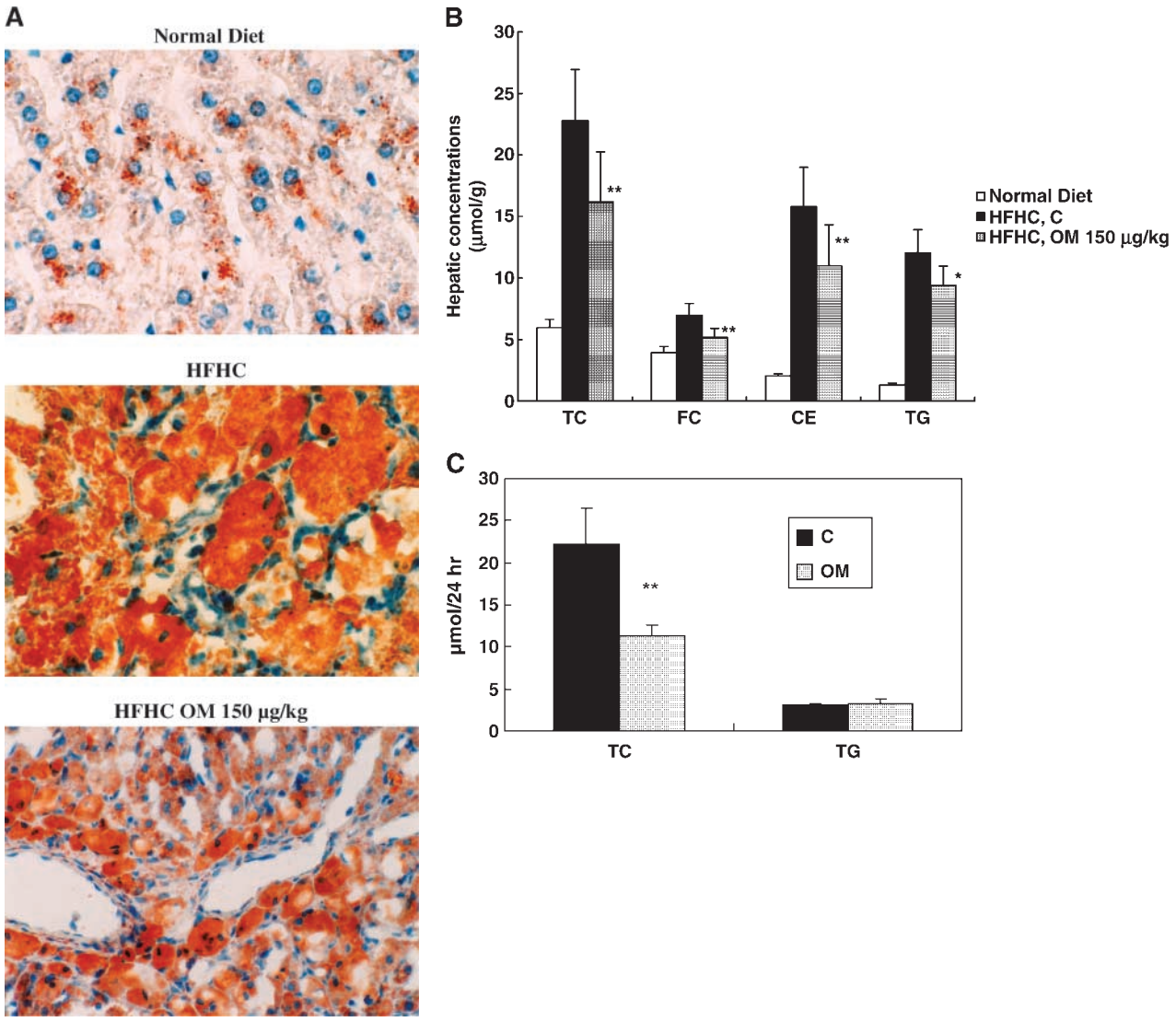

Fig. 3. OM administration reduces hepatic fat storage and cholesterol content of hyperlipidemic hamsters. A: Reduction of hepatic fat storage by OM in HFHC-fed hamsters. Liver tissues were taken from hamsters fed a normal diet (top panel, ×400), a HFHC diet untreated (middle panel, ×400), or a HFHC diet treated with OM for 7 days (bottom panel, ×200) and were immediately frozen for fat staining. The fat on the frozen liver sections was detected with Sudan IV stain, a conventional method for fatty tissue in histology. B: The effects of OM on hepatic cholesterol in hypercholesterolemic hamsters. Hepatic TC, free cholesterol (FC), cholesteryl ester (CE), and TG were measured in hamsters fed a normal diet (n = 3), a HFHC diet for 23 days without OM treatment (C; n = 8), or a HFHC diet for 23 days with OM (150 μg/kg; n = 8) applied for the last 7 days. * $P < 0.05$ and ** $P < 0.01$ compared with the control group. C: The effect of OM on fecal cholesterol in hypercholesterolemic hamsters. The feces of untreated animals (C) and hamsters treated with OM for 6 days under HFHC feeding were collected within a 24 h period, dried, and weighed. Fecal lipids were extracted, and TC and TG were measured as described for hepatic cholesterol contents. ** $P < 0.01$ compared with the control group. The fecal weights of the control and OM-treated groups are not significantly different. Error bars represent means ± SEM of all animals.

creased 3.8-fold (from 5.97 to 22.78 μmol/g), FC was increased 1.8-fold (from 3.93 to 6.98 μmol/g), and TG was increased more than 9-fold (from 1.3 to 12 μmol/g) in HFHC-fed hamsters. OM administration at a daily dose of 150 μg/kg for 7 days reduced hepatic TC by 29% ($P < 0.01$) and FC by 25.7% ($P < 0.01$). In addition, TG hepatic content in OM-treated animals was also significantly reduced by 22.1% ($P < 0.05$). The cholesterol and TG contents were also measured in feces of untreated and OM-treated hamsters. OM treatment reduced fecal TC by 49% ($P < 0.001$) without changing the amount of TG (Fig. 3C).

Because OM treatment reduced the accumulation of fat in hepatocytes, it would be desirable to know whether OM might have some beneficial effect on reversing liver damage that was caused by the high-fat diet. **Figure 4** shows that the activity of aspartate aminotransferase was increased 3-fold and the activity of alanine aminotransferase was increased 7.9-fold by HFHC feeding. OM administration at both low and high doses reduced the enzymatic activities nearly to the baseline levels seen in animals fed the normal diet. Similarly, plasma bilirubin (Bil-T) was increased by the HFHC diet, and OM treatment almost reversed the increase of Bil-T in blood.

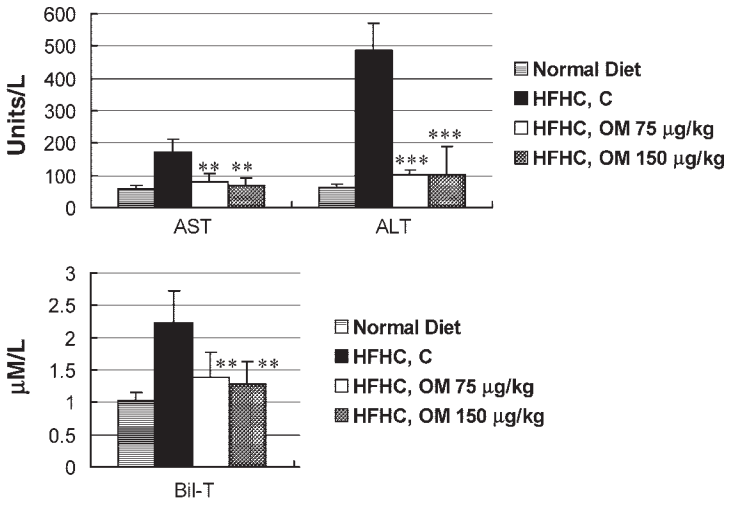

Fig. 4. OM reversed the high-fat diet-induced increase of aminotransferases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] and bilirubin (Bil-T) in hypercholesterolemic hamsters. Enzyme levels from blood samples taken from hamsters fed the normal or the HFHC diet without (C) or with OM treatment for 7 days were measured. ** $P < 0.01$ and *** $P < 0.001$ compared with the control group. Error bars represent means ± SEM of all animals.

Adverse effects associated with OM administration were not detected in hamsters

To determine whether OM administration caused adverse effects, the body weight and food intake of animals were monitored throughout the OM treatment period, and no changes were observed (**Fig. 5**, top and middle panels). The blood levels of creatinine and urea nitrogen were also measured to detect changes in kidney functions, but neither diet nor OM treatment had apparent effects on these two parameters during the course of these experiments (Fig. 5, bottom panel). In hamsters fed a HFHC diet, we also measured liver weight in untreated and OM-treated animals and did not detect significant changes associated with OM treatment. These results together indicate that OM is not toxic to hamsters during short-term treatment; however, the toxicity of OM in long-term treatment awaits further investigation.

OM is a fast-acting hypolipidemic bioagent

Our previous studies in HepG2 cells showed that OM regulates LDLR expression with kinetics much faster than those of lovastatin and other LDLR modulators (16). The LDLR mRNA level peaks at 1 h of OM treatment and is sustained for more than 24 h. To determine whether this

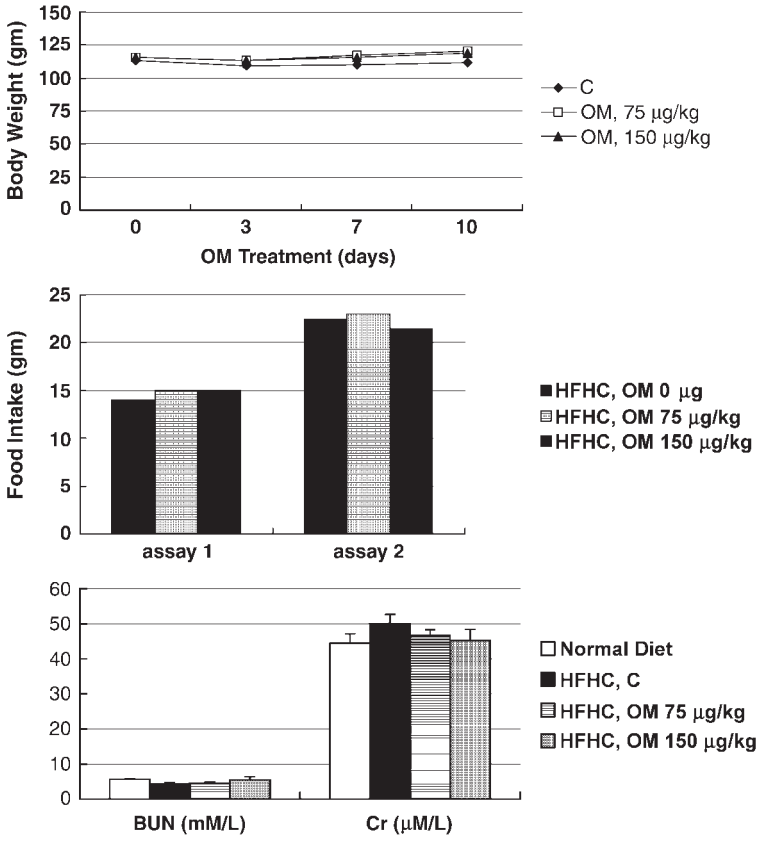

Fig. 5. OM 7 day treatment has no detectable adverse effects. Top, The body weights of hamsters fed a HFHC diet without (C) or with OM treatment at the indicated doses were monitored on alternate days. Middle, Food intake within 24 h was measured two times on different days and are presented as assay 1 and assay 2. Bottom, Creatinine (Cr) and blood urea nitrogen (BUN) levels from blood samples taken from hamsters fed the normal or the HFHC diet without or with OM treatment for 7 days were measured. Error bars represent means ± SEM of all animals.

rapid induction can be reiterated in vivo, resulting in declines of blood lipid levels after a single administration, OM at a single intraperitoneal dose of 300 μg/kg/animal was administered to a group of four hypercholesterolemic hamsters, and another four HFHC-fed hamsters received the OM dilution buffer as a control. Thirty-six hours after OM administration, animals were killed, blood samples were collected, and liver tissues were harvested for RNA preparation. **Figure 6A** shows that a single administration of OM reduced plasma levels of TC by 21% ($P < 0.05$), LDL-C by 15% ($P < 0.05$), and TG by 36% ($P < 0.05$). Quantitative real-time RT-PCR analysis shows that the hepatic LDLR mRNA level was increased 2.1-fold ($P < 0.001$) (Fig. 6B). In addition, the increased aminotransferase activities in the hyperlipidemic hamsters were also reduced substantially after a single OM injection (Fig. 6C).

DISCUSSION

In this study, we demonstrate that OM, a cytokine that upregulates hepatic LDLR expression through a sterol-independent mechanism, increased hepatic LDLR mRNA expression under in vivo conditions of hypercholesterolemia, resulting in the reduction of plasma lipids in hamsters fed a HFHC diet.

The rapid effects of OM in reducing LDL-C levels in hypercholesterolemic animals were clearly demonstrated in two different experiments. In the first experiment, OM was administered twice daily for 7 days, and in the second experiment, a single injection of OM was applied. We show that OM at a daily dose of 150 μg/kg reduced LDL-C by 20% after a 7 day treatment regimen. In the experiment in which hamsters were given a single injection, OM at a dose of 300 μg/kg decreased LDL-C by 15% even after a period of 36 h. Compared with the statin therapies that have shown to maximally decrease LDL-C to 60%, the effects of OM seem to be moderate. However, the regimen used in this in vivo study is a pioneer trial for hypercholesterolemia with a modest dose; larger cholesterol-lowering effects of OM may be achieved by improvement of the treatment protocol with longer durations and higher doses. By performing quantitative real-time RT-PCR, we showed that hepatic LDLR mRNA levels of the high-fat-fed hamsters were increased 2.5- and 2.1-fold by OM treatment in two separate experiments, which likely accounted for the reduced level of LDL-C in the circulation. In contrast, liver mRNA levels of HMG-CoA reductase were not altered in OM-treated animals, confirming that the SRE-1/SREBP pathway was not involved in the actions of OM, as both LDLR and HMG-CoA reductase promoters contain the SRE-1 motif and are targets for SREBP-mediated transcriptional activation (27).

It is noteworthy that the TG levels in hypercholester-

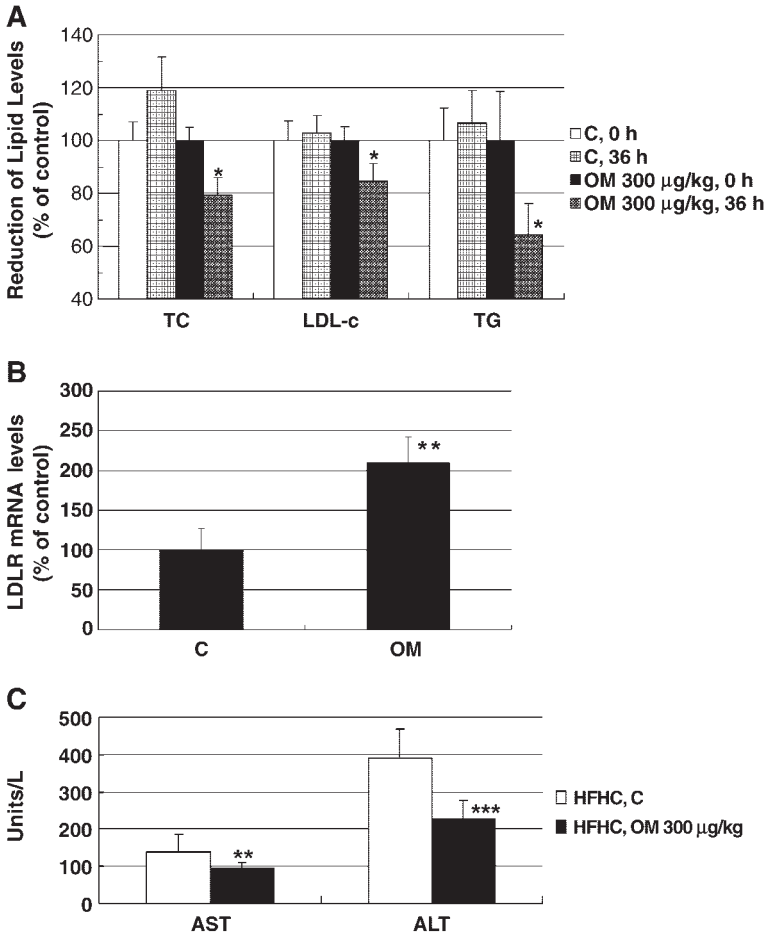

Fig. 6. OM induces a rapid reduction of plasma lipid levels through the upregulation of hepatic LDLR. A: Serum was taken before and 36 h after a single intraperitoneal administration of OM at a dose of 300 μg/kg. Results represent means ± SEM of four animals. * $P < 0.05$ compared with before treatment. B: Thirty-six hours after OM injection, animals from the untreated (C) and treated groups were killed and liver total RNAs were immediately prepared and analyzed for LDLR mRNA by quantitative real-time RT-PCR assay. ** $P < 0.01$ compared with the control group. C: Enzyme levels from blood samples taken from control and OM-treated hamsters were analyzed using standard hospital procedures. ALT, alanine aminotransferase; AST, aspartate aminotransferase. ** $P < 0.01$ and *** $P < 0.001$ compared with the control group.

olemic hamsters were markedly and rapidly reduced by OM administration. Plasma TG has been increasingly recognized as an independent risk factor for coronary heart disease in addition to LDL-C (28). Most plasma TG occurs as VLDL and chylomicron particles. VLDL is derived from liver and chylomicrons are derived from intestinal absorption. VLDL is first remodeled into intermediate density lipoprotein and then converted to LDL, which is taken up by the liver primarily through LDLR. Hence, the increased liver LDLR expression contributes to some of the reduction of plasma TG in OM-treated hypercholesterolemic hamsters. However, because the potency for TG reduction apparently is greater than that for LDL-C reduction, it is possible that the removal of TG by OM may involve additional LDLR-independent mechanisms in this animal model. OM might affect lipoprotein lipase activity or affect the chylomicron or VLDL synthesis and secretion that would contribute to the TG-lowering effect of OM. Further studies are needed to fully characterize the underlying mechanisms responsible for plasma TG reduction by OM in vivo.

Improvement of liver function, as monitored by liver enzyme levels, in the hypercholesterolemic hamster model is an extension of the active lipid-lowering effects of OM. The HFHC diet greatly increased fat storage in hamster livers and led to severe liver tissue damage. As a consequence, aminotransferase levels in blood during fat feeding were increased severalfold. Treating the hypercholesterolemic hamsters with OM for 7 days nearly normalized the enzymatic values back to baseline levels. Additionally, a single injection of OM also substantially reduced the enzyme levels in blood after 36 h. Staining with Sudan IV showed a significant reduction of fat staining in liver sections of OM-treated animals compared with the untreated group. This histological change is in agreement with the lower hepatic levels of TC, cholesteryl ester, and TG in OM-treated hamsters. In addition to decreasing the fat content, hepatic structure and morphology were partially restored in the HFHC group after OM administration. We recognize that the reducing effects of OM in liver fat and cholesterol contents were observed within a relatively short term; consequences of the long-term treatment of hypercholesterolemic hamsters with OM in hepatic lipid contents need to be evaluated to reach a firm conclusion. At present, the direct mechanisms by which OM reduced fat storage in hamster livers and ameliorated tissue damage are unclear. OM has been reported to induce maturation of fetal hepatocytes in primary cell cultures, as shown by causing differentiative morphological changes, expression of differentiation markers, and intracellular glycogen accumulation (29–32). Knockout mice deficient for the OM receptor subunit gp130 showed defective development of fetal hepatocytes (29). The findings reported here that OM treatment partially reversed the tissue damage caused by a high-fat diet provide in vivo evidence supporting a physiological role of OM in hepatic development and normal function.

A study of OM toxicity in mice showed that OM at 1.3 mg/kg/day for 15 days caused a slight weight loss (10% of body weight), which was the only overt sign of toxicity (33). The doses (75 and 150 μg/kg/day) used in this study in hamsters are much lower than those used in the toxicity test, and we did not observe any adverse effect of OM in the current experiments. Food intake and body weights were not changed by OM administration. A previous study to examine OM's effect on inducing an inflammatory response in mice reported that subcutaneous injection of 1 μg of human recombinant OM into mice caused redness and swelling at the injection site that were observed within 6 and 12 h after injection (34). However, this inflammatory response was not seen at all in hamsters injected intraperitoneally with OM.

In conclusion, we have shown the in vivo lipid-lowering effect and the upregulation of hepatic LDLR expression by OM in hypercholesterolemic hamsters without detectable adverse effects within a relatively short period. The long-term effects of OM in regulating lipid metabolism and toxicity await further investigations. This study confirmed the in vitro activity of OM in the regulation of LDLR and further implied that the sterol-independent regulatory mechanism is functional under hypercholesterolemic conditions. With regard to the pharmacological implications of these findings, OM is a multifunctional cytokine. Use of OM in vivo is likely to change the expression of genes involved in many different cellular functions in addition to lipid metabolism. Therefore, other pathways that potentially could be regulated by OM under in vivo conditions must be thoroughly characterized before considering the clinical application of OM in the treatment of hyperlipidemia.

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