

Original Article

Ibrolipim increases ABCA1/G1 expression by the LXR α signaling pathway in THP-1 macrophage-derived foam cells

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Aim: To determine the effects and potential mechanisms of ibrolipim on ATP-binding membrane cassette transporter A-1 (ABCA1) and ATP-binding membrane cassette transporter G-1 (ABCG1) expression from human macrophage foam cells, which may play a critical role in atherogenesis.

Methods: Human THP-1 cells pre-incubated with ox-LDL served as foam cell models. Specific mRNA was quantified using real-time RT-PCR and protein expression using Western blotting. Cellular cholesterol handling was studied using cholesterol efflux experiments and high performance liquid chromatography assays.

Results: Ibrolipim 5 and 50 μ mol/L significantly increased cholesterol efflux from THP-1 macrophage-derived foam cells to apoA-I or HDL. Moreover, it upregulated the expression of ABCA1 and ABCG1. In addition, LXR α was also upregulated by the ibrolipim treatment. In addition, LXR α small interfering RNA completely abolished the promotion effect that was induced by ibrolipim.

Conclusion: Ibrolipim increased ABCA1 and ABCG1 expression and promoted cholesterol efflux, which was mediated by the LXR α signaling pathway.

Keywords: ATP-binding membrane cassette transporter A-1; ATP-binding membrane cassette transporter G-1; ibrolipim; liver X receptor α ; atherosclerosis; RNA interference; high density lipoprotein; apolipoprotein; cholesterol

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Introduction

Coronary artery disease (CAD) is a major cause of mortality in advanced societies^[1, 2]. Multiple factors contribute to the formation of lesions that ultimately lead to CAD. A major event in the progression of atherosclerosis is the differentiation of monocytes to macrophages that accumulate lipoprotein-derived cholesterol to form foam cells within the arterial wall^[3]. Excess unesterified cholesterol (UC) is toxic to cells; therefore, cells have developed several ways to protect themselves against cholesterol toxicity. One key pathway is the efflux of cholesterol to extracellular "acceptors" via cholesterol exporters like ATP-binding membrane cassette transporter A-1 (ABCA1) and ATP-binding membrane cassette transporter G-1

(ABCG1)^[4, 5].

Ibrolipim is an effective lipoprotein lipase (LPL) activator^[6]. It has previously been reported that ibrolipim increases lipoprotein lipase (LPL) mRNA in tissues and LPL activity in post-heparin plasma, resulting in a reduction in plasma triglyceride levels and an elevation of HDL. Previous studies from our laboratory and others have also shown that increasing LPL activity in skeletal muscle results in decreased fat accumulation, and long-term administration of ibrolipim protects against the development of experimental atherosclerosis in animals^[6–8]. However, the detailed mechanism for cholesterol transport proteins induced by ibrolipim is unclear.

Recently, our laboratory revealed that the liver X receptors (LXR) synthetic agonist T0901317 promoted ABCA1 gene and protein levels in the aorta, liver, and small intestine of apoE^{-/-} mice and significantly increased cholesterol efflux from peritoneal macrophages^[9]. We also showed that IFN- γ may first downregulate the expression of LXR α through the

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JAK/STAT1 signaling pathway and then decrease expression of ABCA1 and cholesterol efflux in THP-1 macrophage-derived foam cells^[10]. In addition, eicosapentaenoic acid (EPA) reduces ABCA1 serine phosphorylation and impairs ABCA1-dependent cholesterol efflux through a cAMP/PKA pathway in THP-1 macrophage-derived foam cells^[11]. The objective of this study was to investigate the effects of ibrolipim on the key exporters of macrophage cholesterol efflux ABCA1 and ABCG1, on the nuclear transcription factor LXR α that regulates their expression, and on macrophage cholesterol handling.

Materials and methods

Materials

Ibrolipim ([4-(4-bromo-2-cyano-phenylcarbonyl)-benzyl]-phosphonic acid diethyl ester; Chemical Abstracts Service (CAS) 133208-93-2; lot N_Q C00C99) that was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory (Tokushima, Japan) was provided by Prof Wei-dong YIN. HDL and lipid-free apoA-I were obtained from Calbiochem (Cambridge, MA). Rabbit polyclonal anti-ABCA1, anti-ABCG1, anti-LXR α , and anti- β -actin antibody were obtained from Abcam (Cambridge, UK). CellTiter 96A_{queous} One Solution Reagent was from Promega (Madison, WI). pSilencer2.1-U6-Hygro was obtained from Ambion Company. Lipofectamine™ 2000 was from the Invitrogen Company. 3-[4,5-Dimethylthiazol-2-yl]-2,5-tetrazolium bromide (MTT) and other chemicals of reagent grade were obtained from Sigma (St. Louis, MO).

Cell culture

Human THP-1 cells were cultured in RPMI-1640 supplemented with 0.1% nonessential amino acids, penicillin (100 U/mL), streptomycin (100 μ g/mL), and 20% fetal bovine serum (FBS) at 37 °C in 5% CO₂ at a cell density of 0.2 to 1.0 \times 10⁶/mL. After three to four days, cells were treated with PMA (160 nmol/L) for 24 h, and the medium was then replaced by a serum-free medium containing oxLDL (50 μ g/mL) for 48 h in order for the cells to become fully differentiated macrophages before their use in experiments.

Cell proliferation and cytotoxicity assay

A cell cytotoxicity assay was assessed by measuring the activity of mitochondrial dehydrogenase as described previously^[12]. THP-1 cells were plated in 96-well plates with a density of 1 \times 10⁴/well. The medium was replaced after 24 h. The cells were cultured as indicated above. After treatment with ibrolipim at different concentrations for 24 h, 10 μ L/well of CellTiter 96Aqueous One Solution Reagent [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reagent] was added. After incubation at 37 °C for 1 h in a humidified 5% CO₂ atmosphere, the absorbance at 490 nm was recorded with an ELISA plate reader. "Control" refers to incubations in the presence of vehicle only (0.1% of DMSO or ethanol) and was considered to have 100% viable cells.

Transfection for LXR α silencing

Short-interfering RNA (siRNA) specific for human LXR α (Santa Cruz Biotechnology) and nonsilencing control siRNA were synthesized by the Biology Engineering Corporation in Shanghai, China. The double-stranded DNA for siRNA was ligated with a linearized pSilencer 2.1-U6 siRNA expression vector at *Bam*HI and *Hind*III sites. The products were transformed into competent *Escherichia coli* DH5 α cells and cultured on Luria-Bertani (LB) plates with 100 μ g/mL ampicillin. Ampicillin-resistant colonies were selected by restriction digestion and confirmed by DNA sequencing. For transfection with small interfering RNA (siRNA), foam cells were plated in 96-well plates at a density 2 \times 10⁶ cells per well. The next day, cells were transfected with LXR α siRNA in OPTI-MEM with 5 μ g/mL Lipofectamine 2000 (Invitrogen). The final siRNA concentration was 100 nmol/L. The efficiency of transfection was approximately 50% as assessed with a fluorescein-labeled RNA probe. After 48 h of transfection, ibrolipim (50 μ mol/L) was added to the culture medium. The cells were cultured for an additional 24 h before expression or functional studies were performed. The target sequence for human LXR α siRNA was AAGTACACAGGAGGCCATCTT, and the target sequence for control non-silencing siRNA was AATTCTCCGAACGTGTCACGT. In comparison to the control siRNA, the siRNA of LXR α suppressed the expression of LXR α proteins by 84% according to Western blot analysis.

Western blot analyses

Cellular and whole-tissue proteins were extracted. An equal amount of total proteins (40 μ g) was loaded onto a 10% SDS-polyacrylamide electrophoresis gel and was electrophoresed for 2 h at 100 V in buffer containing 25 mmol/L Tris base, 250 mmol/L glycine and 0.1% SDS. After electrophoresis, the proteins were electrically transferred to an immobilon-P transfer membrane in buffer containing 25 mmol/L Tris, 192 mmol/L glycine, 20% methanol, and 0.005% SDS. After transfer, the membrane was blocked in TBST (20 mmol/L Tris base pH 7.6, 150 mmol/L NaCl, and 0.1% Tween-20) containing 5% skim milk for 4 h at room temperature. The membrane was incubated with antibodies against ABCA1, ABCG1, LXR α , and β -actin in the blocking solution at 4°C overnight. The membrane was then washed three times with TBST for 30 min, incubated with secondary antibody in the blocking solution for 50 min at room temperature, and washed three times with TBST for 30 min. Immunoreactivity was detected by the ECL test. The protein content was calculated by densitometry using Labwords analysis software.

RNA isolation and real-time quantitative PCR analysis

Total RNA from cells was extracted using TRIzol reagent in accordance with the manufacturer's instructions. Real-time quantitative PCR, using SYBR Green detection chemistry, was performed on a Roche light Cycler Run 5.32 Real-Time PCR System. A master mixture for each quadruple was set up in a total reaction volume of 30 μ L with 5 μ L cDNA (in a 1:5 dilution), 15 μ L 2 \times TaqMan Universal Master Mix, No AmpErase

UNG (Applied Biosystems), 5 μ L of diethylpyrocarbonate-treated water (Ambion Ltd., Huntingdon, UK), and 1.5 μ L 20 \times primer/probe set (Assay-on-Demand Assay Mix; Applied Biosystems). From this mixture, an aliquot of 5 μ L was placed in each of the four wells. The thermal cycling conditions were 95 $^{\circ}$ C for 10 min, then 40 cycles of 95 $^{\circ}$ C for 15 s and 60 $^{\circ}$ C for 1 min. Quantitative measurements were determined using the $\Delta\Delta C_t$ method and expression of β -actin was used as the internal control. The sequences of the primers are as shown in Table 1.

Cellular cholesterol efflux experiments

Cells were cultured as indicated above. Then they were labeled with 0.2 μ Ci/mL [3 H]cholesterol. After 72 h, cells were subsequently washed with phosphate-buffered saline (PBS) and incubated overnight in RPMI 1640 medium containing 0.1% (*w/v*) bovine serum albumin (BSA) to allow equilibration of the [3 H]cholesterol in all of the cellular pools. Equilibrated [3 H]cholesterol-labeled cells were washed with PBS and incubated in 2 mL of efflux medium containing RPMI 1640 medium and 0.1% BSA with or without apoA-I (50 μ g/mL) or HDL (μ g/mL). An 150 μ L sample of efflux medium was obtained at the designated times and passed through a 0.45- μ m filter to remove any floating cells. The monolayers were washed twice in PBS, and cellular lipids were extracted with isopropanol. Medium and cell-associated [3 H]cholesterol was then measured by liquid scintillation counting. The percentage of efflux was calculated by the following equation: [total media counts/(total cellular counts+total media counts)] \times 100%.

High performance liquid chromatography assays (HPLC)

HPLC analysis was conducted as described previously^[13]. Briefly, cells were washed with PBS three times. The appropriate volume (usually 1 mL) of 0.5% NaCl was added about 50–200 μ g cellular proteins per mL. Cells were sonicated using an ultrasonic processor for 2 min. The protein concentration in the cell solution was measured using a BCA kit. A 0.1 mL aliquot of cell solution (containing 5–20 μ g of protein) was used to measure the free cholesterol, and another aliquot was used for total cholesterol detection. Free cholesterol was dissolved in isopropanol (1 mg cholesterol/mL) and stored at -20 $^{\circ}$ C as a stock solution. A cholesterol standard calibration solution ranging from 0 to 40 μ g of cholesterol per mL was obtained by diluting the cholesterol stock solution in the same cell lysed buffer.

A 0.1 mL aliquot of each sample (cholesterol standard cali-

bration solutions, or cell solutions) was supplemented with 10 μ L of the reaction mixture, which included 500 mmol/L MgCl₂, 500 mmol/L Tris-HCl (pH 7.4), 10 mmol/L dithiothreitol, and 5% NaCl. A total of 0.4 U of cholesterol oxidase in 10 μ L 0.5% NaCl was added to each tube for free cholesterol determination or 0.4 U cholesterol oxidase plus 0.4 U of cholesterol esterase for a total cholesterol measurement. The total reaction solution in each tube was incubated at 37 $^{\circ}$ C for 30 min, and 100 μ L methanol:ethanol (1:1) was added to stop the reaction. Each solution was kept cold for 30 min to allow protein precipitation and then centrifuged at 1500 r/min for 10 min at 15 $^{\circ}$ C. Ten microliters of supernatant was applied onto a System Chromatographer (PerkinElmer Inc) that included a PerkinElmer series 200 vacuum degasser, a pump, a PerkinElmer series 600 LINK, and a PerkinElmer series 200 UV/vis detector, and a Discovery C-18 HPLC column (Supelco Inc). The column was eluted using isopropanol:*n*-heptane:acetonitrile (35:13:52) at a flow rate of 1 mL/min for 8 min. The absorbance at 216 nm was monitored, and the data were analyzed with TotalChrom software from PerkinElmer.

Statistical analysis

Data are expressed as means \pm SD. Results were analyzed by one-way ANOVA and Student's *t* test using SPSS 13.0 software. *P* values of less than 0.05 were considered statistically significant.

Results

Upregulation of ABCA1 and ABCG1 expression by ibrolipim in THP-1 macrophage-derived foam cells

To investigate whether ibrolipim treatment can cause cell toxicity, the effects of different concentrations of ibrolipim on foam cell viability were compared using MTT reagent. As shown in Figure 1A, the difference in cell viability between different treatments was not statistically significant. These results indicate that the experimental concentrations of ibrolipim did not induce cell proliferation and cell death.

ABCA1 and ABCG1 are key players in reverse cholesterol transport and are critical in regulating cellular cholesterol homeostasis^[14, 15]. To cast light on ibrolipim's effect on ABCA1 and ABCG1, we investigated the expression levels of mRNA and protein for ABCA1 and ABCG1 in THP-1 macrophage-derived foam cells. As shown, ibrolipim increased ABCA1 and ABCG1 expression at both the transcriptional (Figure 1B and 1C) and translational levels (Figure 1D and 1E) in a dose-dependent and time-dependent manner. Used as a positive control, the LXR ligand T0901317 (5 μ mol/L) increased

Table 1. Primer sequences for real-time quantitative PCR.

Gene	GenBank No	Forward primer	Reverse primer
ABCA1	NM_005502	5'-GTCCTCTTTCCCGCATTATCTGG-3'	5'-AGTTCCTGGAAGGTCTTGTTCAC-3'
ABCG1	NM_004915	5'-CGGAGCCCAAGTCGGTGTG-3'	5'-TTTCAGATGTCCATTTCAGCAGGTC-3'
LXR α	NM_013839	5'-AGCGTCCACTCAGAGCAAGT-3'	5'-GGGGACAGAACAGTCATTTCG-3'
β -actin	BC013835	5'-CTGGAACGGTGAAGGTGACA-3'	5'-AAGGGACTTCTGTAAACAATGCA-3'

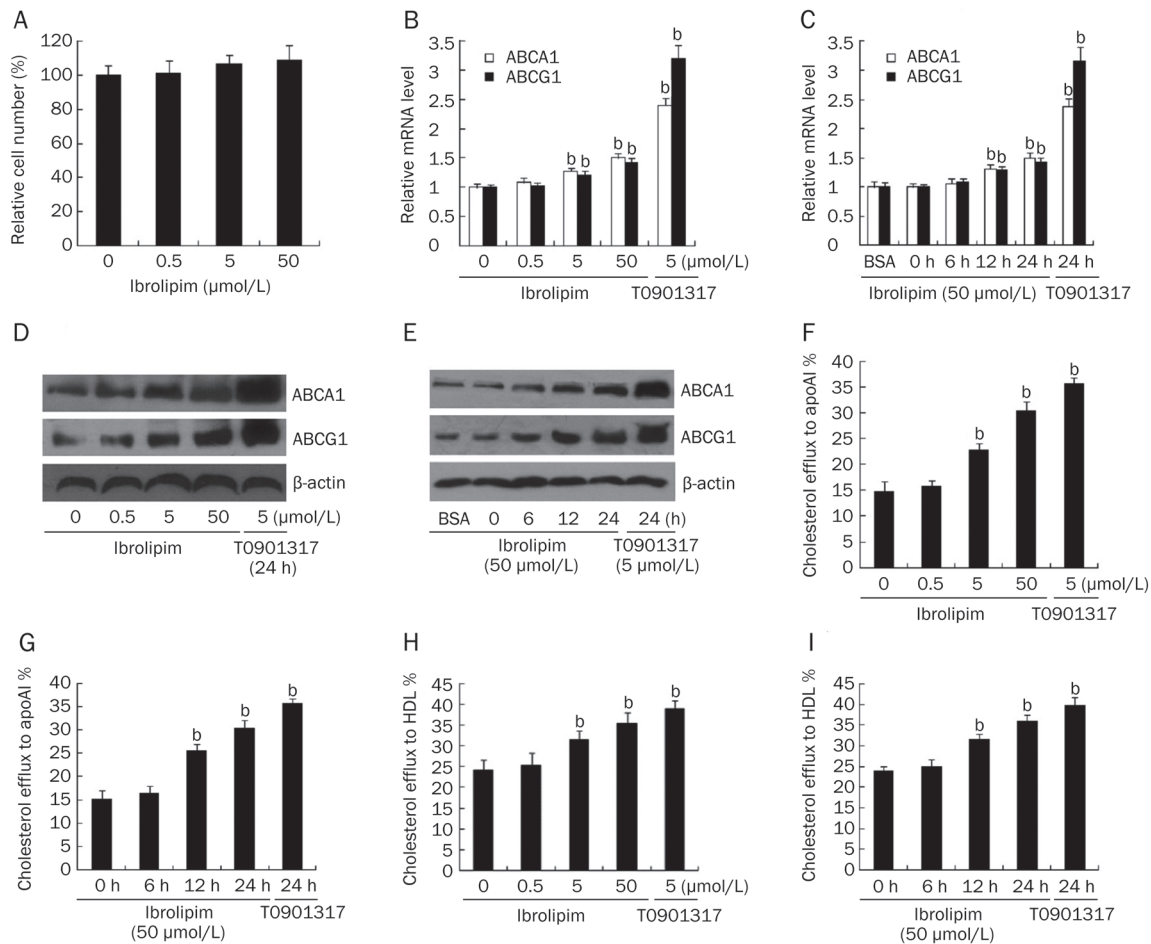


Figure 1. Effects of ibrolipim on the expression of ABCA1 and ABCG1 and cholesterol efflux in THP-1 macrophage-derived foam cells. Cells were treated with ibrolipim at different concentrations or different exposure times as indicated. **A**, The foam cell viability was measured using CellTiter 96Aqueous One Solution Reagent. Control (0 μmol/L) refers to incubations in the presence of vehicle only (0.1% DMSO) and was considered to have 100% viable cells. **B** and **C**, The expression of ABCA1 and ABCG1 were measured at mRNA levels by real-time PCR. **D** and **E**, ABCA1 and ABCG1 protein expressions were measured by Western blot. **F** and **H**, Cholesterol efflux to apoA-I or HDL from foam cells in response to different concentrations of ibrolipim treatment for 24 h. **G** and **I**, Cholesterol efflux to apoA-I or HDL from foam cells in response to ibrolipim at different exposure times. All the results are expressed as mean±SD. ^b*P*<0.05 vs controls. *n*=3.

ABCA1 and ABCG1 expression at 24 h.

Ibrolipim promotes cholesterol efflux in THP-1 macrophage-derived foam cells

Because ABCA1 and ABCG1 were upregulated by ibrolipim, we next determined the effect of ibrolipim on cholesterol efflux from THP-1 macrophage-derived foam cells by measuring ³H-cholesterol and cholesterol content. Significant increases of cholesterol efflux to apoA-I were observed in a concentration-dependent manner in response to ibrolipim treatment (Figure 1F). Next, a time course study was performed, and the differences in cholesterol efflux from foam cells to apoA-I between apoA-I only and ibrolipim-treated groups peaked at 12 and 24 h (Figure 1G). In addition, high performance liquid chromatography was used to measure cellular cholesterol content. The cellular cholesterol content was decreased while cholesterol efflux was increased by ibrolipim (Table 2), suggesting

Table 2. Cholesterol content in THP-1 macrophage-derived foam cells in response to ibrolipim and apoA-I. The results are expressed as mean±SD from three independent experiments, each performed in triplicate. ^b*P*<0.05 vs control group.

Ibrolipim	0 μmol/L	0.5 μmol/L	5 μmol/L	50 μmol/L	T0901317 (5 μmol/L)
TC	437±42	415±35	312±24 ^b	274±26 ^b	252±21 ^b
FC	174±21	166±16	126±18 ^b	117±14 ^b	105±13 ^b
CE	263±22	249±21	186±13 ^b	157±18 ^b	147±16 ^b
CE/TC (%)	60.1	60.0	59.6	57.2	58.3

THP-1 macrophage-derived foam cells were divided into five groups and cultured in medium with apoA-I (50 μg/mL) at 37 °C containing 0, 0.5, 5, and 50 μmol/L ibrolipim or 5 μmol/L T0901317 for 24 h respectively. Cellular cholesterol and cholesterol ester were extracted as described above. And HPLC was performed to determine the cellular total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE).

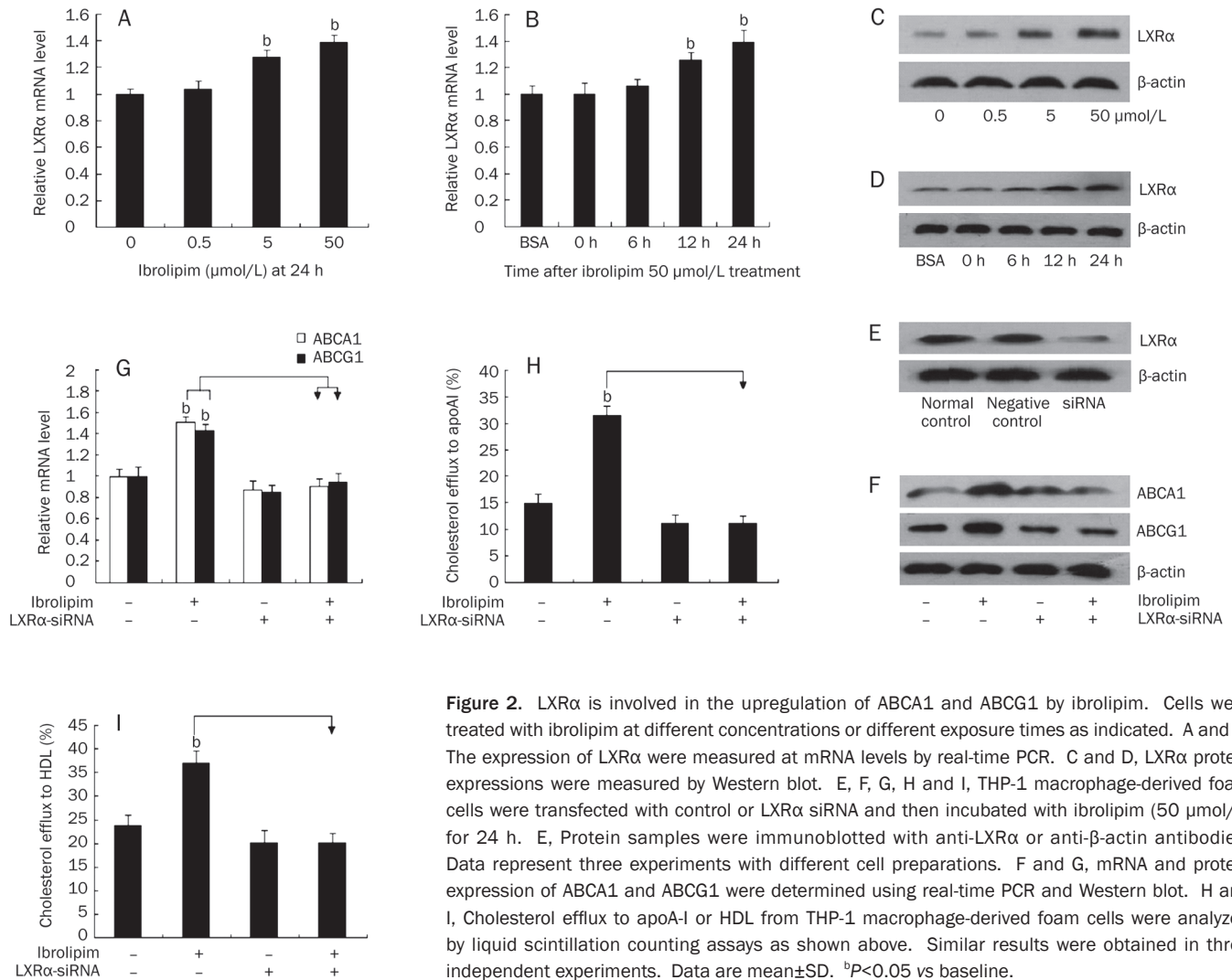


Figure 2. LXRα is involved in the upregulation of ABCA1 and ABCG1 by ibrolipim. Cells were treated with ibrolipim at different concentrations or different exposure times as indicated. A and B, The expression of LXRα were measured at mRNA levels by real-time PCR. C and D, LXRα protein expressions were measured by Western blot. E, F, G, H and I, THP-1 macrophage-derived foam cells were transfected with control or LXRα siRNA and then incubated with ibrolipim (50 μmol/L) for 24 h. E, Protein samples were immunoblotted with anti-LXRα or anti-β-actin antibodies. Data represent three experiments with different cell preparations. F and G, mRNA and protein expression of ABCA1 and ABCG1 were determined using real-time PCR and Western blot. H and I, Cholesterol efflux to apoA-I or HDL from THP-1 macrophage-derived foam cells were analyzed by liquid scintillation counting assays as shown above. Similar results were obtained in three independent experiments. Data are mean±SD. ^bP<0.05 vs baseline.

that ABCA1 expression can be upregulated by ibrolipim in foam cells. HDL, another acceptor of cholesterol and phospholipids transported by ABCG1, was also used to evaluate the effect of ibrolipim on cholesterol efflux in foam cells. Consistent with apoA-I, ibrolipim treatment also induced a significant increase of cholesterol efflux from foam cells to HDL in a dose- and time-dependent manner (Figure 1H and 1I). For directly measuring the cellular cholesterol content, ibrolipim-treated foam cells also showed a decrease compared with the control (HDL only, Table 3). Used as a positive control, the LXR ligand T0901317 (5 μmol/L) significantly increased cholesterol efflux from THP-1 macrophage-derived foam cells to apoA-I or HDL at 24 h.

LXRα is involved in the upregulation of ABCA1 and ABCG1 induced by ibrolipim in THP-1 macrophage-derived foam cells

The LXRα have been shown to regulate the expression of ABCA1 and ABCG1^[16, 17], which serve as free-cholesterol and phospholipid translocators enabling cholesterol efflux from the macrophage to various acceptors, including nascent cholesterol-poor HDL, and thus have a central role in the

Table 3. Cholesterol content in THP-1 macrophage-derived foam cells in response to ibrolipim and HDL. The results are expressed as mean±SD from three independent experiments, each performed in triplicate. ^bP<0.05 vs control group.

Ibrolipim	0 μmol/L	0.5 μmol/L	5 μmol/L	50 μmol/L	T0901317 (5 μmol/L)
TC	405±35	386±38	281±27 ^b	242±23 ^b	221±20 ^b
FC	161±24	154±19	116±21 ^b	98±10 ^b	95±11 ^b
CE	244±18	232±26	165±11 ^b	144±17 ^b	26±13 ^b
CE/TC (%)	60.3	60.1	58.7	59.4	57.2

THP-1 macrophage-derived foam cells were divided into five groups and cultured in medium with apoA-I (50 μg/mL) at 37 °C containing 0, 0.5, 5, and 50 μmol/L Ibrolipim or 5 μmol/L T0901317 for 24 h, respectively. Cellular cholesterol and cholesterol ester were extracted as described above. And HPLC was performed to determine the cellular total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE).

regulation of reverse cholesterol transport. To further confirm whether LXRα expression can be affected by ibrolipim, real-

time quantitative PCR and Western immunoblotting analyses were performed. As shown (Figure 2A–2D), the expression of LXR α mRNA and protein was increased in a dose-dependent and time-dependent manner when cells were treated with ibrolipim. We then examined the effect of LXR α siRNA on the upregulation of ABCA1 and ABCG1, which was induced by ibrolipim. Treatment with siRNA for LXR α down-regulated LXR α protein expression by 88% (Figure 2E) and completely abolished the upregulation of ABCA1 and ABCG1 expression by ibrolipim (Figure 2F and 2G). At the same time, cellular cholesterol efflux to apoA-I (Figure 2H) or HDL (Figure 2I) in cells treated by the combination of LXR α siRNA and ibrolipim was completely reversed when compared with cells treated by ibrolipim alone.

Discussion

Atherosclerosis is a chronic pathological process, and it can take decades for severe atheromatous lesions to develop in humans^[18]. Macrophages play a central role in the formation of arterial lesions by accumulating excessive amounts of lipids, mainly cholesterol ester (CE), through the uptake of modified lipoproteins. This occurs by a variety of mechanisms, including the scavenger receptor pathways such as scavenger receptors A (SR-A) and CD36^[19]. During this process, cholesterol efflux may play a pivotal role in the removal of excess cholesterol from extra hepatic cells including macrophages and vascular smooth muscle cells (VSMCs)^[20]. Thus, decreased cholesterol efflux from the arterial wall may potentially promote the progression of atherosclerosis. The principal molecules involved in the efflux of cholesterol from macrophage foam cells are ABCA1 and ABCG1^[4, 5]. ABCA1, the mutant molecule in Tangier disease, is involved in the efflux of cholesterol from peripheral tissue macrophages to lipid-free apolipoproteins, and in particular to apoA1^[20–22], whereas ABCG1 facilitates cellular cholesterol efflux from macrophages to lipidated particles such as mature HDL, but not to lipid-free apolipoproteins^[23, 24].

It has previously been reported that ibrolipim increased LPL mRNA levels, LPL protein mass, and LPL activity in post-heparin plasma and reduced plasma TG levels with concomitant increases of HDL-C levels in animals with lipid disorder. Kusunoki *et al* have reported that ibrolipim treatment suppresses fat accumulation in a high-fat diet-fed rat model. Tsutsumi *et al* have demonstrated that increasing LPL activity results in the elevation of high-density lipoprotein cholesterol and that long-term administration of ibrolipim protects against the development of atherosclerosis in rats fed with an atherogenic diet^[6–8]. Recently, our laboratory revealed that ibrolipim upregulates the expression of NPC1 through the LXR α pathway in THP-1 macrophage-derived foam cells^[25]. Moreover, we also showed that ibrolipim upregulated ABCA1 and inhibited diet-induced atherosclerosis in Chinese Bama minipigs^[8]. To further investigate the detailed mechanism for cholesterol transport proteins induced by ibrolipim, we first demonstrated that ibrolipim could upregulate the expression of ABCA1 and ABCG1 as well as promote cholesterol efflux in THP-1 macrophage-derived foam cells.

Expression of both ABCA1 and ABCG1 may be suppressed by the zinc finger protein 202 (ZNF202) and upregulated by cholesterol loading or by treatment with specific oxysterols including 25-, 20(S)-, and 22(R)-hydroxycholesterol^[16, 17, 26]. It is clear that induction of both ABCA1 and ABCG1 by these specific oxysterols depended on activation of LXR^[16, 17, 26]. To further understand the role of LXR α in the upregulation of ABCA1 and ABCG1 expression by ibrolipim, we investigated the effect of ibrolipim on the expression of LXR α to find out whether the expression of LXR α is changed during the course of treatment. It turned out that LXR α expression was also increased by ibrolipim in THP-1 macrophage-derived foam cells. LXR α siRNA was then used to see whether LXR α is involved in the upregulation of ABCA1 and ABCG1 induced by ibrolipim. LXR α siRNA completely abolished the upregulation of ABCA1 and ABCG1 expression by ibrolipim. Cellular cholesterol efflux promoted by ibrolipim was also reversed by LXR α siRNA. Therefore, we speculate that LXR α was involved in the upregulation of ABCA1 and ABCG1 induced by ibrolipim in THP-1 macrophage-derived foam cells.

In summary, the results of our study indicate that ibrolipim upregulates ABCA1 and ABCG1 expression as well as cholesterol efflux in THP-1 macrophage-derived foam cells. Expression of LXR α is also increased by ibrolipim treatment. However, enhanced ABCA1 and ABCG1 expression that was induced by ibrolipim was completely abolished by LXR α small interfering RNA. All of these findings suggest that ibrolipim may first upregulate the expression of LXR α and then increase the expression of ABCA1 and ABCG1 as well as cholesterol efflux in THP-1 macrophage-derived foam cells. These observations may provide new insights into the treatment of atherosclerosis.

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Author contribution

Si-guo CHEN, Ji XIAO, and Chao-ke TANG designed the research; Si-guo CHEN, Ji XIAO, Jin JIANG, Li-bao CUI, and Mei-mei LIU performed the research; Wei-dong YIN, Guo-jun ZHAO, and Chao-ke TANG contributed new analytical reagents and tools; Xie-hong LIU, Zhong-cheng MO, Kai YIN, and Chun-zhi TAN analyzed data; Ji XIAO wrote the paper.

Abbreviations

ABCA1: ATP-binding cassette transporter A1; ABCG1: ATP-binding cassette transporter G1; As: atherosclerosis; CAD: Coronary artery disease; LXRs: liver X receptors; RXR: retinoid X receptor; ox-LDL: oxidized low density lipoprotein; PMA: phorbol 12-myristate 13-acetate; TC: total cholesterol; CE:

cholesterol ester; FC: free cholesterol; RCT: reverse cholesterol transport; LDL: low density lipoprotein; HDL: high density lipoprotein; apoA1: apolipoprotein A-I; IFN- γ : interferon- γ ; GAS: interferon- γ activation sequence; JAK: janus kinase; STAT: signal transducer and activator of transcription; EPA: eicosapentaenoic acid; UC: unesterified cholesterol; LPL: lipoprotein lipase; cAMP: cyclic AMP; PKA: protein kinase A; siRNA: short-interfering RNA; SR-A: scavenger receptors A; NPC1: Niemann-Pick type C; ZNF202: zinc finger protein 202.

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