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Original Contribution

α-Lipoic acid ameliorates foam cell formation via liver X receptor α-dependent upregulation of ATP-binding cassette transporters A1 and G1

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ABSTRACT

α-Lipoic acid (α-LA), a key cofactor in cellular energy metabolism, has protective activities in atherosclerosis, yet the detailed mechanisms are not fully understood. In this study, we examined whether α-LA affects foam cell formation and its underlying molecular mechanisms in murine macrophages. Treatment with α-LA markedly attenuated oxidized low-density lipoprotein (oxLDL)-mediated cholesterol accumulation in macrophages, which was due to increased cholesterol efflux. Additionally, α-LA treatment dose-dependently increased protein levels of ATP-binding cassette transporter A1 (ABCA1) and ABCG1 but had no effect on the protein expression of SR-A, CD36, or SR-BI involved in cholesterol homeostasis. Furthermore, α-LA increased the mRNA expression of ABCA1 and ABCG1. The upregulation of ABCA1 and ABCG1 by α-LA depended on liver X receptor α (LXRα), as evidenced by an increase in the nuclear levels of LXRα and LXRE-mediated luciferase activity and its prevention of the expression of ABCA1 and ABCG1 after inhibition of LXRα activity by the pharmacological inhibitor geranylgeranyl pyrophosphate (GGPP) or knockdown of LXRα expression with small interfering RNA (siRNA). Consistently, α-LA-mediated suppression of oxLDL-induced lipid accumulation was abolished by GGPP or LXRα siRNA treatment. In conclusion, LXRα-dependent upregulation of ABCA1 and ABCG1 may mediate the beneficial effect of α-LA on foam cell formation.

Accumulation of lipid-laden macrophage foam cells in the intima is the major hallmark of early stage atherosclerotic lesions [1–3]. Regulation of cholesterol metabolism by these macrophages is a critical step in the initiation and progression of atherosclerosis [2,3]. The formation of these foam cells is mainly due to uncontrolled uptake of modified low-density lipoprotein (LDL) or impaired cholesterol efflux in macrophages, resulting in excessive lipoprotein-derived cholesterol, which is processed, stored, and accumulated inside the cells [4,5]. Scavenger receptors (SRs), class A SR (SR-A) and CD36, are responsible for internalization of modified LDL [6,7]. In contrast, the efflux of accumulated cholesterol in macrophages is mediated by reverse cholesterol transporters (RCTs), including SR-BI and the ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1) [8–10]. Thus, the cellular lipid content in foam cells is dynamically regulated by these SRs and RCTs. Ample evidence has demonstrated that dietary supplementation with antioxidants decreases the expression of SRs or increases that of RCTs, thus leading to the suppression of cholesterol accumulation in macrophages and retardation of atherosclerotic progression [11–13].

α-Lipoic acid (α-LA), widely existing in prokaryotic and eukaryotic cells, is an essential cofactor of the multienzyme complexes that are associated with the mitochondrial electron transport reactions in cellular energy metabolism [14,15]. α-LA and its dithiol form dihydrolipoic acid (DHLA) are also considered potent free radical scavengers and have been used to prevent or reduce reactive oxygen species (ROS)-induced damage. Moreover, the α-LA–DHLA system recycles the antioxidant potency of glutathione (GSH), vitamin C, vitamin E, and coenzyme Q10, thereby maintaining the cellular reduced state and countering oxidative stress [16–18]. Over the past decade, much evidence has confirmed the benefits of α-LA in this antioxidant capacity in various diseases, including type II diabetes, neurodegeneration, and atherosclerosis in humans and experimental

Abbreviations: α-LA, α-lipoic acid; ABCA1, ABCG1, ATP-binding cassette transporter A1, G1; GGPP, geranylgeranyl pyrophosphate; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDLR, low-density lipoprotein receptor; Luc, luciferase; LXRα, liver X receptor α; LXRE, LXR binding element; oxLDL, oxidized low-density lipoprotein; RCT, reverse cholesterol transporter; siRNA, small interfering RNA; SR-A, class A scavenger receptor; SR-BI, class B scavenger receptor type I; SREBP2, sterol-response element binding protein 2.

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animals [19–25]. Additionally, α-LA has excellent potent cytoprotective and anti-inflammatory actions in endothelial cells and macrophages [26–28]. Despite extensive studies on the protective effects of α-LA on cardiovascular diseases, whether α-LA affects the expression of SRs and RCTs during the development of macrophage foam cells remains unclear.

This study was conducted, first, to investigate the effects of α-LA on oxidized LDL (oxLDL)-induced foam cell formation; second, to delineate the effects of α-LA on the expression of SR-A, CD36, SR-BI, ABCA1 and ABCG1; and third, to explore the molecular mechanisms involved in α-LA-mediated modulation of lipid accumulation. Our results demonstrate that α-LA suppresses the oxLDL-mediated lipid accumulation through a liver X receptor α (LXRα)-dependent upregulation of the ABCA1 and ABCG1 genes.

Materials and methods

Reagents

Rabbit anti-CD36, goat anti-SR-A, goat anti-LDL receptor (LDLR), goat anti-3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), and anti-histone H1 antibodies; ABCA1 small interfering RNA (siRNA); and ABCG1 siRNA were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-sterol-response element binding protein 2 (SREBP2) antibody was from BD Biosciences (San Jose, CA, USA). Rabbit anti-LXRα antibody was from ABR (Golden, CO, USA). Mouse anti-ABCA1 antibody was from Abcam (Cambridge, MA, USA). Rabbit anti-SR-BI and anti-ABCG1 were from Novus Biologicals (Littleton, CO, USA). Mouse anti-α-tubulin antibody, racemic *R,S*-mixture of α-LA, human LDL, Sephadex G-50, butylated hydroxytoluene (BHT), geranylgeranyl pyrophosphate (GGPP), Tri reagent, and phorbol myristate acetate (PMA) were from Sigma Chemical (St. Louis, MO, USA). Lipofectamine reagent was from Invitrogen (La Jolla, CA, USA). Control and LXRα siRNA were from Thermo Fisher Scientific (Lafayette, CO, USA). LXR agonist TO901317 and 3-dodecanoyl-NBD–cholesterol were from Cayman (Ann Arbor, MI, USA). The cholesterol assay kit was from Randox (Antrim, UK). DiO-oxLDL was from Biomedical Technologies (York, UK).

Cell culture

Murine macrophage J774.A1 cells (American Type Culture Collection, TIB-67) and human monocytic THP-1 cells (Bioresource Collection and Research Center, Hsinchu, Taiwan) were cultured in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml). THP-1 cells were induced with 50 nM PMA for 7 days to differentiate into macrophages.

Preparation and modification of low-density lipoprotein

In addition to the source of LDL from Sigma Chemical, we also freshly isolated human LDL from plasma as native LDL. Briefly, lipoproteins from the plasma were separated by sequential ultracentrifugation at 40,000 rpm for 48 h at 4 °C in KBr in the presence of BHT. The collected LDL was concentrated with the use of a Kwik Spin tube and then desalted by Sephadex G-50 gel filtration with 150 mM NaCl, 20 mM Hepes buffer (pH 7.4). The purified LDL was stored at 4 °C under nitrogen. The oxidation of LDL was prepared as described [29]. The LDL from Sigma Chemical was exposed to 5 μM $CuSO_4$ for 24 h at 37 °C, and Cu^{2+} was then removed by extensive dialysis. The extent of modification was determined by measuring the thiobarbituric acid-reactive substances (TBARS). OxLDL containing approximately 30–60 nmol of TBARS defined as malondialdehyde equivalents per milligram of LDL protein was used for experiments.

Oil red O staining

Cells were fixed with 4% paraformaldehyde and then stained with 0.5% oil red O. Hematoxylin was used as counterstaining. The density of the lipid content was evaluated by alcohol extraction after oil red O staining. The absorbance at 540 nm was measured by use of a microplate reader (BioTek Instruments, Winooski, VT, USA).

DiO–oxLDL binding assay

DiO–oxLDL, copper-oxidized LDL, labeled with green fluorescence, has been used for experiments involving oxLDL uptake or binding to scavenger receptors of macrophages [30]. In this study, J774.A1 macrophages were treated with or without α-lipoic acid (12.5–50 μM) for 24 h and then 10 μg/ml DiO–oxLDL at 4 °C for 4 h. The cells were washed and lysates were analyzed by fluorimetry (Molecular Devices, Sunnyvale, CA, USA) with 540 nm excitation and 590 nm emission.

Cholesterol efflux assay

We previously applied NBD–cholesterol, a fluorescently tagged cholesterol derivative with the hydrophilic NBD fluorophore, to study cholesterol efflux in macrophages [31]. Briefly, macrophages were treated with various concentrations of α-LA for 12 h, followed by equilibration with NBD–cholesterol (1 μg/ml) for an additional 6 h in the presence of α-LA. NBD–cholesterol-labeled cells were washed with phosphate-buffered saline and incubated in RPMI 1640 medium for 6 h. The fluorescence-labeled cholesterol released from the cells into the medium was measured with a multilabel counter (PerkinElmer, Waltham, MA, USA). Cholesterol efflux was expressed as a percentage of fluorescence in the medium relative to the total amount of fluorescence (cells and medium).

Cholesterol measurement

Cellular cholesterol was extracted with hexane/isopropanol (3/2, v/v). After cellular debris was removed, the supernatant was dried under nitrogen flush. The level of cholesterol was measured using cholesterol and triglyceride assay kits.

Small interfering RNA transfection

Macrophages were transfected with scramble, ABCA1, ABCG1, or LXRα siRNA by the use of Lipofectamine for 24 h for the indicated experiments.

RT-PCR

Total RNA was isolated from cells using the Tri reagent and converted into cDNA by reverse transcriptase (Biolabs, Ipswich, MA, USA), with oligo(dT) used as the primer. The cDNAs obtained were then used as the templates for semiquantitative PCR. PCR was performed in a DNA thermal cycler (Biometra Tpersonal, Horsham, PA, USA). The PCR program was 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min; then 1 cycle of 72 °C for 10 min. The nucleotide sequences of the primers were as follows: ABCA1, sense 5′-CAGGAGGTGATGTTTCTGACCA-3′, antisense 5′-TTGGCTGTTCTCCATGAAGGTC-3′; ABCG1, sense 5′-CCGGGTTGGAACTGTTCATTTCCT-3′, antisense 5′-CTGTCTGCATTGTGTTGCATTGC-3′; GAPDH, sense 5′-TGTTCCAGTATGACTCCACTC-3′, antisense 5′-TCCACCACCCTGTTGCTGTA-3′.

Preparation of nuclear extracts

The nuclear extracts were prepared as described previously [32]. Cells were lysed in 10 mM Hepes, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$,

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0.5% Nonidet P-40, 1 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride. Nuclei were pelleted at 5000 *g* for 5 min at 4 °C, and the resulting supernatants were used as the cytosolic fraction. Nuclei were resuspended in 50 mM Tris, pH 7.5, 300 mM NaCl, 1% Triton X-100, 5 mM ethylenediaminetetraacetate, 1 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride; sheared for 15 s with a microprobe sonicator; and incubated on ice for 5 min. After centrifugation at 12,000 *g* for 5 min at 4 °C, the supernatant was collected as the nuclear extract.

Western blot analysis

Cells were lysed with phosphate-buffered saline containing 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 1 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride on ice. After sonication, crude extracts underwent centrifugation at 12,000 *g* for 5 min at 4 °C. The supernatant was collected as the cell lysate. All protein concentrations were determined by protein assay. Aliquots (50 μg) of cell lysates or nuclear extracts were separated on 8% SDS–PAGE and then transblotted onto Immobilon-P membrane (Millipore, Bedford, MA, USA). After being blocked with 5% skim milk, the blots were incubated with various primary antibodies and then secondary antibodies. The protein bands were detected by using an enhanced chemiluminescence kit (ECL; PerkinElmer Life Sciences) and quantified by ImageQuant 5.2 software (Healthcare Bio-Sciences, Philadelphia, PA, USA).

Transient transfection and luciferase reporter assay

Cells were transfected with the plasmids 3×LXRE-Luc, a reporter construct containing three copies of LXRE; phABCA1 (−928)-Luc, a reporter plasmid for the human ABCA1 promoter, or phABCA1-DR4m-Luc, a reporter plasmid with a mutation in the LXRE (kindly provided by Dr. A.R. Tall, Division of Molecular Medicine, Department of Medicine, Columbia University, New York, NY, USA); and the hABCA1 promoter containing the DR4 mutant, by using TurboFect (Fermentas, Glen Burnie, MD, USA). The pGL3-Renilla plasmid was cotransfected as a transfection control. At 24 h after transfection, the cells were treated with α-LA for another 12 h. The cells were then lysed for Luc and *Renilla* activity assays.

Statistical analyses

The experiments were performed at least three times. Results are presented as means ± SEM. Data were analyzed using the Kruskal–Wallis test followed by the Dunnett multiple comparisons or Mann–Whitney *U* test. Differences were considered statistically significant at $p<0.05$.

Results

α-LA suppresses the formation of foam cells

Abundant lipid-laden macrophage foam cells are found in fatty streaks and atherosclerotic plaques, which implies their importance in atherosclerosis. Modified LDL, in particular oxLDL, has several proatherogenic properties for the formation of foam cells and progression of atherosclerosis [1–3]. We thus explored the effects of α-LA on oxLDL-induced foam cell formation. Treatment with α-LA markedly reduced the oxLDL-induced lipid accumulation in macrophages, as revealed by oil red O staining (Figs. 1A and B) or by measurement of cellular cholesterol content (Fig. 1C). To investigate the mechanisms underlying the reduced foam cell formation by α-LA, we assessed the effect of α-LA on cholesterol uptake and efflux. α-LA treatment did not affect DiO–oxLDL-induced cholesterol uptake (Fig. 2A) but significantly promoted cholesterol efflux (Fig. 2B). Additionally, α-LA had no effect on the protein expression of cholesterol synthesis-related genes, including SREBP2, HMGCR, and LDLR (Fig. 2C), which implies that de novo lipid synthesis is not involved in α-LA-mediated reduction in foam cell formation. These results indicate that the modulation of cholesterol efflux is a critical regulation in the suppressive effect of α-LA during the formation of macrophage foam cells.

Effect of α-LA on expression of SR-A, CD36, SR-BI, ABCA1, and ABCG1

Previous studies demonstrated that SR-A, CD36, SR-BI, ABCA1, and ABCG1 play the most critical roles in cholesterol homeostasis during the formation of foam cells [6–10]. We investigated whether expression of these receptors and transporters is involved in the antiatherogenic property of α-LA in the development of foam cells. As shown in Fig. 3, treating macrophages with α-LA (12.5, 25, or 50 μM) for 24 h significantly increased the protein levels of ABCA1 and ABCG1 in a dose-dependent manner but did not affect the protein expression of SR-A, CD36, or SR-BI. Combined treatment with α-LA and oxLDL further increased the oxLDL-induced increase in protein expression of ABCA1 and ABCG1 (Fig. 3F). Additionally, transfection with ABCA1 and ABCG1 siRNA for gene knockdown prevented the suppressive effect of α-LA on oxLDL-mediated cholesterol accumulation (Fig. 4). However, treatment with 50 μM α-LA significantly enhanced the mRNA expression of ABCA1 and ABCG1 in a time-dependent manner (Fig. 5A). These results suggest that upregulation of ABCA1 and ABCG1 by α-LA may be regulated at the transcriptional level.

α-LA induces activation of LXRα in macrophages

To address whether the key transcription factor LXRα is involved in α-LA-increased gene expression of ABCA1 and ABCG1, we determined the nuclear protein level of LXRα in α-LA-treated and vehicle-treated macrophages. α-LA increased the level of nuclear LXRα in a time-dependent manner (Fig. 5B). Furthermore, to explore whether the increased expression of LXRα in α-LA-treated macrophages is associated with transcriptional activation of LXRα, cells were transfected with 3×LXRE-Luc and then treated with α-LA for LXR activation assays. The LXR agonist TO901317 was used as a positive control. Incubation with α-LA or TO901317 markedly increased LXRE-mediated luciferase activity by 3.1- and 5.2-fold, respectively (Fig. 5C). Moreover, the α-LA-mediated induction of ABCA1 promoter activity was abrogated on transfection with ABCA1 and DR4m (the reporter plasmid with a mutation in the LXRE; Fig. 5D). To specifically examine the role of LXRα, we performed additional experiments to demonstrate that the increase in ABCA1 and ABCG1 protein expression caused by α-LA was mainly due to LXRα activation. Inhibition of LXRα activation by treatment with the LXRα-specific inhibitor GGPP or LXRα siRNA diminished the effect of α-LA, increasing the protein expression of ABCA1 and ABCG1 without altering the protein expression of SR-A, CD36, or SR-BI (Figs. 6A and C). More importantly, the suppressive effect of α-LA on oxLDL-mediated cholesterol accumulation was totally abrogated by GGPP or LXRα siRNA treatment (Figs. 6B and D). These results indicate the essential role of LXRα activation in α-LA-regulated gene expression of ABCA1 and ABCG1, which may contribute to the suppressive effect of α-LA in foam cell transformation in vitro.

Effect of α-LA on expression of SRs and reverse cholesterol transporters and foam cell formation in human macrophages

Because α-LA has been widely used as a dietary supplement in clinical or experimental trials, we thus assessed the effect of α-LA on human THP-1 macrophages. α-LA showed a similar inhibitory effect on oxLDL-induced lipid accumulation in THP-1 macrophages treated with α-LA (Fig. 7A). Furthermore, the protein levels of ABCA1 and

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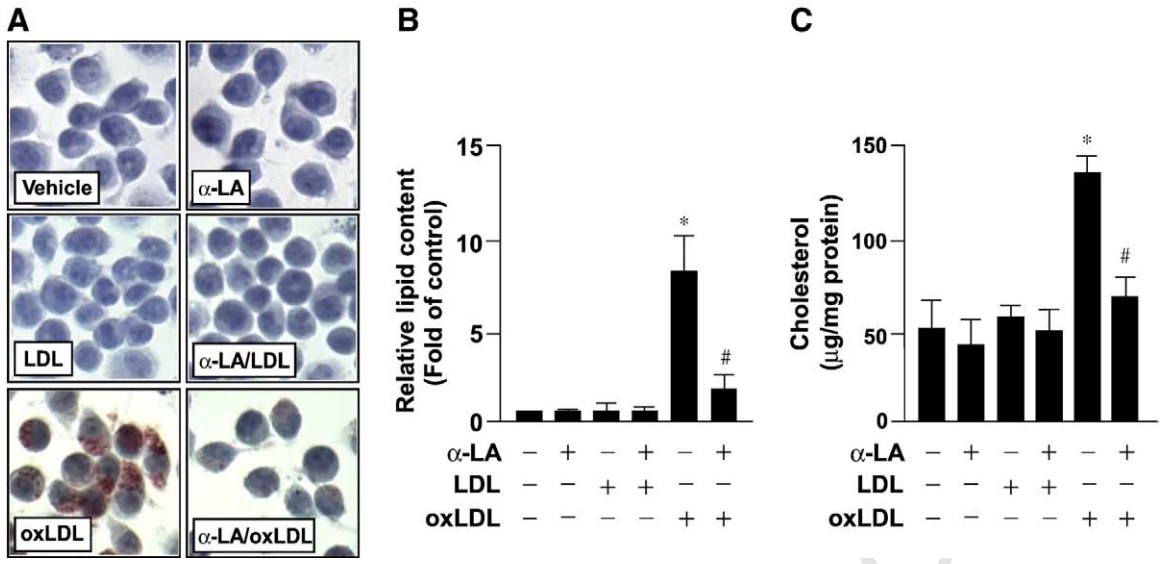

Fig. 1. α-LA attenuates the oxLDL-induced lipid accumulation in macrophages. (A) J774.A1 cells were incubated with vehicle (ethanol), LDL (50 μg/ml), oxLDL (50 μg/ml), α-LA (50 μM), α-LA/LDL, or α-LA/oxLDL for 24 h and fixed and stained with oil red O to detect intracellular lipid content. Cellular nuclei were stained with hematoxylin. Original magnification 400×. (B) Lipid content measured by alcohol extraction of oil red O-stained lipid, with absorbance at 540 nm. (C) The intracellular cholesterol was determined by an enzymatic method. Data shown are means ± SEM from three independent experiments. $^{*}p<0.05$ vs control; $^{\#}p<0.05$ vs oxLDL alone.

ABCG1 were elevated in α-LA-treated THP-1 macrophages, whereas α-LA had no effect on the protein expression of SR-A, CD36, or SR-BI (Fig. 7B). In parallel, the oxLDL-mediated lipid accumulation reduced by α-LA in THP-1 macrophages was abolished by pretreatment with GGPP (Fig. 7C).

Discussion

Ample research has suggested that α-LA is a potent antioxidant and provides protection against cardiovascular diseases in human and experimental rodent models. However, the exact molecular mechanisms underlying the benefits of α-LA are not fully understood. The atheroprotective nature of α-LA has been extensively investigated. For instance, α-LA effectively inhibits the tumor necrosis factor α-mediated expression of adhesion molecules and apoptosis and improves the dysfunction of endothelial nitric oxide synthase in endothelial cells [33–35]. α-LA also profoundly inhibits the proliferation of smooth muscle cells in vitro and in vivo [36,37]. Moreover, α-LA is known to attenuate the inflammatory responses in macrophages [38,39]. However, the efficacy of α-LA on cholesterol metabolism in macrophages has never been investigated. Lipid-laden foam cell accumulation is a key feature of early stage atherosclerotic lesions. Although the precise mechanisms are still equivocal and most probably multifactorial, uncontrolled oxLDL uptake or dysregulation of cholesterol efflux in macrophages is thought to be the major contributor [4–6]. In this study, we demonstrated for the first time that α-LA ameliorates the oxLDL-induced lipid accumulation during the formation of macrophage foam cells. From this observation, we further investigated the mechanism underlying the α-LA-mediated suppression on foam cells using this cell culture system.

The intracellular lipid homeostasis of foam cells is dynamically regulated by oxLDL uptake and cholesterol efflux. Moreover, these processes are tightly controlled by SRs and RCTs, respectively [6–10]. SR-A and CD36 are well known to be responsible for internalization of oxLDL, which promotes the cellular accumulation of cholesterol [4,5]. Macrophages from mice lacking SR-A and/or CD36 have impaired capacity for oxLDL internalization and are less prone to foam cell formation [4–6]. However, ABCA1, ABCG1, and SR-BI, the three major transporters of cholesterol efflux in foam cells [7–10], are critical in the cholesterol homeostasis of macrophages. In vivo experiments

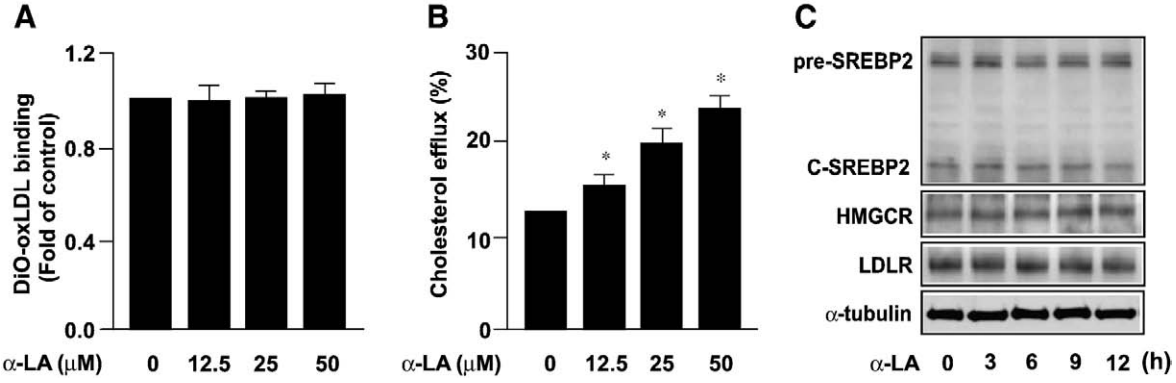

Fig. 2. α-LA promotes cholesterol efflux in macrophages. (A) For DiO–oxLDL binding assay, macrophages were treated with vehicle (ethanol) or the indicated concentrations of α-LA for 24 h and then 10 μg/ml DiO–oxLDL at 4 °C for 4 h. Cellular lysates were analyzed by fluorimetry. (B) Macrophages were treated with the indicated concentrations of α-LA for 12 h and then NBD–cholesterol (1 μg/ml) for an additional 6 h in the presence of α-LA. Cholesterol efflux is expressed as a percentage of fluorescence in the medium relative to the total amount of fluorescence. (C) Macrophages were treated with vehicle (ethanol) or α-LA (50 μM) for the indicated times. The protein levels of SREBP2 precursor (pre-SREBP2), C-terminus SREBP2 (C-SREBP2), hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), LDL receptor (LDLR), or α-tubulin were determined by Western blot analysis. $^{*}p<0.05$ versus untreated group. Data shown are means ± SEM from three independent experiments.

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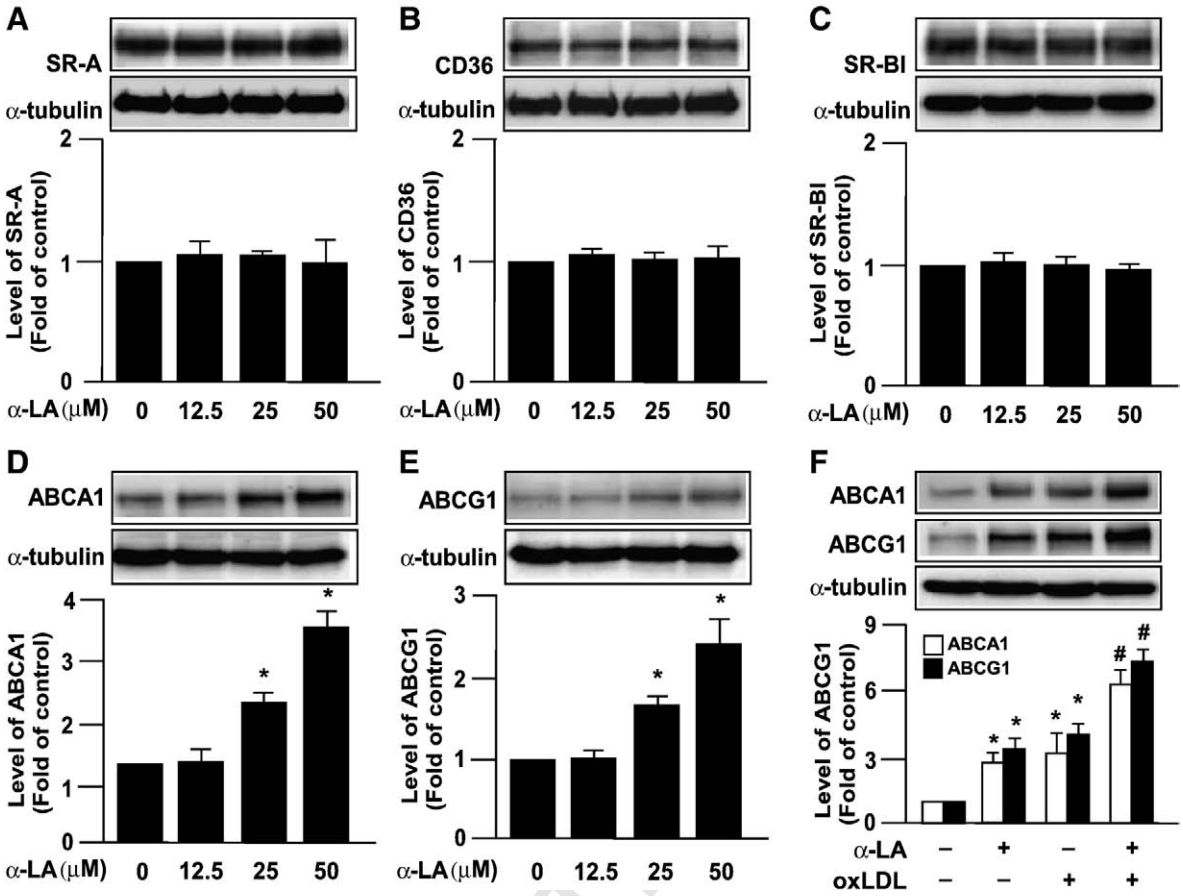

Fig. 3. Effects of α-LA on expression of scavenger receptors and ATP-binding cassette transporters. J774.A1 cells were treated with vehicle (ethanol) or indicated concentrations (12.5, 25, 50 μM) of α-LA for 24 h, and cell lysates underwent Western blot analysis of the protein levels of SR-A, CD36, SR-BI, ABCA1, ABCG1, or α-tubulin. The fold induction is defined as the level of protein at the indicated times relative to the untreated cells set as 1. (F) Effects of treatment with α-LA and oxLDL on ABCA1 and ABCG1 protein expression. Data shown are means ± SEM from three independent experiments. $^{*}p<0.05$ versus untreated groups.

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demonstrated that foam cell accumulation and atherosclerotic lesions are significantly increased in individual transporter-deficient mice [40–42]. Recent evidence points to reduced expression of SRs or elevated function of RCTs in macrophages, leading to reduced deposition of cholesterol in macrophages, by several dietary flavonoids with antiatherogenic actions, such as procyanidin, (−)-epicatechin gallate, and resveratrol [43–45]. Therefore, we theorized that α-LA administration might regulate the expression of SRs or RCTs to affect the formation of foam cells. α-LA did not affect the protein level of SR-A, CD36, or SR-B1 but increased the mRNA and protein expression of ABCA1 and ABCG1, which indicates that the upregulation of ABCA1 and ABCG1 by α-LA probably contributes to inhibition of foam cell formation. These data suggest that α-LA attenuates lipid accumulation in macrophages possibly through escalating cholesterol efflux but not lessening the

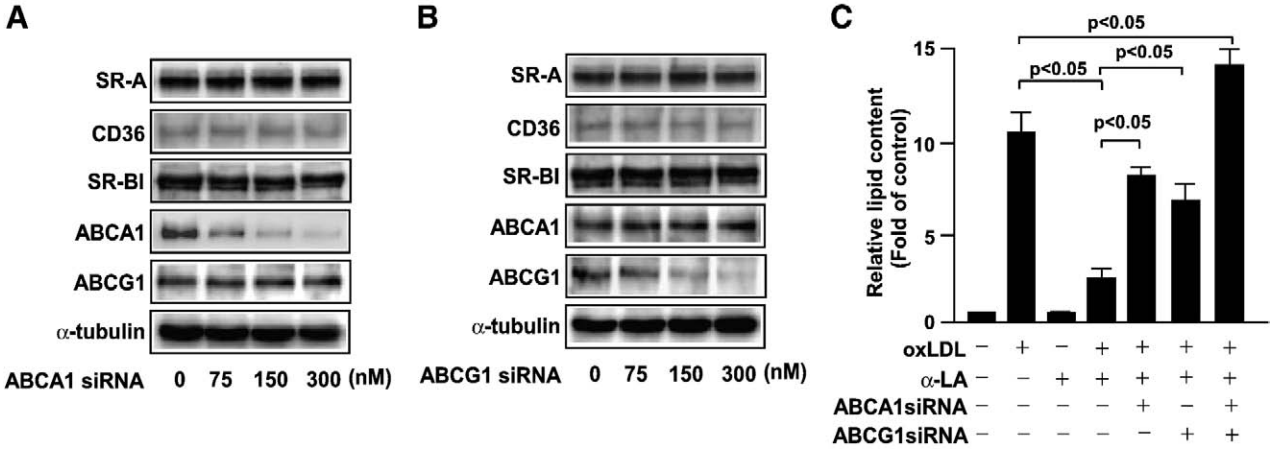

Fig. 4. Knockdown of ABCA1 and ABCG1 expression diminishes the α-LA-mediated suppressive effect on lipid accumulation. Macrophages were transfected with various concentrations of siRNA of SR-A, CD36, SR-B1, and (A) ABCA1 or (B) ABCG1 (B) for 24 h, and cellular lysates underwent Western blot analysis of these protein levels and that of α-tubulin. (C) Macrophages were incubated with siRNA of ABCA1 (300 nM), ABCG1 (300 nM), or both ABCA1 and ABCG1 for 24 h and then α-LA (50 μM), oxLDL (50 μg/ml), or α-LA/oxLDL for an additional 24 h. Intracellular lipid content was measured by alcohol extraction. Data are means ± SEM from three independent experiments.

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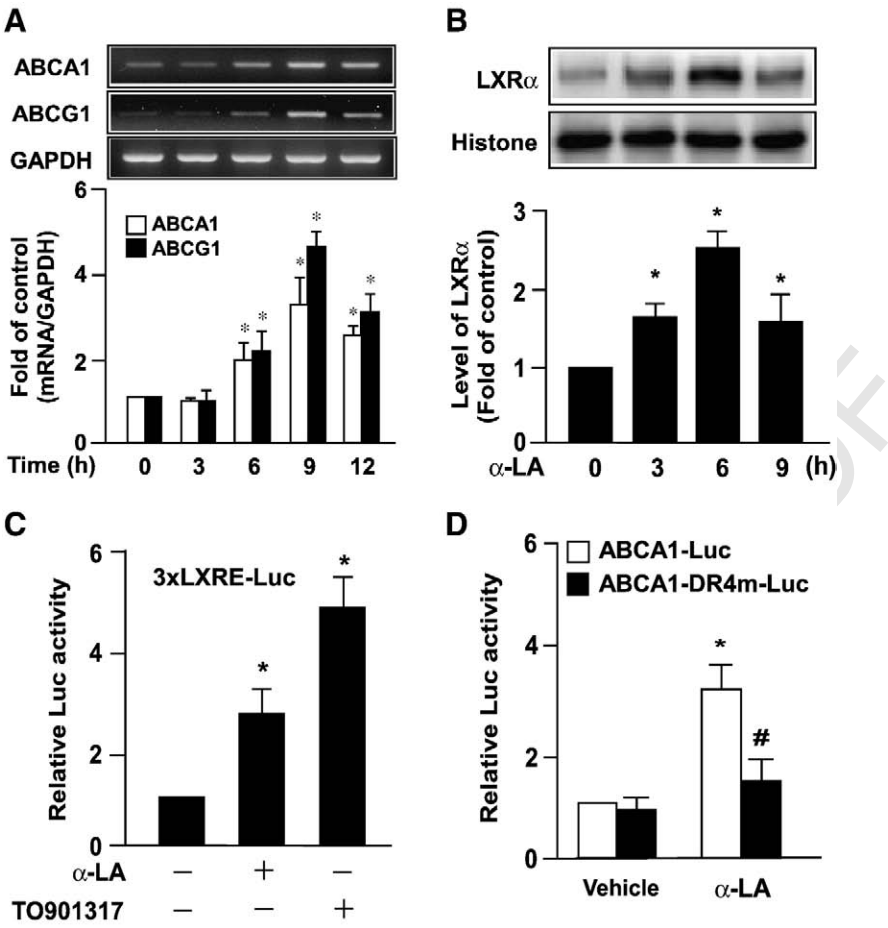

Fig. 5. α-LA induces the mRNA expression of ABCA1 and ABCG1 and activates LXRα in macrophages. (A) J774.A1 cells were incubated with 50 μM α-LA for the indicated times to determine the mRNA level of ABCA1, ABCG1, or GAPDH. (B) J774.A1 cells were incubated with 50 μM α-LA for the indicated times and then the nuclear lysates were subjected to Western blot analysis of the protein level of LXRα or histone H1. (C and D) J774.A1 cells were transfected with plasmid 3 × LXRE-Luc reporter and (C) T0901317, an LXR agonist, or (D) phABCA1-Luc or phABCA1-DR4m-Luc for 12 h and then underwent α-LA treatment for an additional 12 h. The pGL3-Renilla plasmid was cotransfected as a transfection control. The results are relative luciferase activity. Data are means ± SEM from three independent experiments. $^{*}p<0.05$ vs control group; $^{\#}p<0.05$ vs α-LA-treated phABCA1-Luc group.

oxLDL uptake during the formation of foam cells. This finding is consistent with previous studies showing that flavonoid-induced induction of ABCA1 or ABCG1 contributed to the mitigation of lipid accumulation in foam cells [43–45]. We additionally showed that the α-LA-induced upregulation of ABCA1 and ABCG1 was accompanied by an increase in nuclear LXRα and LXRE-mediated luciferase activity. This notion was further reinforced in promoter activation assays showing that α-LA-induced increase in promoter activity was totally abrogated in macrophages transfected with the LXRE mutant (phABCA1-DR4m-Luc). Moreover, the inhibition of LXRα activation by GGPP or LXRα siRNA diminished the α-LA-mediated upregulation of ABCA1 and ABCG1. These results suggest that the α-LA induction of ABCA1 and ABCG1 expression requires LXRα-mediated transcriptional regulation.

Despite the unique pathway we discovered, the detailed mechanisms of α-LA affecting cholesterol efflux merit further investigation. In functional analyses inhibiting LXRα activation, the effect of α-LA suppressing intracellular lipid accumulation was totally abolished, even more than with oxLDL treatment alone. One possible explanation is that oxLDL itself is known to upregulate ABCA1 and ABCG1 via an LXRα-dependent mechanism [46]. These results imply the essential role of LXRα activation in α-LA-induced gene expression of ABCA1 and ABCG1, which may contribute to its suppression of the formation of macrophage foam cells in vitro.

α-LA has been suggested to have antioxidant functions in cardiovascular diseases [23–25]. Although some in vitro findings suggest that α-LA may directly scavenge ROS [47,48], many other studies indicate that its beneficial effects are mediated through indirect antioxidant effects or nonantioxidant effects. For example, the anti-inflammatory actions of α-LA in monocytes or smooth muscle cells are mediated by upregulation of heme oxygenase-1 (an enzyme with antioxidant function) via MAPK/nuclear factor-related factor 2 (Nrf2) [28,49]. Additionally, the beneficial effects of α-LA may also rely on an Nrf2-dependent phase II detoxification response such as upregulation of GSH and γ-glutamylcysteine ligase [50,51]. In this study, we found that α-LA attenuates the lipid accumulation in foam cells via LXRα-mediated gene expression of ABCA1 and ABCG1. Whether α-LA may activate these signaling pathways related to antioxidant functions and they, in turn, participate in the protective action of α-LA against lipid accumulation in macrophages requires further investigation.

In summary, our study provides a new insight into the antiatherogenic properties of α-LA—reducing lipid accumulation in the formation of foam cells via upregulation of ABCA1 and ABCG1. The upregulation by α-LA is via transcriptional regulation involving LXR activation. The findings of this study provide a novel explanation for the antiatherogenic action of α-LA and the possible molecular mechanism in potential therapeutic interventions in atherosclerosis.

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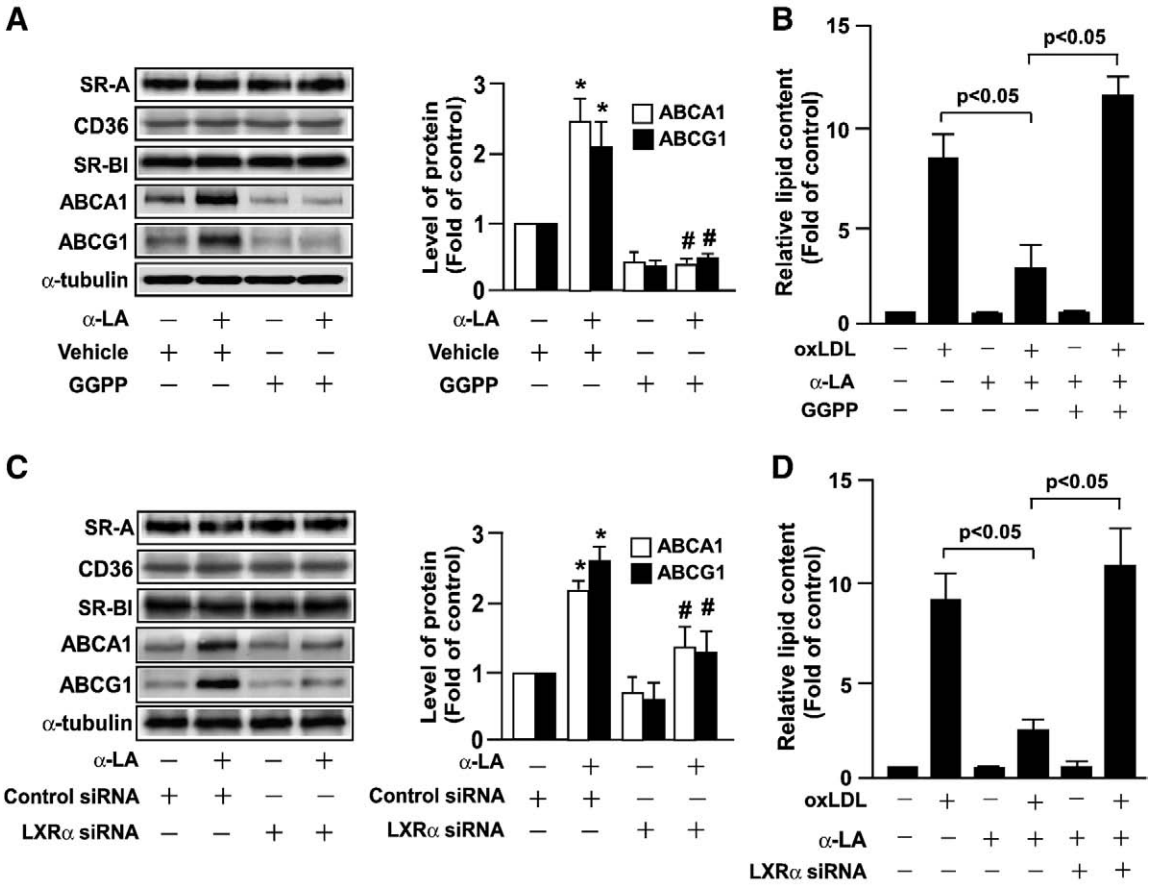

Fig. 6. Inhibition of LXR activation abolishes α-LA-mediated increase in the expression of ABCA1 and ABCG1 and attenuation of lipid accumulation. (A and C) J774.A1 cells were pretreated with or without 20 μM geranylgeranyl pyrophosphate (GGPP) for 2 h or transfected with control siRNA (600 nM) or LXRα siRNA (600 nM) for 24 h and then incubated with vehicle (ethanol) or α-LA (50 μM) for another 24 h. Protein levels of SR-A, CD36, SR-B1, ABCA1, and ABCG1 in cell lysates were determined by Western blot analysis. (B and D) After incubation with (B) GGPP or (D) LXRα siRNA, macrophages were treated with vehicle (ethanol), α-LA (50 μM) or α-LA/oxLDL (50 μg/ml) for 24 h. Density of lipid content was measured by oil red O staining, with absorbance at 540 nm. Data are means ± SEM from three independent experiments. $^{*}p<0.05$ vs control group; $^{\#}p<0.05$ vs α-LA alone.

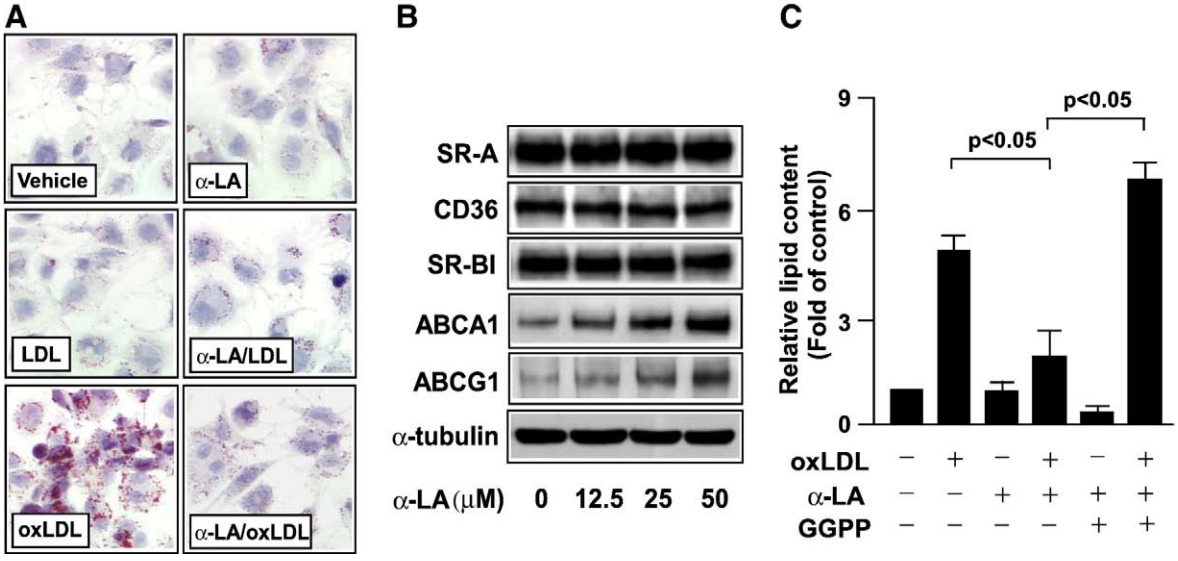

Fig. 7. α-LA increases the protein expression of ABCA1 and ABCG1 and attenuates lipid accumulation in human THP-1 macrophages. THP-1 cells were incubated with PMA (50 ng/ml) for 7 days to differentiate into macrophages and then incubated with vehicle (ethanol), LDL (50 μg/ml), oxLDL (50 μg/ml), α-LA (50 μM), α-LA/LDL, or α-LA/oxLDL for 24 h and stained with oil red O to detect intracellular lipid content. Original magnification 400×. (B) THP-1 macrophages were incubated with 12.5, 25, or 50 μM α-LA for 24 h, and cell lysates underwent Western blot analysis of the protein level of SR-A, CD36, SR-BI, ABCA1, ABCG1, or α-tubulin. (C) Cells were pretreated with 20 μM GGPP for 2 h and then incubated with 50 μM α-LA, 50 μg/ml oxLDL, or α-LA/oxLDL for an additional 24 h. Density of lipid content was measured by oil red O staining, with absorbance at 540 nm. Data are means ± SEM from three independent experiments.

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