European Review for Medical and Pharmacological Sciences 2016; 20: 3945-3952

Quercetin upregulates ABCA1 expression through liver X receptor alpha signaling pathway in THP-1 macrophages

Y. LU, Y.-P. JIA

Department of Cardiology, The first Hospital of Shanxi Medical University, Taiyuan, China

Abstract. – OBJECTIVES: Quercetin has been reported to have the activities of antioxidant, anti-inflammatory, anti-virus, anti-cancer and so on. Many studies showed that quercetin could lower blood pressure and improve blood capillary elasticity, and it can also reduce LDL oxidation and prevent atherosclerosis. Although quercetin has been recognized to have the function of preventing atherosclerosis, little is known about its underlying mechanism. In this study, we try to explore whether quercetin up-regulates LXRα-mediated ABCA1 expression.

MATERIALS AND METHODS: THP-1 cells were cultured. The expression of ABCA1 and LXRα were detected by real-time PCR and western blot. Cellular cholesterol efflux from THP-1 macrophages was analyzed using liquid scintillation counting assays.

RESULTS: Real-time PCR and Western blot showed quercetin increased the expression of ABCA1 and LXRα at both the mRNA and protein levels in a concentration-dependent and time-dependent manner in THP-1 macrophages. Liquid scintillation counting assays indicated quercetin increased the cholesterol efflux and decreased the cellular cholesterol content. Furthermore, the expression of LXRα was decreased after THP-1 macrophage transfected with LXRα siRNA. Meanwhile, the expression of ABCA1 was also recovered after incubated with the combination of LXRα siRNA and quercetin compared with quercetin alone.

CONCLUSIONS: Quercetin could increase ABCA1 expression and cholesterol efflux through LXRα pathway to eventually promote RCT in the THP-1 macrophage.

Key Words

Atherosclerosis (AS), Quercetin, Reverse cholesterol transport (RCT), ATP-binding cassette transporter A1 (ABCA1), LXRα pathway.

Introduction

Atherosclerosis (AS), which has the feature of slow development, is a vascular disease. Recent years, the morbidity and mortality rates of AS has been near the top in global and it has serious damage to health[1]. It is a process of multiple factors involved[2]. A large number of lipid accumulates in macrophages under vascular endothelial and the formation of foam cells are the hallmark of its early lesions. Foam cells eventually disintegrated to release intracellular cholesterol and the intracellular cholesterol becomes the main component of atherosclerotic plaque. This process promotes the progress of atherosclerosis[3,4]. Therefore, it is significant to promote cholesterol efflux from macrophages *in vivo* and to reverse cholesterol transport (reverse cholesterol transport, RCT) in inhibiting the formation of foam cells in the vessel wall lipid deposition and the prevention of atherosclerotic disease. ATP-binding cassette transporter A1 (ABCA1), which is one key protein of RCT, has been reported to have the function of promoting cholesterol efflux from macrophage and preventing the formation of foam cells[5-7]. It is believed that promoting the expression of ABCA1 has an important significance of inhibiting the development of atherosclerosis. Liver X receptors alpha (LXRα) is a member of super nuclear receptor protein family. Recent years, it has been reported by adjusting the target gene of ABCA1 expression can regulate reverse cholesterol transport[7-9].

Quercetin is a kind of natural flavonoid compounds, which widely exists in the plant. Quercetin widely exists in the human diet, and two-third of human daily dietary intake of flavonoids substances is quercetin and its related derivatives. In recent years, the biological activity of quercetin has been received widespread attention. Quercetin has been reported to have the activities of antioxidant, anti-inflammatory, anti-virus, anti-cancer and so on[10-13]. Many studies[14,15] showed that quercetin could lower blood pressure and improve blood capillary elasticity, and it can also reduce LDL oxidation and prevent atherosclerosis. Although quercetin has been recognized to have the function of preventing atherosclerosis, little is

Corresponding Author: Yongping Jia, MD, Ph.D; e-mail: doctoryongping@163.com

known about its underlying mechanism. Whether quercetin has an influence on the expression of ABCA1 and ABCA1-dependent cholesterol efflux in THP-1 macrophage is still unclear. Meanwhile, whether LXRα pathway involved in it is also unknown. Based on these, we try to explore whether quercetin up-regulates LXRα-mediated ABCA1 expression.

In this study, we explore the underlying mechanism of quercetin preventing atherosclerosis. Results indicated that quercetin increased ABCA1 expression and cholesterol efflux through LXRα pathway to eventually promote RCT in the THP-1 macrophage. Therefore, quercetin has great potential in the treatment of cardiovascular disease.

Materials and Methods

Materials and Reagents

The THP-1 cell line was purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). RPMI 1640 and fetal bovine serum (FBS) were purchased from GIBCO (Grand Island, NY). Quercetin and apoA-I were obtained from Sigma-Aldrich (St. Louis, MO, USA). TRIzol Reagent was obtained from Invitrogen (Carlsbad, CA, USA). BCA Protein Assay Kit was purchased from Pierce Chemical (Rockford, IL, USA). Enhanced chemiluminescence kit was purchased from Peirce (Tottenham, UK). Antibodies against ABCA1, ABCG1 and β-actin were obtained from Abcam (Cambridge, UK). siRNAs were purchased from the GenePharma Co. (Shanghai, China).

Cell Culture

The THP-1 cell line was purchased from the Cell Bank of the Chinese Academy of Science. THP-1 cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were maintained in a humidified atmosphere with 5% CO_2 at 37 °C.

RNA Isolation and Real-time Polymerase Chain Reaction (PCR)

The THP-1 cells were seeded in 6-well plates at a density of 1×10^6 cells/well and differentiated into macrophages by the addition of 100 nM PMA for 48 h. The macrophages were induced with serum-free medium containing ox-LDL (20 μg/mL) for 48 h into foam cells. Then the foam cells were incubated with 25 μg/mL apoA-1 and quercetin (5, 10, 15 μM) for 24 h respectively or with apoA-1 (25 μg/mL) for 24 h and quercetin (15 μM) for 0, 6, 12, 24 h. Total RNA from cells was extracted using TRIzol reagent according to the manufacturer's protocol. Real-time quantitative PCR, using SYBR Green detection chemistry, was carried out on an ABI 7300 Real-time PCR System, and then using the following conditions: 95 °C denaturation for 2 minutes, followed by 35 cycles of 95 °C for 30 seconds and 60 °C for 30 seconds.

The targeted genes and primer sequences are as follows:

ABCA1:
Forward primer:
5'-AACAGTTTGTGGCCCTTTTG-3',
Reverse primer:
5'-AGTTCCAGGCTGGGGTACTT-3';
LXRα:
Forward primer:
5′-TCCTCAGTCTGCTCCACC-3′,
Reverse primer:
5′-TGCTCTCCGAGATCTGGG-3′;
GAPDH:
Forward primer:
5'-AACTTTGGCATTGTGGAAGG-3',
Reverse primer:
5'-ACACATTGGGGGTAGGAACA-3'.

Quantitative measurements were determined using the ΔΔCt method, and the expression of GAPDH was used as the internal control.

Western Blot Analyses

The THP-1 cells were into 60 mm dishes (1×10^6 cells/dish) and differentiated into macrophages by the addition of 100 nM PMA for 48 h. The macrophages were induced with serum-free medium containing ox-LDL (20 μg/mL) for 48 h into foam cells. Then the foam cells were incubated with 25 μg/mL apoA-1 and quercetin (5, 10, 15 μM) for 24 h respectively or with apoA-1 (25 μg/mL) for 24h and quercetin (15 μM) for 0, 6, 12, 24 h. Treated cells were harvested and lysed with Western lysis buffer containing 1% PMSF for 15 min and centrifuged at 13000 rpm. Protein concentration was detected with a BCA protein kit. An equal amount of protein (80 μg) was loaded on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and the protein was transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were then blocked in 5% non-fat dry milk for 1 h. The membranes were incubated with anti-ABCA1, anti-LXRα and anti-GAPDH overnight at 4 °C. Then, the membranes were incubated with the appropriate HRP-conjugated secondary antibody. Finally, the protein was detected with ECL Western blotting detection system.

Characterization of Cellular Cholesterol Efflux Experiments

Cells were washed with phosphate-buffered saline (PBS) and incubated overnight in RPMI 1640 medium containing 0.1% (w/v) bovine serum albumin (BSA). The macrophages were cultured and labeled with [^{3}H]-cholesterol (0.2 μCi/mL) for 72h. Then, the macrophages were plated in 12-well plates at a density of 2×10^5cells/well in RPMI 1640 with 10% (v/v) FBS and 1% penicillin/streptomycin. Equilibrated [^{3}H]-cholesterol-labelled cells were washed with PBS, and incubated in efflux medium containing RPMI 1640 medium and 0.1% BSA with 50 lg/mL human plasma apoA-1 or HDL for 6 h. Monolayers were washed twice in PBS, and cellular lipids were extracted with isopropanol. The radioactivity in 40 μL of the supernatant was determined by liquid scintillation counting. At the end of the incubation, cells were lysed in 400 μl of lysis buffer (PBS containing 1% (v/v) Triton X-100) for 30 min at room temperature and radioactivity was measured in 40 μl cell lysate. The percentage of secreted [14C] cholesterol was calculated by dividing the medium-derived counts by the sum of the total counts present in the culture medium and the cell lysate. Medium and cell-associated [^{3}H]-cholesterol were measured by liquid scintillation counting. Percent efflux was calculated by the following equation: [total media counts/(total cellular counts + total media counts)] ×100%.

Macrophages Transfection with LXRα-RNA

For transfection with siRNA, macrophages were plated in 12-well plate at a density of 5×10^5 cells/well. Cells were transfected with LXRα siRNA in Opti-MEM with 5 μg/mL Lipofectamine® RNAiMAX (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The LXRα-siRNA sequence used for the target was AAGTACACAGG-AGGCCATCTT, and the target sequence for control non-silencing siRNA was AATTCTCCGAACGTCTCACGT. All siRNA transfections were performed for 24 h preceding subsequent macrophages measurements.

Statistical Analysis

All data were performed using the SPSS 17.0 software. Student's *t*-test was used for two groups. Statistical significance of differences was analyzed with one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. A value of less than 0.05 ($p < 0.05$) and 0.01 ($p < 0.01$) were considered significant and highly significant, respectively.

Results

Quercetin Increases ABCA1-mediated Cholesterol Efflux in THP-1 Macrophages

To detect whether quercetin had effect on ABCA1 expression in THP-1 macrophages, the THP-1 macrophages were incubated with 25 μg/mL apoA-1 and quercetin (5, 10, 15 μM) for 24 h respectively or with apoA-1 (25 μg/mL) for 24h and quercetin (15 μM) for 0, 6, 12, 24 h and then the expression of ABCA1 was detected by real-time PCR and Wstern blot. As shown in Figure 1, quercetin increased ABCA1 expression at both the mRNA and protein levels in a dose-dependent (Figure 1A and C) and time-dependent manner (Figure 1B and D). ABCA1 has been reported to play an important role in regulating cellular cholesterol homeostasis. Based on the above studies, the effect of quercetin on apoA-1 specific cholesterol efflux and cholesterol content in THP-1 macrophage were determined by liquid scintillation counting and high performance liquid chromatography (HPLC). The results showed quercetin increased the cholesterol efflux and decreased the cellular cholesterol content (Figure 1E and F, Tables I and II). All above results indicated quercetin up-regulated ABCA1-mediated cholesterol efflux in the THP-1 macrophages.

Quercetin Increases the Expression of LXRα in THP-1 Macrophage

Liver X receptors (LXRs), which are important in regulating cholesterol efflux from macrophages, are ligand-activated transcription factors[16,17]. To detect whether quercetin could increase the expression of LXRα, THP-1 macrophage were treated with 25 μg/mL apoA-1 and quercetin (5, 10, 15 μM) for 24 h respectively or with apoA-1 (25 μg/mL) for 24h and quercetin (15 μM) for 0, 6, 12, 24 h. Similarly, the expression of LXRα was detected by real-time PCR and Western blot. The results showed the expression of LXRα was up-regulated in a concentration-dependent (Figure 2A and C) and time-dependent manner (Figure 2B and D) at both the mRNA and protein levels. These results indicated quercetin increased the expression of LXRα in THP-1 macrophage.

LXRα is Involved in Quercetin Up-regulation ABCA1 Expression i n THP-1 Macrophages

To verify further whether quercetin increases ABCA1 expression through LXRα pathway, THP-1 macrophage was treated with LXRα siRNA and

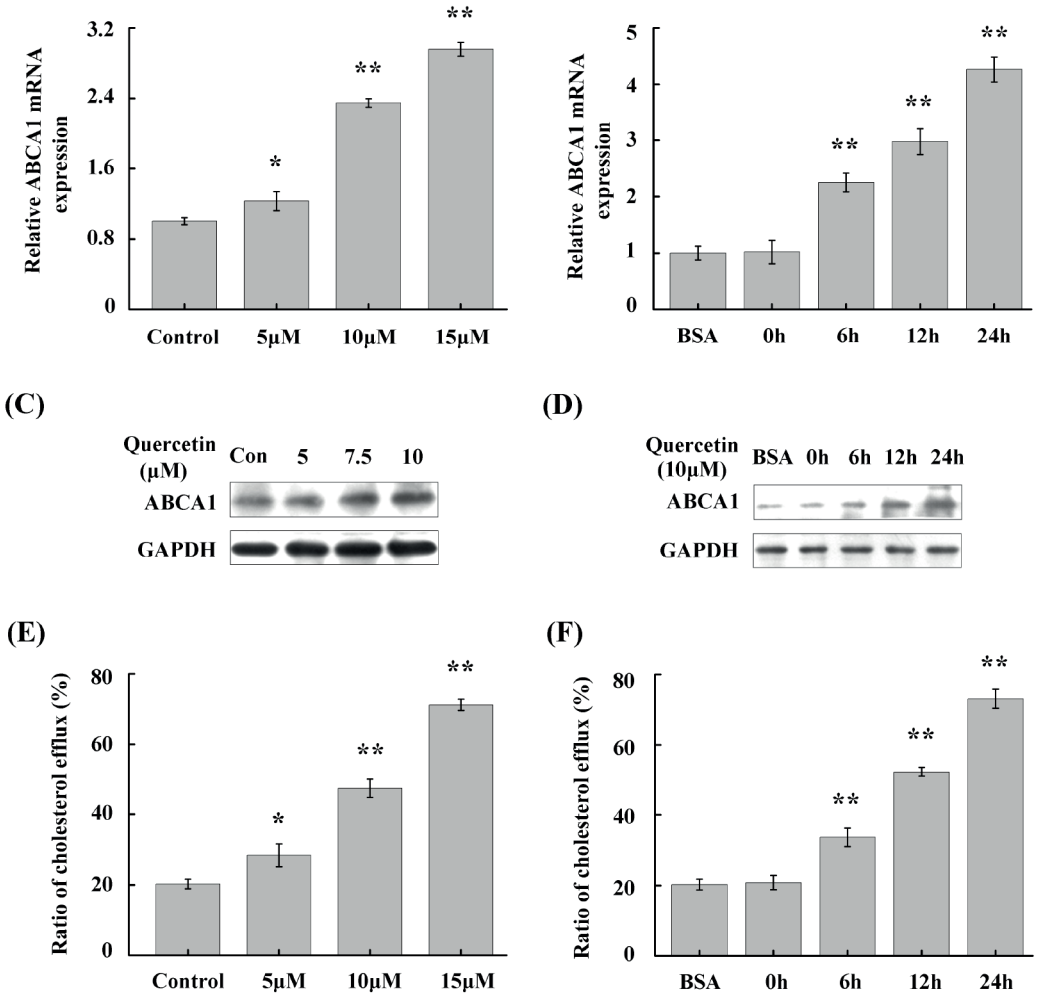

Figure 1. Quercetin induced increasing of ABCA1 expression and the cholesterol efflux from THP-1 macrophages. **A**, **C** and **E**: The THP-1 macrophages treated with 25 μg/mL apoA-I and with 5 μM, 10 μM and 15 μM quercetin for 24 h respectively. **B**, **D** and F: The THP-1 macrophages were incubated with 25 μg/mL apoA-I and 5 mg/mL BSA for 24 h or incubated with 25 μg/mL apoA-I and 15 μM quercetin for 0 h, 6 h, 12 h, 24 h, respectively. **A** and **B**: The mRNA level of ABCA1 was determined by real-time PCR. **C** and **D**: The protein level of ABCA1 was measured by Western blot. **E** and **F**: Cellular cholesterol efflux from THP-1 macrophages was analyzed with liquid scintillation counting assays. Data are the mean ± SD of three independent experiments. *$p < 0.05$ and **$p < 0.01$ compared to control.

then incubated with apoA-I (25 μg/mL) and quercetin (15 μM) for 24 h respectively. The expression of ABCA1 was detected using real-time PCR and western blot and cellular cholesterol efflux from THP-1 macrophages was analyzed by liquid scintillation counting assays. Results showed the expression of LXRα was decreased after THP-1 macrophage transfected with LXRα siRNA (Figure

Table I. Effect of quercetin on cellular cholesterol content at different concentrations in THP-1 macrophages.

Quercetin	0 (μM)	5 (μM)	10 (μM)	15 (μM)
TC (mg/dL)	452 ± 30	398 ± 24*	290 ± 22**	248 ±23**
FC (mg/dL)	181 ± 17	159 ± 8*	116 ± 15**	100 ± 11**
CE (mg/dL)	271 ± 11	239 ± 18*	174 ± 10**	148 ± 14**
CE/TC (%)	60	60	60	59.7

The THP-1 macrophages were treated with 25 μg/mL apoA-I and with 5 μM, 10 μM and 15 μM quercetin for 24 h respectively. The cellular total cholesterol and free cholesterol were determined by HPLC. Data are the mean ± SD of three independent experiments. *$p < 0.05$ and **$p < 0.01$ compared to control.

Table II. Effect of quercetin on cellular cholesterol content at different time in THP-1 macrophages.

Quercetin	BSA (24h)	0h	6h	12h	24h
TC (mg/dL)	480 ± 32	456 ± 27	360 ± 21**	285±23**	242 ± 18**
FC (mg/dL)	190 ± 13	184 ± 12	144 ± 15**	114 ± 10**	97 ± 8**
CE (mg/dL)	290 ± 11	272 ± 15	216 ± 16**	171 ± 12**	145 ± 13**
CE/TC (%)	60	59.6	60	60	59.9

The THP-1 macrophages were incubated with 25 μg/mL apoA-I and 5 mg/mL BSA for 24 h or incubated with 25 μg/mL apoA-I and 15μM quercetin for 0 h, 6 h, 12 h, 24 h, respectively. The cellular total cholesterol and free cholesterol were determined by HPLC. Data are the mean ± SD of three independent experiments. *$p < 0.05$ and **$p < 0.01$ compared to control.

3A). Meanwhile, the expression of ABCA1 was decreased after treated by the combination of LXRα siRNA and quercetin compared with quercetin alone (Figure 3 B and C) and the cellular cholesterol efflux and cholesterol content from THP-1 macrophages was also abolished (Figure 3D, Table III). All above results suggested quercetin increased ABCA1 expression through LXRα pathway.

Discussion

Atherosclerosis is a process of multiple factors that involved in. In the progression of atherosclerosis, monocytes are differentiated from macrophages, and foam cells are formed in the vessel wall[18,19]. Therefore, it is important to reverse cholesterol transport (RCT). ATP-binding cassette transporter A1 (ABCA1) has been reported to promote cholesterol efflux from macrophage, and it is one key protein in reversing cholesterol transport. Meanwhile, it has been demonstrated to mediate foam cells and other peripheral cellular free cholesterol efflux to apoA-1[20,21]. ABCA1 is important to inhibit the development of atherosclerosis. Liver X receptors alpha (LXRα), which is a member of the nuclear receptor family, has been reported to promote reverse cholesterol transport via adjusting the target gene of ABCA1 expression[22,23].

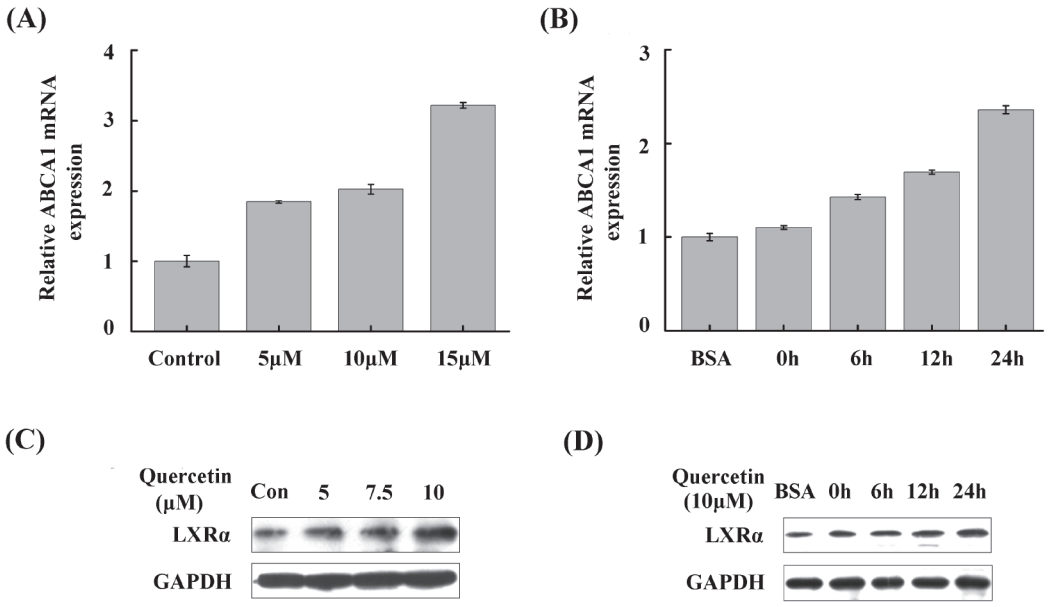

Figure 2. Quercetin induced increasing of LXRα expression in THP-1 macrophages. **A** and **C**: The THP-1 macrophages treated with 25 μg/mL apoA-I and with 5 μM, 10 μM and 15 μM quercetin for 24 h respectively. **B** and **D**: The THP-1 macrophages were incubated with 25 μg/mL apoA-I and 5 mg/mL BSA for 24 h or incubated with 25 μg/mL apoA-I and 15 μM quercetin for 0 h, 6 h, 12 h, 24 h, respectively. **A** and **B**: The mRNA level of LXRα was determined by real-time PCR. **C** and **D**: The protein level of LXRα was measured by Western blot. Data are the mean ± SD of three independent experiments. *$p < 0.05$ and **$p < 0.01$ compared to control.

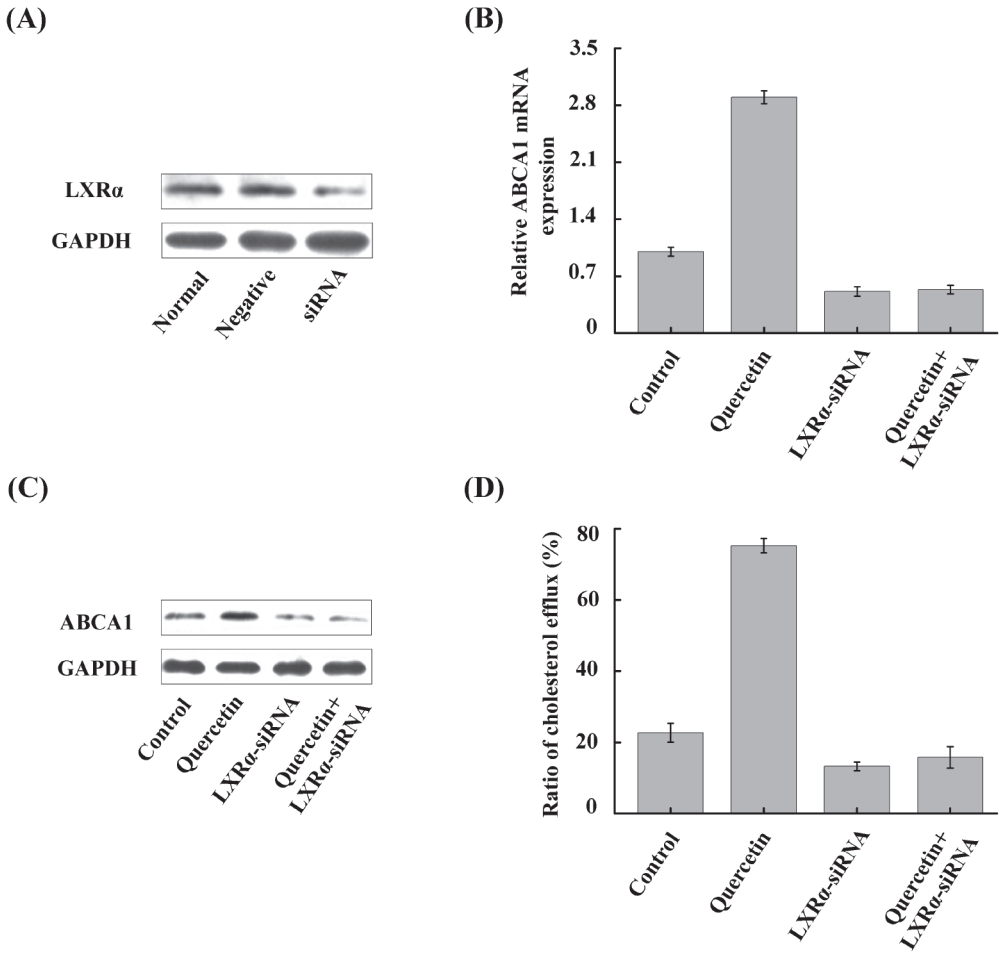

Figure 3. Quercetin up-regulated ABCA1 expression by LXRα pathway in THP-1 macrophages. **A**: THP-1 macrophage was treated with LXRα siRNA. The protein level of LXRα was measured by Western blot. **B**, **C** and **D**. THP-1 macrophage was treated with LXRα siRNA and then incubated with apoA-I (25 μg/mL) and quercetin (15 μM) for 24 h respectively. **B**: The mRNA level of ABCA1 was determined by real-time PCR. **C**: The protein level of ABCA1 was measured by Western blot. **D**: Cellular cholesterol efflux from THP-1 macrophages was analyzed with liquid scintillation counting assays. Data are the mean ± SD of three independent experiments. *$p < 0.05$ compared to control. #$p < 0.05$ compared with the quercetin group.

Many reports[24,25] indicated that natural phenolic compounds have the biological activities of anti-inflammatory, anti-aging, curing cardiovascular disease and so on. Flavonoid compounds, which are considered to be safe and good for your health in food additives, are the most common class of the natural phenolic compounds[26]. Quercetin is a kind of natural flavonoid compounds and has been

Table III. Effect of LXRα siRNA on cellular cholesterol content at different time in THP-1 macrophages.

Quercetin	Control	Quercetin	LXRα-siRNA	Quercetin+ LXRÐ-siRNA
TC (mg/dL)	463 ± 28	215 ± 16*	629 ± 34*	638 ± 30* #
FC (mg/dL)	185 ± 11	86 ± 6*	252 ± 12*	256 ± 10* #
CE (mg/dL)	278 ± 13	129 ± 15*	377 ± 20*	382 ± 18* #
CE/TC (%)	60	60	59.9	59.9

The THP-1 macrophages were tranfected with control or LXRα siRNA, and then incubated with 25 μg/mL apoA-I and 15 μM quercetin for 24 h. The cellular total cholesterol and free cholesterol were determined by HPLC. Data are the mean ± SD of three independent experiments. *$p < 0.05$ compared to control. #$p < 0.05$ compared with the quercetin group.

reported to have the function of lowering blood pressure and improving blood capillary elasticity[27]. At the same time, it can decrease LDL oxidation and prevent atherosclerosis[14,28]. In this study, quercetin increased the expression of ABCA1 were found at both the mRNA and protein levels in a dose- and time-dependent manner. Quercetin also increased the cholesterol efflux and decreased the cellular cholesterol content. Further studies observed that quercetin could increase the expression of LXRα. LXRα has been reported to regulate RCT to maintain the cholesterol level by promoting the expression of ABCA1. Then, we verified whether quercetin increased the expression of ABCA1 via up-regulating the expression of LXRα. Results showed the expression of LXRα was decreased after THP-1 macrophage transfected with LXRα siRNA. At the same time, the expression of ABCA1 was decreased after treated with composite processing compared with quercetin alone, and also the cellular cholesterol efflux and cholesterol content from THP-1 macrophages were reversed. These results implied that quercetin could increase ABCA1 expression and cholesterol efflux through LXRα pathway in THP-1 cells.

Conclusions

The effects of quercetin on the expression of ABCA1 and LXRα were detected by real-time PCR and Western blot. Results indicated quercetin increased the expression of ABCA1 and LXRα in a concentration- and time-dependent manner at both mRNA and protein level. More importantly, quercetin increased the expression of ABCA1 and cholesterol efflux by promoting LXRα expression. This finding suggests that quercetin could promote the cholesterol efflux from THP-1, and this study has great significance in the clinical treatment of atherosclerosis.

Conflicts of interest

The authors declare that no conflicts of interest exist.

References

1) Eder L, Gladman DD. Atherosclerosis in psoriatic disease: latest evidence and clinical implications. Ther Adv Musculoskelet Dis 2015; 7: 187-195.
2) Bevc S, Sabic S, Hojs R. Atherosclerosis in hemodialysis patients--the role of microinflammation. Ren Fail 2008; 30: 1012-1016.
3) Upmacis RK. Atherosclerosis: a link between lipid intake and protein tyrosine nitration. Lipid Insights 2008; 2008(2): 75.
4) Reina SA, Llabre MM, Allison MA, Wilkins JT, Mendez AJ, Arnan MK, Schneiderman N, Sacco RL, Carnethon M, Delaney JA. HDL cholesterol and stroke risk: The Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2015; 243: 314-319,
5) Xiao Z, Wang J, Chen W, Wang P, Zeng H, Chen W. Association studies of several cholesterol-related genes (ABCA1, CETP and LIPC) with serum lipids and risk of Alzheimer's disease. Lipids Health Dis 2012; 11: 163.
6) Wehmeier KR, Kurban W, Chandrasekharan C, Onstead-Haas L, Mooradian AD, Haas MJ. Inhibition of ABCA1 protein expression and cholesterol efflux by TNF alpha in MLO-Y4 osteocytes. Calcif Tissue Int 2016; 98: 586-595.
7) Liang B, Wang X, Bian Y, Yang H, Liu M, Bai R, Yang Z, Xiao C. Angiotensin-(1-7) upregulates expression of adenosine triphosphate-binding cassette transporter A1 and adenosine triphosphate-binding cassette transporter G1 through the Mas receptor through the liver X receptor alpha signalling pathway in THP-1 macrophages treated with angiotensin-II. Clin Exp Pharmacol Physiol 2014; 41: 1023-1030.
8) Ikhlef S, Berrougui H, Kamtchueng Simo O, Khalil A. Paraoxonase 1-treated oxLDL promotes cholesterol efflux from macrophages by stimulating the PPARgamma- LXRalpha-ABCA1 pathway. FEBS Lett 2016; 590: 1614-1629.
9) Zhao JF, Jim Leu SJ, Shyue SK, Su KH, Wei J, Lee TS. Novel effect of paeonol on the formation of foam cells: promotion of LXRalpha-ABCA1-dependent cholesterol efflux in macrophages. Am J Chin Med 2013; 41: 1079-1096.
10) Nishimura Fde C, de Almeida AC, Ratti BA, Ueda-Nakamura T, Nakamura CV, Ximenes VF, Silva Sde O. Antioxidant effects of quercetin and naringenin are associated with impaired neutrophil microbicidal activity. Evid Based Complement Alternat Med 2013; 2013: 795916.
11) Wang L, Wang B, Li H, Lu H, Qiu F, Xiong L, Xu Y, Wang G, Liu X, Wu H, Jing H. Quercetin, a flavonoid with anti-inflammatory activity, suppresses the development of abdominal aortic aneurysms in mice. Eur J Pharmacol 2012; 690: 133-141.
12) Wu W, Li R, Li X, He J, Jiang S, Liu S, Yang J. Quercetin as an antiviral agent inhibits influenza A virus (IAV) entry. Viruses 2016; 8: pii: E6.
13) Rivera Rivera A, Castillo-Pichardo L, Gerena Y, Dharmawardhane S. Anti-breast cancer potential of quercetin via the Akt/AMPK/mammalian target of rapamycin (mTOR) signaling cascade. PLoS One 2016; 11: e0157251.
14) Garelnabi M, Mahini H, Wilson T. Quercetin intake with exercise modulates lipoprotein metabolism and reduces atherosclerosis plaque formation. J Int Soc Sports Nutr 2014; 11: 22.
15) Juzwiak S, Wojcicki J, Mokrzycki K, Marchlewicz M, Bialecka M, Wenda-Rozewicka L, Gawronska-Szklarz B, Drozdzik M. Effect of quercetin on experimental hyperlipidemia and atherosclerosis in rabbits. Pharmacol Rep 2005; 57: 604-609.

16) Bischoff ED, Daige CL, Petrowski M, Dedman H, Pattison J, Juliano J, Li AC, Schulman IG. Non-redundant roles for LXRalpha and LXRbeta in atherosclerosis susceptibility in low density lipoprotein receptor knockout mice. J Lipid Res 2010; 51: 900-906.
17) Mao MJ, Hu JP, Wang C, Zhang YY, Liu P. Effects of Chinese herbal medicine Guanxinkang on expression of PPARgamma-LXRalpha-ABCA1 pathway in ApoE-knockout mice with atherosclerosis. Zhong Xi Yi Jie He Xue Bao 2012; 10: 814- 820.
18) Alexandroff AB, Pauriah M, Lang CC, Struthers AD, Armstrong DJ. Atherosclerosis as a systemic feature of psoriasis. Clin Exp Dermatol 2011; 36: 451-452.
19) Yatsuya H, Folsom AR, Investigators A. Risk of incident cardiovascular disease among users of smokeless tobacco in the Atherosclerosis Risk in Communities (ARIC) study. Am J Epidemiol 2010; 172: 600-605.
20) Francone OL, Aiello RJ. ABCA1: regulation, function and relationship to atherosclerosis. Curr Opin Investig Drugs 2002; 3: 415-419.
21) Huang L, Fan B, Ma A, Shaul PW, Zhu H. Inhibition of ABCA1 protein degradation promotes HDL cholesterol efflux capacity and RCT and reduces atherosclerosis in mice. J Lipid Res 2015; 56: 986-997.
22) Sha YH, Hu YW, Gao JJ, Wang YC, Ma X, Qiu YR, Li SF, Zhao JY, Huang C, Zhao JJ, Lu JB. Lipoxin A4 promotes ABCA1 expression and cholesterol efflux through the LXRalpha signaling pathway in THP-1 macrophage-derived foam cells. Int J Clin Exp Pathol 2015; 8: 6708-6715.
23) Ozasa H, Ayaori M, Iizuka M, Terao Y, Uto-Kondo H, Yakushiji E, Takiguchi S, Nakaya K, Hisada T, Uehara Y, Ogura M. Pioglitazone enhances cholesterol efflux from macrophages by increasing ABCA1/ABCG1 expressions via PPARgamma/LXRalpha pathway: findings from in vitro and ex vivo studies. Atherosclerosis 2011; 219: 141-150.
24) Criquet J, Rodriguez EM, Allard S, Wellauer S, Salhi E, Joll CA, von Gunten U. Reaction of bromine and chlorine with phenolic compounds and natural organic matter extracts--Electrophilic aromatic substitution and oxidation. Water Res 2015; 85: 476-486.
25) Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr Cancer 2010; 62: 1-20.
26) Qiu Y, Liu Q, Beta T. Antioxidant activity of commercial wild rice and identification of flavonoid compounds in active fractions. J Agric Food Chem 2009; 57: 7543-7551.
27) Sugantha Priya E, Selvakumar K, Bavithra S, Elumalai P, Arunkumar R, Raja Singh P, Brindha Mercy A, Arunakaran J. Anti-cancer activity of quercetin in neuroblastoma: an in vitro approach. Neurol Sci 2014; 35: 163-170.
28) Lu Y, Wang RH, Guo BB, Jia YP. Quercetin inhibits angiotensin II induced apoptosis via mitochondrial pathway in human umbilical vein endothelial cells. Eur Rev Med Pharmacol Sci 2016; 20: 1609-1616.