Histol Histopathol (2025) 40: 585-596

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE Open Access

The Lian-Dou-Qing-Mai Formula activates the PPARγ-LXRα-ABCA1/ABCG1 pathway by regulating IL-10, leading to the promotion of cholesterol efflux and a reduction in atherosclerotic plaques

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Summary. Background. To observe the effect of the Lian-Dou-Qing-Mai (LDQM) formula on lipid metabolism in mice and explore its mechanism from the perspective of regulating the PPARγ/LXRα/ABCA1 signaling pathway.

Methods. THP-1 cells were induced to transform into foam cells with ox-LDL. Atherosclerosis (AS) models were constructed using a high-fat diet in ApoE$^{-/-}$ mice. Detection kits were used to evaluate triglyceride (TG) and total cholesterol (TC) content; TNF-α, MCP-1, MMP-9, TMP-1, PPARγ, LXRα, ABCA1, and ABCG1 mRNA and protein expression were identified using real-time PCR and western blot. Aortic plaque development and lipid deposition were seen using hematoxylin and eosin (HE) and oil red O staining, respectively.

Results. In the cell model, LDQM could inhibit the formation of THP-1 macrophage-derived foam cells and the expression of inflammatory factors, promote macrophage cholesterol efflux, increase the expression of IL-10, and activate the PPARγ-LXRα-ABCA1/ABCG1 pathway. Additional IL-10 treatment further promotes LDQM-induced cholesterol efflux in THP-1 cells. In *in vivo* models, LDQM inhibited the area of atherosclerotic lesions, aortic lipid deposition, and inflammation levels in ApoE$^{-/-}$ mice through IL-10, and activated the expression level of the PPARγ-LXRα-ABCA1/ABCG1 pathway.

Conclusion. LDQM may affect the PPARγ/LXRα/ABCA1 signaling pathway through IL-10, regulate lipid metabolism, reduce serum inflammatory expression and lipid deposition, and improve the formation of atheroplaques.

Key words: Lian-Dou-Qing-Mai, PPARγ-LXRα-ABCA1/ABCG1, IL-10, Atherosclerosis

Introduction

Currently, atherosclerosis (AS) ranks among the primary contributors to mortality and morbidity on a global scale (Kobiyama and Ley, 2018). The development of foam cells that originate from macrophages is a distinctive characteristic of AS, which is governed by the processes of cholesterol absorption, intracellular metabolism, and efflux (Yu et al., 2013, Guerrini and Gennaro, 2019). The pathway involving PPARγ-LXRα-ABCA1/ABCG1 is of significant importance in the regulation of cholesterol efflux and holds potential as a viable treatment target for AS, as evidenced by various studies (Wang et al., 2018; Zheng et al., 2021; Hu et al., 2022). The PPARγ-LXRα-ABCA1/ABCG1 pathway exhibits potential in the regulation of cholesterol efflux, however, the therapeutic potential of PPARγ agonist therapy may be restricted by its systemic effects. Activation of PPARγ within adipose tissue has the potential to result in weight gain, insulin resistance, and fluid retention, thereby elevating the

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www.hh.um.es. DOI: 10.14670/HH-18-803

Abbreviations. LDQM, Lian-Dou-Qing-Mai; TG, triglyceride; TC, total cholesterol; IL-10, Interleukin-10; HE, hematoxylin and eosin; AS, atherosclerosis; TCM, Traditional Chinese Medicine; PMA, phorbol-12-myristate-13-acetate; ox-LDL, oxidized low-density lipoprotein; LSC, liquid scintillation counting; cDNA, complementary DNA.

likelihood of cardiovascular events (Wang et al., 2016; Darbre, 2017). Consequently, it is imperative to devise PPARγ agonists that are more discerning and focused, while exhibiting fewer unfavorable consequences.

The cytokine interleukin-10 (IL-10) is widely recognized for its anti-inflammatory properties and is considered to play a crucial role in safeguarding against the development of AS, as evidenced by the relevant literature (Jiang et al., 2016; Orecchioni et al., 2022). Moreover, according to scholarly investigations, IL-10 that originates from macrophages has the potential to induce cholesterol uptake and cholesterol efflux, consequently mitigating inflammation and apoptosis in AS (Han et al., 2010). In addition, IL-10 can regulate the immune response through its capacity to hinder the activation and proliferation of pro-inflammatory cells, including macrophages and T cells, while simultaneously promoting the differentiation and activation of anti-inflammatory cells, such as regulatory T cells and M2 macrophages (Ouyang and O'Garra, 2019; Saraiva et al., 2020). The protective function of IL-10 in AS may be attributed to its immune-modulatory impacts.

The use of Traditional Chinese Medicine (TCM) in the management of AS has garnered growing acknowledgment and implementation (Meng et al., 2022). Several TCM remedies, including Danshen (Zhang et al., 2020), Ginkgo biloba (Tian et al., 2019), and Red yeast rice (Banach et al., 2019), have demonstrated therapeutic advantages in the prevention and management of AS. The Lian-Dou-Qing-Mai (LDQM) formula has garnered significant attention in contemporary times within the field of TCM. LDQM is a composite botanical blend comprising six distinct herbs, namely Salvia miltiorrhiza, Astragalus membranaceus, and Lonicera japonica, among others. Research has demonstrated that this mixture has a favorable impact on atherosclerotic plaques (Zhu et al., 2012, 2014). Empirical evidence suggests that LDQM has the potential to decrease the dimensions of atherosclerotic plaques (Li et al., 2022b), enhance the stability of susceptible plaques (Behling-Rosa, 2017), and ameliorate vascular endothelial function (Zhu et al., 2012). The molecular mechanisms responsible for the therapeutic effects of LDQM in AS are yet to be fully understood. Research findings indicate that the anti-atherosclerotic impacts of LDQM may be attributed to its anti-inflammatory (Zhu et al., 2012, 2014) characteristics, alongside its capacity to regulate lipid metabolism (Zhu et al., 2010). Additional investigation is required to clarify the specific molecular targets and mechanisms of action of LDQM in relation to AS.

Based on the available evidence, we hypothesize that the LDQM formula may exert its anti-atherosclerotic effects by upregulating the expression of IL-10 and activating the PPARγ-LXRα-ABCA1/ABCG1 pathway, which promotes cholesterol efflux and reduces atherosclerotic plaque formation.

Materials and methods

Preparation of LDQM formula

The LDQM Formula comprises a prescription composition consisting of Forsythia (15 g), Coptis (3 g), Wild Bean (15 g), Peony Peel (10 g), Zhimu (10 g), Red Peony (10 g), and Lai Zhu Seed (10 g). The medicinal substances are sourced in the granular dosage form from Jiangyin Tianjiang Pharmaceutical Co., Ltd.

Serum preparation and detection of Total Cholesterol (TC) / Total Triglyceride (TG)

Blood samples were obtained from the specimens and permitted to coagulate at room temperature. The coagulated blood was subsequently subjected to centrifugation to isolate the serum from the clot and cellular constituents. TC/TG was detected using a serum sample and detection kit (BC1985 and BC0625, Solarbio, Beijing, China).

Cell culture and foam cell model

THP-1 cells were maintained in RPMI-1640 medium enriched with 10% fetal bovine serum (10437028, Thermo Fisher, MA, USA). These cells are then differentiated into macrophages by exposing them to 100 ng/mL phorbol-12-myristate-13-acetate (PMA, 16561-29-8, Sigma, MO, USA) for 24 hours. After differentiation, THP-1 macrophages are subjected to treatment with oxidized low-density lipoprotein (ox-LDL, L34358, Thermo Fisher) at a concentration range of 50-100 μg/mL for 24-48 hours, thereby facilitating the induction of foam cell formation. To verify the development of foam cells, the aforementioned cells were subjected to staining with Oil Red O (Sigma, Deisenhofen, Germany).

Following ox-LDL treatment, the LDQM Formula was administrated at a dose of 250 μg/ml (LQ) or 500 μg/ml (HQ) diluted in distilled water. The cells were treated with the relative concentration of LDQM for 10h.

Recombinant human IL-10 (rIL-10, Cat: 573204, Biolegend, CA, USA) was reconstituted in sterile endotoxin-free PBS at 100 μg/mL. The concentration of rIL-10 used in cells was 10 ng/mL (Roth and Fisher, 1999). For the LDQM/IL-10 group, cells were treated with LDQM for 10h, followed by rIL-10 for 1h (Tilg et al., 2002).

Cell Transfection and Treatment

IL-10 siRNA (si-IL-10) and a negative control (si-NC) were synthesized by GenePharma (Shanghai, China). To introduce si-IL-10 and si-NC into THP-1 cells, Lipofectamine 3000 (L3000001, ThermoFisher) was employed as the transfection reagent. After 48 hours

of transfection, cells were harvested for further experimental procedures.

Cholesterol efflux assays

THP-1 cells were cultured in six-well plates at a density of 1×10^6 cells per well. The cells were then radiolabeled with 5 μCi/ml [^{3}H]-cholesterol for 48 hours in a media containing 0.2% BSA. Subsequently, cells were subjected to a 24-hour incubation period in the presence of apoA-1 (10 μg/ml) or high-density lipoprotein (HDL) (50 μg/ml). The quantification of the medium and cell-associated [^{3}H]-cholesterol was performed using liquid scintillation counting (LSC).

qRT-PCR

Total RNA from aortic tissues and cell lines was extracted using TRIzol® reagent (Thermo Fisher Scientific, MA, USA). Subsequently, the PrimeScriptTM RT reagent Kit (Takara, Dalian, China) was employed to generate complementary DNA (cDNA) from the RNA samples. The SYBR® Green Real-Time PCR master mix (Thermo Fisher Scientific, MA, USA) was utilized, and the ABI StepOnePlusTM Real-Time PCR System (Applied Biosystems, CA, USA) was used for the analysis. The primer sequences are presented in Table 1. Importantly, GAPDH and HPRT (Hypoxanthine guanine phosphoribosyl transferase) were used as reference genes. Specifically, HPRT was used as the internal control for IL-10 analysis. The $2^{-\Delta\Delta Ct}$ method was utilized to calculate the relative mRNA expression level (Li et al., 2022c).

Animal experiments

The study utilized 10 C57BL/6 wild-type mice and 50 mutant mice with the ApoE$^{-/-}$ genotype, aged between 42 to 48 days and weighing between 18 to 20 g. The mice were procured from Beijing Wetong Lihua Laboratory Animal Technology Co., Ltd. The experimental animals were housed in the SPF-level animal room of the Institute of Laboratory Animals of the Guizhou University of Chinese Medicine, and the experimental procedures were conducted in strict adherence to the relevant regulations and guidelines of the Animal Experiment Ethics Committee of the hospital. The study also underwent ethical review and approval by the Ethics Committee of the hospital. After all mice were adaptively fed for seven days, ApoE$^{-/-}$ mice were fed daily with high-fat feed (containing 0.15% cholesterol, 21% fat) for eight weeks, and normal group mice were fed normal feed. Mice had unrestricted access to water. The body weight was measured weekly using a precision scale. The high-fat feed with rat LDQM Formula was prepared at a drug-to-high-fat feed ratio of 2:18. Following the consumption of the drug feed, any inadequacy was compensated for by the intake of high-fat feed. Recombinant mouse IL-10 (rIL-10, Cat: 417-ML-025, R&D Systems, MN, USA) was reconstituted in sterile endotoxin-free PBS at 100 μg/mL. Ten μg of rIL-10 was administered via intraperitoneal injection every three days continuously for eight weeks.

HE staining

HE staining was performed to visualize the pathological morphological alterations of the mouse aortic arch and liver. A 4% paraformaldehyde solution was utilized for fixation. Tissue samples were prepared by fixing and embedding them in paraffin, followed by sectioning into 4-μm slices. The sections were then subjected to HE staining using a kit (G1100, Solarbio, Beijing, China) and subsequently dried using a neutral gum seal. Then, the samples were observed and imaged using a light microscope (BX60, Olympus, Tokyo, Japan). Finally, the lesion area was quantified using Image-Pro Plus 7.0 software.

Oil Red O Staining

The cells, aorta, or aortic root slides were subjected to fixation using a 4% formaldehyde solution, followed by staining with Oil Red O (Sigma, Deisenhofen, Germany) for 30 minutes. Subsequently, the cells and sections underwent a five-minute rinse with 75% ethanol. Following PBS washing, the sections were promptly subjected to imaging and quantified using Image-Pro Plus 7.0 software.

Western blot

Total protein was extracted by lysing cells in RIPA

Table 1. Primer sequences for qRT-PCR.

Gene		Sequences (5'-3')
IL-10	Forward	GCTCTTACTGACTGGCATGAG
	Reverse	CGCAGCTCTAGGAGCATGTG
PPARγ	Forward	GCAGCTACTGCATGTGATCAAGA
	Reverse	GTCAGCGGGTGGGACTTTC
LXRα	Forward	AGGAGTGTCGACTTCGCAAA
	Reverse	CTCTTCTTGCCGCTTCAGTTT
ABCA1	Forward	CCCAGAGCAAAAAGCGACTC
	Reverse	GGTCATCATCACTTTGGTCCTTG
TNF-α	Forward	CTGTAGCCCACGTCGTAGC
	Reverse	GGTTGTCTTTGAGATC
MCP-1	Forward	TCACTGAAGCCAGCTCTCTCT
	Reverse	GTGGGGCGTTAACTGCAT
MMP-9	Forward	TTCTGGCACACGCCTTTC
	Reverse	CCATAGTAAGTGGGGATCACG
TIMP-1	Forward	TACGCCTACACCCCAGTCAT
	Reverse	-ATGTGCAAATTTCCGTTCCT
GAPDH	Forward	GGAGCGAGATCCCTCCAAAAT
	Reverse	GGCTGTTGTCATACTTCTCATGG

buffer (Servicebio, Wuhan, China) supplemented with protease inhibitors (Beyotime, Shanghai, China). The protein was equally partitioned and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes. The following protocol involved blocking with 5% skim milk powder. The membrane was subjected to incubation with primary antibodies, including IL-10 (ab133575, Abcam), PPARγ (ab178860, Abcam), LXRα (ab176323, Abcam), ABCA1 (ab18180, Abcam), ABCG1 (ab52617, Abcam), and GAPDH (#8884, Cell Signaling Technology) at 4°C overnight. Subsequently, the membrane was incubated with HRP-conjugated secondary antibody (1:2000, Abcam, Cambridge, UK) for 2 hours at room temperature. Finally, the bands were subjected to imaging using a Gel Imaging System (Life Science, CA, USA) and analyzed using ImageJ software.

Statistical analysis

SPSS 19.0 software was used for statistical analysis, the measurement data were expressed as $\bar{x}\pm s$ (mean ±standard deviation), the comparison between multiple groups was using one-way ANOVA, and the difference was statistically significant at $P<0.05$.

Results

LDQM formula inhibits the formation of THP-1 macrophage-derived foam cells and the expression of inflammatory factors and promotes macrophage cholesterol efflux

Foam cells derived from THP-1 macrophages were effectively established through the induction of THP-1 monocytes with PMA for 24 hours. Following a 48-hour co-cultivation of oxLDL with macrophages and subsequent staining with Oil Red O, a significant quantity of red lipid droplets was detected within the cytoplasm of the cells. These observations were consistent with the morphological features of foam cells, as depicted in Figure 1A. The study found that the administration of LDQM Formula resulted in a significant reduction of lipid droplets in THP-1

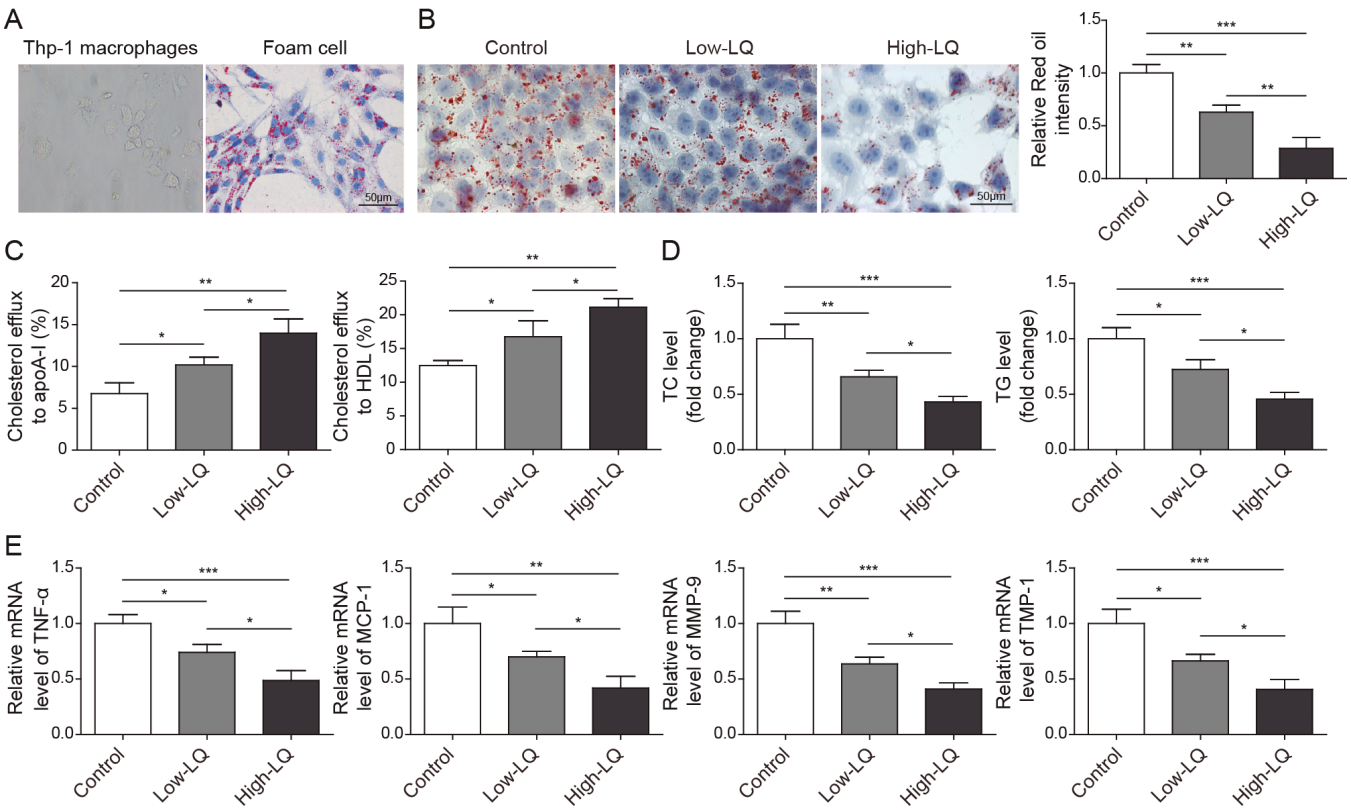

Fig. 1. LDQM formula inhibits the formation of THP-1 macrophage-derived foam cells and the expression of inflammatory factors and promotes macrophage cholesterol efflux. **A.** Representative images of THP-1 macrophages and induced foam cells under the light microscope. **B.** Induced THP-1 cells were treated with saline (control), low dose, and high dose LDQM. Representative images of Oil Red O staining are shown. **C.** LSC assays were conducted to identify the presence of apoA-1 or HDL-mediated [^{3}H]-cholesterol efflux. **D.** TC and TG were examined by detection kits. **E.** Inflammatory factors TNF-α, MCP-1, MMP-9, and TMP-1 were detected by qRT-PCR. The data is reported as mean ± standard error of the mean (SEM) from three separate experiments, with each experiment conducted in triplicate. Statistical significance was determined using a threshold of $^*P<0.05$, $^{**}P<0.01$, $^{**}P<0.001$. Scale bars: A, B, 50 μm.

macrophages, and this effect was observed to be dependent on the dosage administered, as depicted in Figure 1B. Furthermore, we observed that LDQM Formula facilitated the process of cholesterol efflux to apolipoprotein A-I and HDL, and this effect was found to be dependent on the dosage administered, as depicted in Figure 1C. Furthermore, the administration of LDQM Formula demonstrated an inhibitory effect on both the TC and TG levels, as evidenced by the results presented in Figure 1D. Additionally, the expression of various inflammation factors, including TNF-α, MCP-1, MMP-9, and TMP-1, was also suppressed upon treatment with LDQM Formula, as depicted in Figure 1E. The study found that LDQM Formula had a collective inhibitory effect on the formation of foam cells and the expression of inflammatory factors in THP-1 macrophages. Additionally, it was observed to promote cholesterol efflux in macrophages.

LDQM enhances the expression of IL-10 and activates the PPARγ-LXRα-ABCA1/ABCG1 pathway in vitro

Subsequently, the quantification of *IL-10*, *PPARγ*, *LXRα*, *ABCA1*, and *ABCG1* gene expression was performed using qRT-PCR, and western blot techniques. The findings of the study indicated that the administration of LDQM Formula resulted in a dose-dependent upregulation of IL-10 expression and activation of the PPARγ-LXRα-ABCA1/ABCG1 pathway, as demonstrated in Figure 2A,B.

IL-10 enhances the cholesterol efflux triggered by LDQM in THP-1 cells

Next, an in-depth analysis was conducted on the roles and functionalities of LDQM Formula and IL10. The lipid droplets in THP-1 macrophages exhibited a significant reduction under the administration of LDQM Formula. The results depicted in Figure 3A indicate that rIL-10 exerted additional inhibitory effects on the formation of lipid droplets in THP-1 macrophages. The LDQM Formula treatment facilitated the process of cholesterol efflux to apoA-I and HDL, and this effect was subsequently expedited by rIL-10, as depicted in Figure 3B. The administration of LDQM Formula resulted in the inhibition of TC and TG levels and the expression of NF-α, MCP-1, MMP-9, and TMP-1. The trend was further enhanced by the administration of rIL-10, as depicted in Figure 3C,D. Furthermore, qRT-PCR and western blot techniques were employed to measure the levels of *PPARγ*, *LXRα*, *ABCA1*, and *ABCG1* gene expression. The administration of LDQM Formula activated the PPARγ-LXRα-ABCA1/ABCG1 pathway, and the effects were further augmented by rIL-10, as depicted in Figure 3E,F. Moreover, we also knocked down IL-10 using siRNAs to confirm the effect of IL-10 on the PPARγ-LXRα-ABCA1/ABCG1 pathway (Fig. 4A-F). The findings of this study indicated that the promotion of cholesterol efflux in THP-1 cells induced by LDQM Formula was further enhanced by IL-10. Notably, the knockdown efficiency of si-IL-10 is

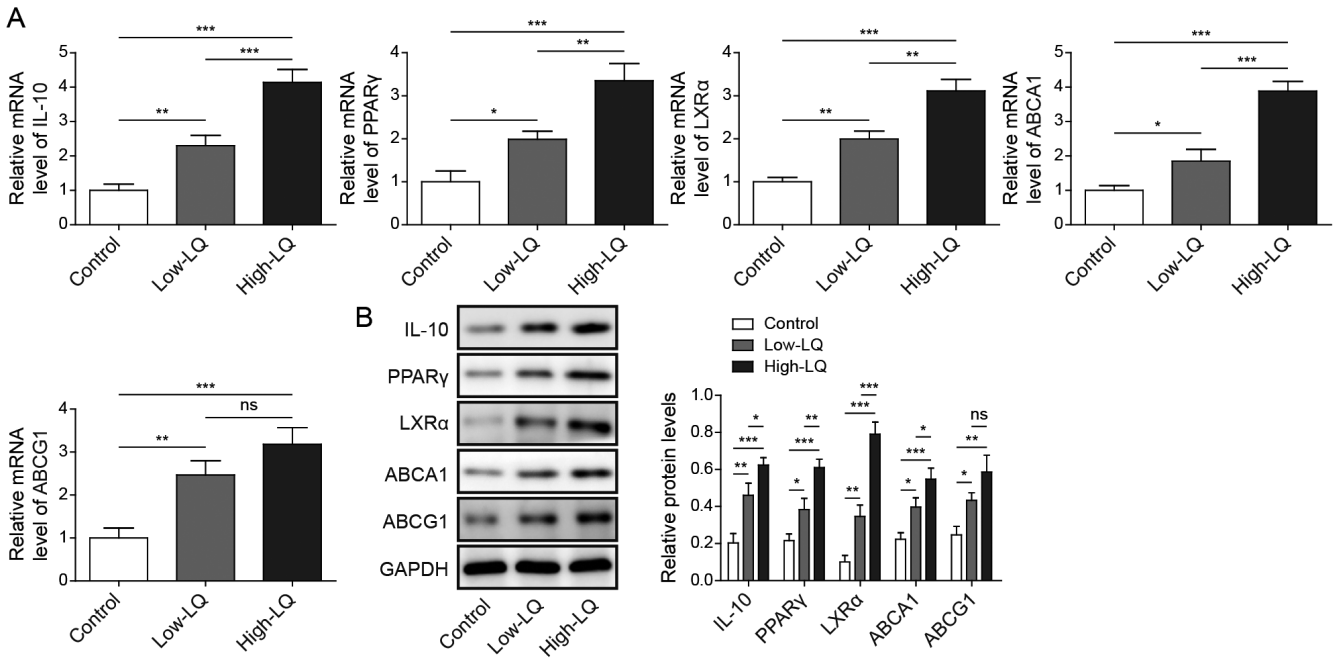

Fig. 2. LDQM enhances the expression of IL-10 and activates the PPARγ-LXRα-ABCA1/ABCG1 pathway *in vitro*. **A.** Levels of IL-10, PPARγ, LXRα, ABCA1, and ABCG1 were detected by qRT-PCR. **B.** Levels of IL-10, PPARγ, LXRα, ABCA1, and ABCG1 were detected by western blot. The data is reported as mean ± standard error of the mean (SEM) from three separate experiments, with each experiment conducted in triplicate. Statistical significance was determined using a threshold of **P*<0.05, ***P*<0.01, ***P*<0.001.

presented in Figure 4G.

LDQM reduces the atherosclerotic lesion area, aortic lipid deposition, and inflammation levels in ApoE$^{-/-}$ mice

The animal models employed in the study were C57BL/6 mice and ApoE$^{-/-}$ mice. Oil Red O staining of the aortic surface was conducted to quantify the extent of atherosclerotic lesions in the arteries. The administration of LDQM Formula resulted in a significant reduction in the development of atherosclerotic plaques in the aorta. Additionally, the

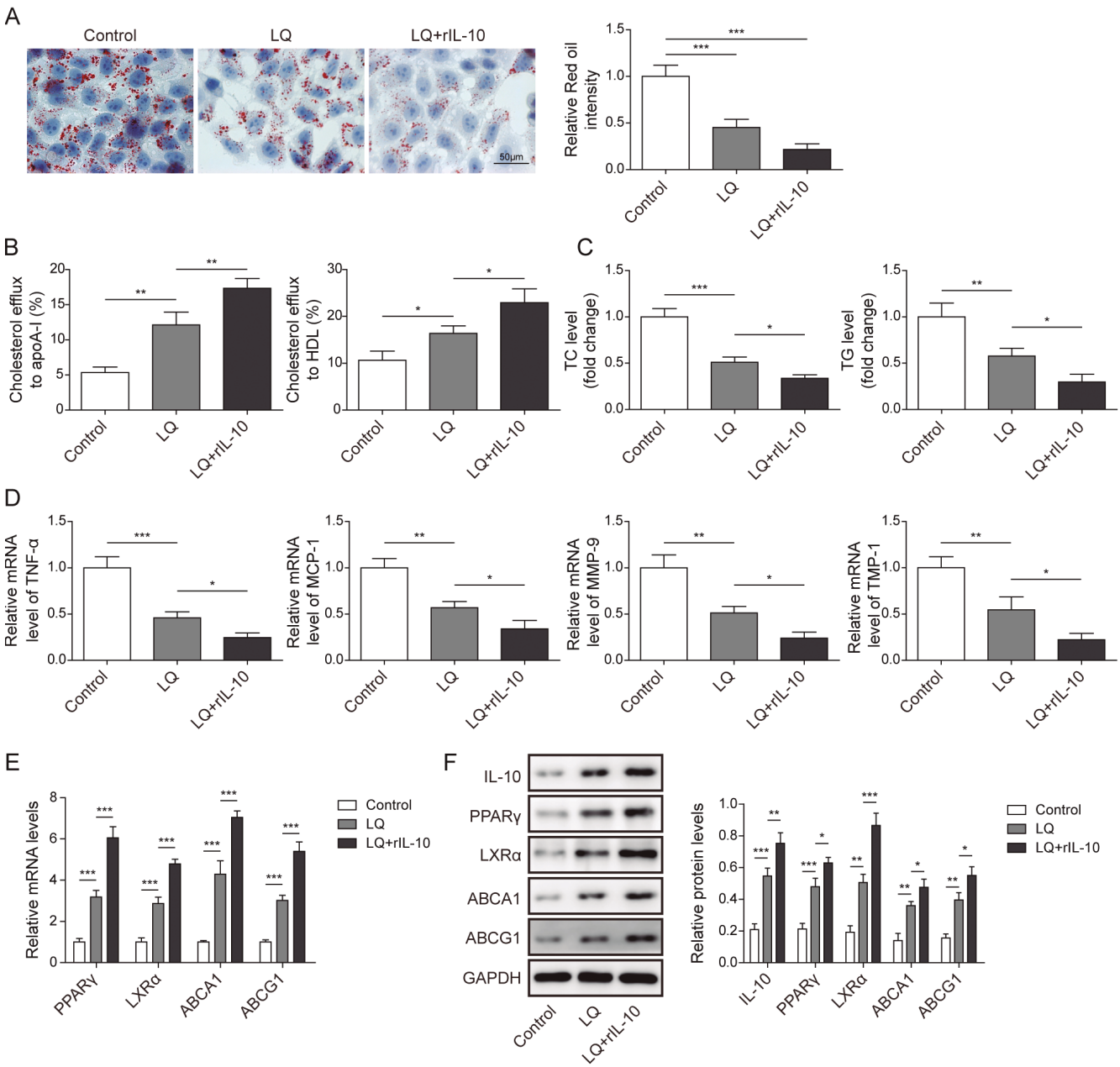

Fig. 3. IL-10 enhances the cholesterol efflux triggered by LDQM in THP-1 cells. THP-1 cells were treated with saline (control), LQ LDQM, or LQ LDQM plus rIL-10. **A.** Cell fat of each group was detected by Oil Red O staining. **B.** Cholesterol efflux was detected by LSC assays. **C.** TC and TG levels were detected by detection kits. **D.** Inflammatory factors TNF-α, MCP-1, MMP-9, and TMP-1 were detected by qRT-PCR. **E-F.** Levels of IL-10, PPARγ, LXRα, ABCA1, and ABCG1 were detected by qRT-PCR and western blot. The data is reported as mean ± standard error of the mean (SEM) from three separate experiments, with each experiment conducted in triplicate. Statistical significance was determined using a threshold of **P*<0.05, ***P*<0.01, ***P*<0.001. Scale bar: A, 50 μm.

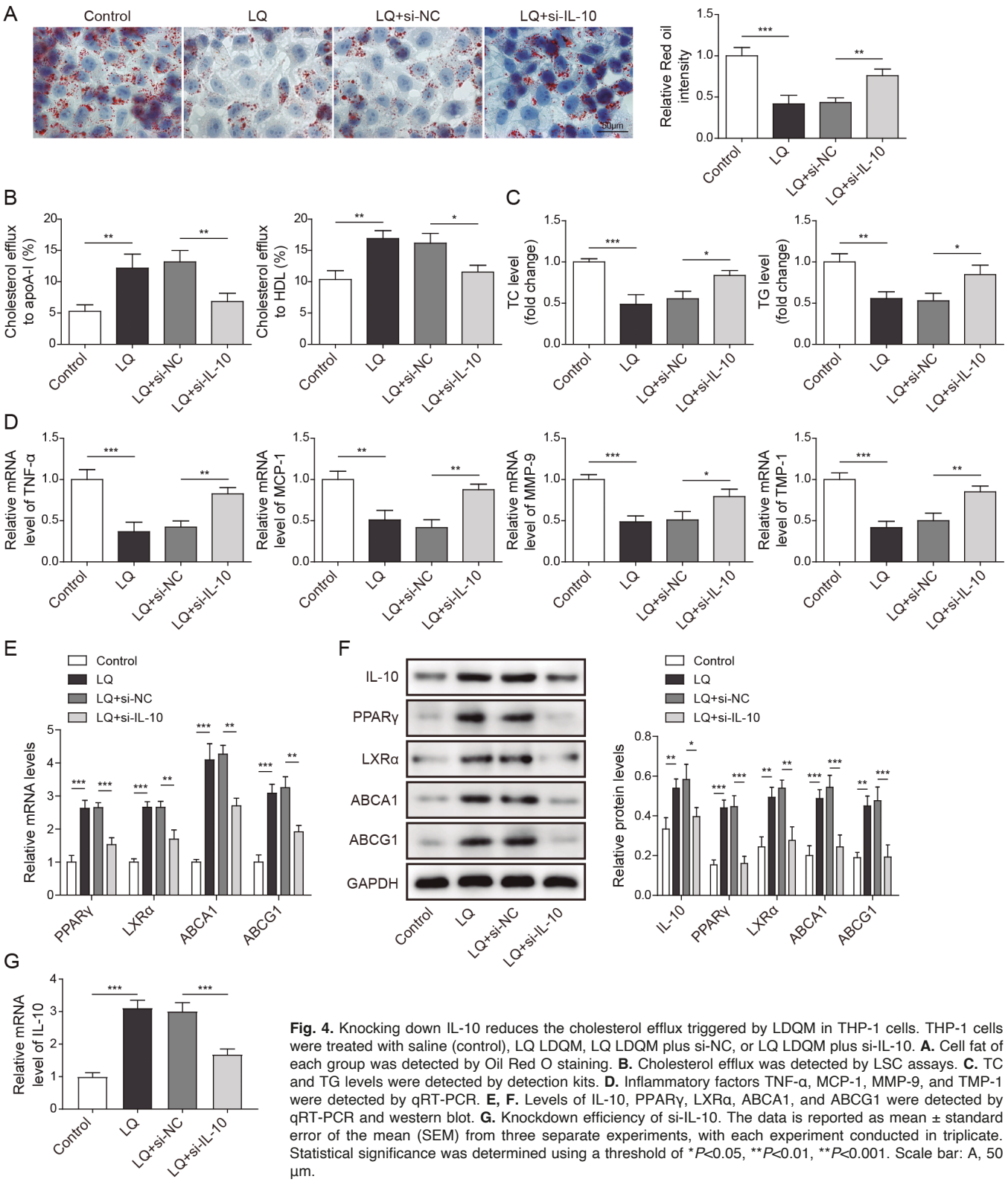

Fig. 4. Knocking down IL-10 reduces the cholesterol efflux triggered by LDQM in THP-1 cells. THP-1 cells were treated with saline (control), LQ LDQM, LQ LDQM plus si-NC, or LQ LDQM plus si-IL-10. **A.** Cell fat of each group was detected by Oil Red O staining. **B.** Cholesterol efflux was detected by LSC assays. **C.** TC and TG levels were detected by detection kits. **D.** Inflammatory factors TNF-α, MCP-1, MMP-9, and TMP-1 were detected by qRT-PCR. **E, F.** Levels of IL-10, PPARγ, LXRα, ABCA1, and ABCG1 were detected by qRT-PCR and western blot. **G.** Knockdown efficiency of si-IL-10. The data is reported as mean ± standard error of the mean (SEM) from three separate experiments, with each experiment conducted in triplicate. Statistical significance was determined using a threshold of **P*<0.05, ***P*<0.01, ***P*<0.001. Scale bar: A, 50 μm.

application of rIL-10 further decreased the size of atherosclerotic plaques, as depicted in Figure 5A. The results of the study indicate that the administration of LDQM Formula in mice resulted in smaller plaques at the root of the aorta, as evidenced by representative images of HE and Oil Red O staining. Additionally, the introduction of rIL-10 further decreased plaque areas in mice aortas, as depicted in Figure 5B. The administration of LDQM Formula resulted in a significant decrease in serum levels of TG, TC, and LDL-C, while also promoting LDL-H levels in mice. The effect of LDQM Formula was further enhanced by rIL-10, as depicted in Figure 5C. The administration of LDQM Formula resulted in significant inhibition of inflammatory factors, such as TNF-α, MCP-1, MMP-9, and TMP-1, in the aortic tissue of mice. Moreover, the effect of LDQM Formula was further enhanced by rIL-10, as depicted in Figure 5D. To summarize, the administration of LDQM Formula exhibited a significant preventive effect against AS, while the addition of rIL-10 further enhanced the anti-atherosclerotic effect in ApoE$^{-/-}$ mice.

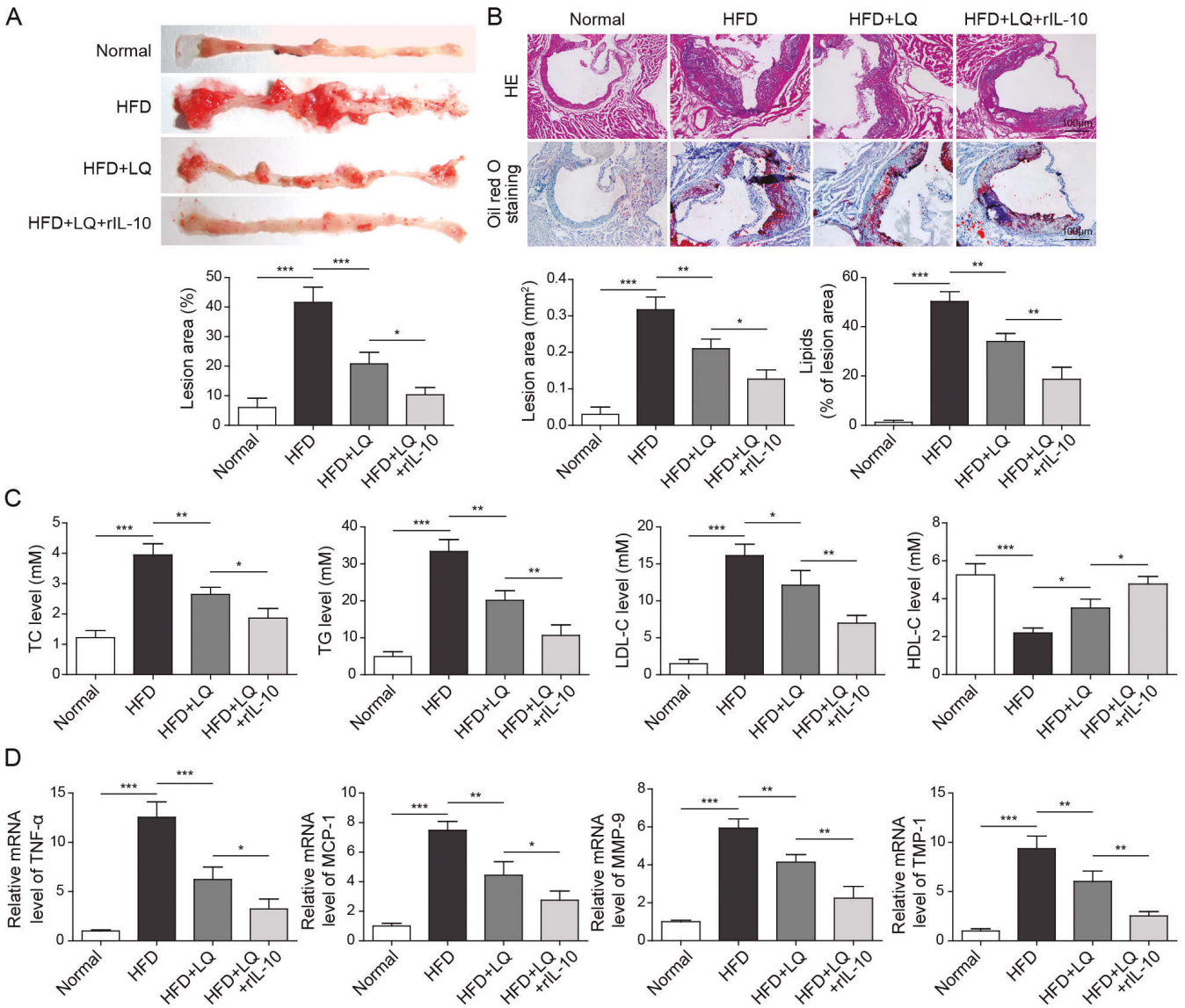

Fig. 5. LDQM reduces the atherosclerotic lesion area, aortic lipid deposition, and inflammation level in ApoE$^{-/-}$ mice. ApoE$^{-/-}$ mice were fed with high-fat feed for eight weeks and treated with saline (control), LDQM (21.04 g/kg), or LDQM plus rIL-10 (10 animals per group). Aortic surface Oil Red O staining. The area of atherosclerotic lesions is quantified as a percentage of the total. **B.** Representative images of aortic root HE and Oil Red O staining. **C.** Total cholesterol and triglycerides, LDL-C, and HDL-C levels were evaluated by detection kit. D. TNF-α, MCP-1, MMP-9, and TMP-1 were detected by qRT-PCR. The data is reported as mean ± standard error of the mean (SEM). Statistical significance was determined using a threshold of **P*<0.05, ***P*<0.01, ***P*<0.001. Scale bar: B, 100 μm.

LDQM activates the PPARγ-LXRα-ABCA1/ABCG1 pathway by regulating IL-10 in vivo

The PPARγ-LXRα-ABCA1/ABCG1 pathway was further investigated in animal models. The findings of the study indicated that the administration of LDQM Formula resulted in the activation of the PPARγ-LXRα-ABCA1/ABCG1 pathway, as evidenced by the results obtained from qRT-PCR, and western blot analyses. Additionally, the effect of LDQM Formula was further enhanced by rIL-10, as demonstrated in Figure 6A,B. Thus, it can be inferred that the administration of LDQM Formula resulted in the activation of the PPARγ-LXRα-ABCA1/ABCG1 pathway through the regulation of IL-10 expression *in vivo*. Additionally, the schematic diagram illustrating the mechanism of this study is displayed in Figure 6C, which visually presents the key components and interactions involved in the proposed mechanism.

Discussion

According to recent research, AS is a chronic vascular inflammatory disease that arises from a disorder in lipid metabolism (Wolf and Ley, 2019). In instances where the body is in a pathological condition, there is an elevation in the levels of inflammation factors and adhesion molecules within the body. Monocytes exit the bloodstream and infiltrate diverse tissues and organs through receptor-mediated mechanisms, prompted by various factors, and subsequently undergo differentiation into macrophages (Kloc et al., 2022). The process by which macrophages internalize ox-LDL via CD36 and subsequently differentiate into foam cells (Cao et al., 2019) is known to contribute to the formation of initial atherosclerotic plaques. Plaque rupture is a significant contributor to cardiovascular and cerebrovascular events, as noted in a recent publication (Vergallo and Crea, 2020). The consequences of AS-related illnesses, including coronary heart disease, myocardial infarction, cerebral infarction, and lower extremity arterial occlusion, have emerged as significant contributors to morbidity and mortality. Consequently, the development of anti-atherosclerotic therapy has become a pressing clinical concern.

The efficacy of TCM in disease prevention has been widely acknowledged and supported by contemporary research. Research has indicated that grape seed proanthocyanidins possess the ability to hinder the progression of AS in the aortic wall during the initial stages of AS (Odai et al., 2019). Danshen has been found to regulate the expression of Bcl-2/Bax and inhibit the formation of AS plaques, as reported in a study (Zhang et al., 2019). Similarly, turmeric has been

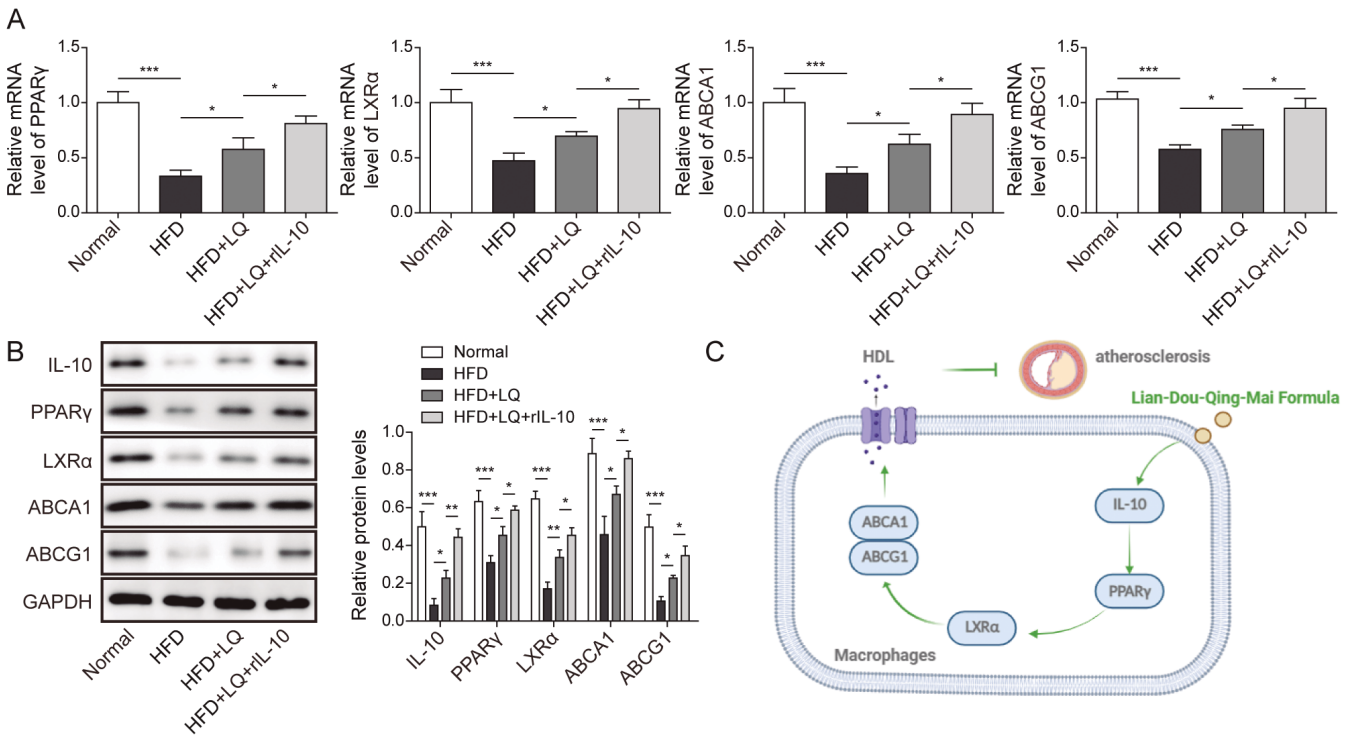

Fig. 6. LDQM activates the PPARγ-LXRα-ABCA1/ABCG1 pathway by regulating IL-10 *in vivo*. ApoE$^{-/-}$ mice were fed with high-fat feed for eight weeks and treated with saline (control), LDQM (21.04g/kg), or LDQM plus rIL-10. **A.** Levels of IL-10, PPARγ, LXRα, ABCA1, and ABCG1 were detected by qRT-PCR. **B.** Levels of IL-10, PPARγ, LXRα, ABCA1, and ABCG1 were detected by western blot. **C.** The schematic diagram illustrates the mechanism of this study. The data is reported as mean ± standard error of the mean (SEM). **P*<0.05, ***P*<0.01, ***P*<0.001.

observed to inhibit macrophage mRNA synthesis and prevent arteriosclerosis (Singh et al., 2015), while Gynostemma has been found to have a similar intervention effect on high-cholesterol feed-fed rats AS and simvastatin, according to another study (Megalli et al., 2005). The saponins found in Panax notoginseng exhibit a preventive effect against AS and other related conditions (Duan et al., 2017). Nonetheless, the existing body of research literature regarding the utilization of compound remedies in TCM for preventing AS is relatively scarce.

The pathogenesis of "phlegm and turbidity" that is caused by the combination of heat, phlegm, and stasis is closely linked to the occurrence of AS, as per previous studies (Feng, 2020; Li et al., 2022a). LDQM, a medicinal composition comprising forsythia, huanglian, wild bean, peony peel, zhimu, red peony, laijizi, and other drugs, is believed to possess the ability to clear heat and tonify kidney turbidity (Zhu et al., 2012). As such, it is expected to have a positive impact on the prevention of AS. LDQM Formula has been studied for its pharmacological properties in modern Chinese medicine. It has been found to possess antibacterial, anti-inflammatory, antihypertensive, lipid-regulating, and antioxidant effects (Zhu et al., 2010, 2012, 2014). The findings of this investigation additionally validated the safeguarding impact of LDQM Formula in individuals with AS. In summary, the LDQM formula was found to inhibit the development of THP-1 macrophage-derived foam cells and the release of inflammatory mediators, while also enhancing macrophage cholesterol efflux.

IL-10 is a soluble cytokine produced by a range of cell types, such as macrophages and regulatory T cells. IL-10 plays a pivotal role in modulating inflammatory reactions by reducing the magnitude of tissue injury. The utilization of IL-10 in a therapeutic context has exhibited the capacity to alleviate the progression of AS, as evidenced by various studies (Pinderski Oslund et al., 1999; Kamaly et al., 2016). The findings of our investigation demonstrate that LDQM Formula can stimulate the expression of IL-10, which in turn promotes cholesterol efflux and diminishes the atherosclerotic lesion area, aortic lipid deposition, and inflammation levels in ApoE-/- mice.

The PPARγ/LXRα/ABCA1 signaling pathway is well-established in lipid metabolism and has been shown to have anti-atherosclerotic effects. The regulation of downstream effector molecules by PPARγ can expedite the lipid excretion process by macrophages and hinder the occurrence of macrophage foaming. As a result, PPARγ can play a role in the regulation of the formation and progression of AS, as indicated by previous studies (Fu et al., 2014; He et al., 2016). The results of the present study indicated that LDQM intervention led to an increase in PPARγ mRNA and protein expression, a reduction in blood lipid levels, and a decrease in liver lipid deposition and aortic plaques in AS mice. The LXR receptor has been observed to detect cholesterol metabolism and impede the development of macrophage-derived foam cells. Additionally, it has been found to interact with the PPARγ pathway to modulate lipid metabolism (Zeng et al., 2018). ABCA1 and ABCG1 are integral membrane proteins that function as semitransporters. These proteins are positively regulated by LXR and are responsible for maintaining cholesterol homeostasis in the body. They facilitate the outflow of cholesterol from liver cells and macrophages, as evidenced by previous research (Khovidhunkit et al., 2003; Zeng et al., 2018). The findings of the study indicated that following LDQM intervention, there was a notable increase in the expression of PPARγ mRNA and protein, as well as an increase in the expression of LXRα, ABCA1, and ABCG1 mRNAs and proteins. This resulted in the restoration of cholesterol and lipid metabolism, a reduction in serum inflammatory expression, liver lipid deposition, and aortic plaque formation. The findings indicated that LDQM can modulate the expression of PPARγ and its associated downstream molecules, namely LXRα, ABCA1, and ABCG1, in a murine AS model.

The findings of this study suggest that LDQM has the potential to modulate the PPARγ/LXRα/ABCA1 signaling pathway through the regulation of IL-10, resulting in the suppression of lipid metabolism and inflammatory response. This, in turn, may mitigate lipid deposition and potentially serve as a mechanism for the prevention of AS. The findings obtained from the experiment have the potential to generate novel insights for the clinical management and prophylaxis of AS.

Acknowledgements. We thank everyone, who supported us in completing this study.

Ethics approval. This study was supported by the Ethics Committee of Xuzhou City Hospital of Traditional Chinese Medicine (No. 2022024).

Data accessibility statement. All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Conflict of interest. The authors declare that they have no conflicts of interest.

Funding. None.

Authors' contributions. Guarantor of integrity of the entire study: Shu Lu Study concepts: Wenqi Liao; Study design: Wenqi Liao; Definition of intellectual content: Wenqi Liao, You Li; Literature research: You Li; Clinical studies: You Li; Experimental studies: Wenqi Liao, Haoyan Zhao; Data acquisition: Haoyan Zhao; Data analysis: Haoyan Zhao; Statistical analysis: Wenqi Liao, Shu Lu; Manuscript preparation: Wenqi Liao, You Li; Manuscript editing: Wenqi Liao, Haoyan Zhao; Manuscript review: Wenqi Liao, Shu Lu; All authors have read and approved the final version of this manuscript to be published.

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Accepted August 19, 2024