Dendrobium nobile Lindl. alkaloids improve lipid metabolism by increasing LDL uptake through regulation of the LXRα/IDOL/LDLR pathway and inhibition of PCSK9 expression in HepG2 cells

JIAN SUN[1,2*], HAO-RUI LIU[2*], YA-XIN ZHU[2*], WEI ZHANG[2], JING-SHAN SHI[1], QIN WU[1] and RUI-XIA XU[2]

[1]Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, Guizhou 563006, P.R. China; [2]Cardiometabolic Center, State Key Laboratory of Cardiovascular Disease, National Center for Cardiovascular Diseases, Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, P.R. China

Received October 16, 2023; Accepted October 11, 2024

DOI: 10.3892/etm.2025.12796

Abstract. *Dendrobium nobile* Lindl. alkaloids (DNLA) are active ingredients that can be extracted from the traditional Chinese herb *Dendrobium Nobile* Lindl. DNLA exhibits hypoglycemic and antihyperlipidemia effects. However, to the best of our knowledge, the specific molecular mechanism by which DNLA can regulate lipid metabolism remains unclear. The aim of the present study was to investigate the effect of DNLA on lipopolysaccharide (LPS)-induced lipid metabolism in HepG2 cells and its potential mechanism. HepG2 cells were treated with LPS with or without different concentrations of DNLA (0, 0.035, 0.35 and 3.5 μg/ml) for 48 h. Cell viability was then detected using the Cell Counting Kit-8 assay. The 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanideperchlorate-low-density lipoprotein (LDL) uptake assay was used to examine LDL uptake. In addition, possible mechanisms were explored using western blot analysis. The effect of the combination of DNLA with rosuvastatin calcium on the expression levels of the LDL receptor (LDLR) and proprotein convertase subtilisin/Kexin type 9 (PCSK9) was examined. The results indicated that LPS stimulation reduced the uptake of LDL by HepG2 cells, decreased the intracellular LDLR content, and increased the expression levels of inducible degrader of the LDLR (IDOL) and liver X receptor (LXR)α. DNLA intervention reversed all of the aforementioned LPS-induced effects in HepG2 cells. Additional mechanistic experiments revealed that DNLA exerted its effects mainly by regulating the LXRα/IDOL/LDLR pathway. It was shown that DNLA also reduced the expression levels of PCSK9, sterol regulatory element binding protein 2 and hepatocyte nuclear factor 1α. In addition, DNLA decreased the expression levels of PCSK9 in rosuvastatin calcium-induced HepG2 cells. Notably, DNLA was able to decrease 3-hydroxy-3-methylglutaryl-coenzyme A reductase and increase cytochrome p450 7A1 expression at the protein level, which are rate-limiting enzymes in cholesterol synthesis and metabolism. Collectively, these data suggested that DNLA could enhance LDL uptake of HepG2 cells by increasing LDLR expression through the LXRα/IDOL/LDLR pathway to alleviate the effects induced by LPS, suggesting the potential benefit of DNLA in improving lipid metabolism disorders.

Correspondence to: Professor Qin Wu, Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, 1 Campus Road, Xinpu, Zunyi, Guizhou 563006, P.R. China
E-mail: wuqinzmc@163.com

Professor Rui-Xia Xu, Cardiometabolic Center, State Key Laboratory of Cardiovascular Disease, National Center for Cardiovascular Diseases, Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 167 Beilishi Road, Xicheng, Beijing 100037, P.R. China
E-mail: ruixiaxu@sina.com

*Contributed equally

Key words: Dendrobium nobile Lindl. alkaloids, HepG2 cells, lipopolysaccharide, low-density lipoprotein receptor, proprotein convertase subtilisin/Kexin type 9, liver X receptor α/inducible degrader of the low-density lipoprotein receptor/low-density lipoprotein receptor signaling pathway

Introduction

Atherosclerosis forms the pathological basis of atherosclerotic cardiovascular disease (ASCVD), which can lead to ischemic heart disease, stroke and peripheral vascular disease (1). Elevated levels of low-density lipoprotein (LDL) cholesterol (LDL-C) have been previously shown to increase the risk of ASCVD (2). By contrast, cholesterol homeostasis is mainly regulated by the dynamic balance among its biosynthesis, uptake, export and metabolism (3). The liver is an important organ for cholesterol synthesis and metabolism (4). The LDL

receptor (LDLR) is a cell surface protein that is mainly expressed in the liver and mediates the clearance of >70% of plasma LDL-C through endocytosis (5,6). Therefore, strategies to increase cellular LDLR expression and stability to reduce plasma LDL-C levels may be effective in preventing the development of ASCVD. Recently, several drugs, such as proprotein convertase subtilisin/Kexin type 9 (PCSK9) inhibitor and ezetimibe, which target individual regulatory factors involved in the pathogenesis of ASCVD, have been developed (7,8). Anti-PCSK9 is a monoclonal antibody that functions as an inhibitor to prevent PCSK9 binding to LDLR on the cell surface, which inhibits LDLR degradation induced by PCSK9. Anti-PCSK9 can enhance the clearance of LDL particles from the circulation mediated by LDLR to subsequently reduce plasma LDL-C levels (9,10).

At the transcriptional level, LDLR gene expression is controlled by a cholesterol-responsive negative feedback mechanism through sterol regulatory element (SRE)-binding proteins (SREBPs), which bind to the SRE region of the LDLR promoter and enhance mRNA transcription, thereby increasing the expression of hepatocyte surface LDLR (11-13). Post-translational regulation of LDLR can be achieved through two major pathways, namely PCSK9 and inducible degrader of the LDLR (IDOL) (14). PCSK9 is a plasma protein that is mainly produced and secreted by the liver (15). It is transcriptionally regulated by SREBP-2 and hepatocyte nuclear factor 1α (HNF1α) (16). Inflammatory pathological stimuli can increase PCSK9 expression (16,17). PCSK9 binds to the extracellular structural domain of LDLR on the cell surface, interfering with LDLR endocytosis, followed by the intracellular degradation of endocytosed LDLR by lysosomes, which results in reduced LDLR recycling back to the cell surface (18,19). IDOL is an E3 ubiquitin ligase that triggers the ubiquitination of LDLR cytoplasmic domain and promotes lysosomal degradation, reducing the abundance of LDLR on the cell surface (14). Unlike PCSK9, IDOL gene expression is induced only by the liver X receptor (LXR), which belongs to a key family of nuclear receptors required for the transcription of genes involved in cholesterol and lipid metabolism (20). These regulators collectively function to control the abundance of LDLR on hepatocytes (21,22).

Dendrobium nobile Lindl. alkaloids (DNLA) are active ingredients that can be extracted from *Dendrobium nobile* Lindl., a traditional Chinese herbal medicine with a long history of use in China (23). A previous study has shown that DNLA reduced carbon tetrachloride-induced liver injury in mice by reducing mitochondrial oxidative stress, which was evidenced by the decrease in mitochondrial malondialdehyde production and a marked increase in manganese superoxide dismutase activity (24). Another recent study reported that DNLA exerted neuroprotective effects against lipopolysaccharide (LPS)-induced neuronal damage and cognitive impairment by attenuating NOD-, LRR- and pyrin domain-containing protein 3-mediated pyroptosis in mice (25). In addition, DNLA has also been documented to effectively ameliorate streptozotocin (STZ)-induced elevation of blood glucose and lipid levels to protect against STZ-induced fatty liver degeneration (26). DNLA has been suggested to exert a beneficial effect on hepatic lipid homeostasis (27). Treatment of diabetic mice with DNLA has been reported to confer beneficial effects on glucose and lipid metabolism (28). However, the mechanisms underlying the DNLA-mediated improvement of lipid metabolism remain to be fully elucidated. To address this, the present study investigated the effects of DNLA on lipid metabolism and the associated mechanisms in HepG2 cells following treatment with LPS.

Materials and methods

Chemicals. Dendrobium Nobile Lindl. (DNL) was purchased from Xintian Traditional Chinese Medicine Industry Development Co., Ltd. DNLA was isolated from DNL based on our previous research methods (29,30), and analyzed by Thermo Fisher Q-Exactive UPLC-Q/Orbitrap MS (Thermo Fisher Science, Inc.). Alkaloids accounted for 79.8% of DNLA, with 92.6% dendrobium ($C_{16}H_{25}O_2N$) as the major compound based on liquid chromatography-mass spectrometry/mass spectrometry analysis as described previously (31-33). Minimum Essential Medium (MEM) was purchased from Procell Life Science & Technology Co., Ltd., and FBS was obtained from Gibco; Thermo Fisher Scientific, Inc.

Cell culture. The HepG2 human liver cancer cell line was used. The cell line was provided by China Infrastructure of Cell Line Resources, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China) with a statement of authentication using the short tandem repeat profiling method. HepG2 cells were incubated in MEM containing 10% FBS, 100 units/ml penicillin and 100 mg/ml streptomycin at 37˚C in a 5% CO_2 incubator.

In LPS (Beijing Solarbio Science & Technology Co., Ltd.) stimulation experiments, HepG2 cells were grown to 80% confluence and the medium without FBS was subsequently replaced. The HepG2 cells were then co-incubated with both LPS (5 μg/ml) and different concentrations of DNLA for 48 h at 37˚C. For LXR agonist experiments, following replacement with 0% FBS medium, the cells were treated with 5 μM T0901317 (Selleck Chemicals) with or without 3.5 μg/ml DNLA for 48 h at 37˚C. For statin experiments, the medium was replaced with medium without FBS and the cells were treated with LPS alone or LPS and 1 μM rosuvastatin calcium (Beijing Solarbio Science & Technology Co., Ltd.) and/or 3.5 μg/ml DNLA for 48 h at 37˚C. The HepG2 cells in the control group were treated with PBS of the same volume as DNLA or LPS in the other groups. The reagents were dissolved in DMSO and diluted with medium to a maximum final DMSO concentration of ≤0.025%.

Cell viability assay. The cytotoxic effects of DNLA and LPS were evaluated in HepG2 cells using the Cell Counting Kit-8 (CCK-8) assay.

HepG2 cells were seeded into 96-well plates ($1x10^4$ cells/well) for 24 h. Following the incubation period at 37˚C, the cells were treated with or without DNLA (0.035, 0.35 and 3.5 μg/ml) for 24 and 48 h at 37˚C, respectively.

HepG2 cells were seeded into 96-well plates ($1x10^4$ cells/well) for 24 h at 37˚C. Following the incubation period, the cells were treated with or without LPS (1, 2, 5 and 10 μg/ml) for 48 h at 37˚C.

Following the treatment period, 10 μl CCK-8 (Dojindo Molecular Technologies, Inc.) was added for incubation at 37˚C for 2 h before the absorbance values were measured at 450 nm using a microplate reader.

Reverse transcription-quantitative PCR (RT-qPCR) analysis. A total of 5x10^5 HepG2 cells per well were seeded into 12-well plates and then treated with DNLA (0.035, 0.35 and 3.5 μg/ml) at 37˚C for 48 h in the presence of LPS (5 μg/ml) . To quantify gene expression, the total RNA was extracted from HepG2 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and then converted to cDNA using the PrimeScript™ RT Master Mix (cat. no. RR036A; Takara Bio, Inc.). Reverse transcription was performed at 37˚C for 15 min and then 85˚C for 5 sec, holding at 4˚C. qPCR was performed in triplicate using SYBR Green qPCR master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and the cDNA was amplified using a Vii7 Real-Time PCR System. The qPCR thermocycling conditions were as follows: 95˚C for 3 min, followed by 40 cycles of 95˚C for 15 secs and 60˚C for 60 sec, with a 4˚C hold. The expression of the target genes in each group were compared using the $2^{-\Delta\Delta Cq}$ method (34) and normalized using statistical analysis. The primers for the reactions were as follows: LDLR forward, 5'-CAGCTACCCCTCGAGACAGA-3' and reverse, 5'-GCAGGCAATGCTTTGGTCTT-3'; and GAPDH forward, 5'-CATGAGAAGTATGACAACAGCC-3' and reverse, 5'-AGTCCTTCCACGATACCAAAG-3'.

Western blot analysis. A total of 1x10^6 HepG2 cells were seeded into 6-well plates and treated as aforementioned. The cells were lysed in RIPA buffer (Thermo Fisher Scientific, Inc.) containing 1 mM phenylmethylsulfonyl fluoride, homogenized on ice, allowed to stand for 30 min and centrifuged at 13,500 x g for 15 min at 4˚C. The protein concentrations were determined using a bicinchoninic assay kit (Thermo Fisher Scientific, Inc.). Total proteins (30 μg from each sample) were then separated using 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Subsequently, the membranes were blocked with 5% skimmed dry milk in Tris-buffer solution with 0.1% Tween-20 (TBST) at room temperature for 2 h before being incubated overnight at 4˚C with the following primary antibodies (1:1,000): Rabbit anti-LDLR (cat. no. ab30532; Abcam), rabbit anti-PCSK9 (cat. no. ab185194; Abcam), rabbit anti-IDOL (cat. no. bs-9674R; BIOSS), rabbit anti-SREBP2 (cat. no. ab30682; Abcam), rabbit anti-LXRα (cat. no. bs-10311R; BIOSS), rabbit anti-HNF1α (cat. no. 89670S; Cell Signaling Technology, Inc.), rabbit anti-3-hydroxy-3-methyl glutaryl-coenzyme A reductase (HMGCR; cat. no. ab242315; Abcam), rabbit anti-cytochrome P450 (CYP) 7A1 (cat. no. bs-21430R; BIOSS) and mouse anti-GAPDH (cat. no. 97166S; Cell Signaling Technology, Inc.). The membranes were then washed with TBST three times and subsequently probed with the appropriate HRP-conjugated secondary antibodies (1:2,000) (cat. nos. SA00001-1 abd SA00001-2; Proteintech Group, Inc.) for 1 h at room temperature. All protein bands were visualized using an electrochemiluminescence kit (Thermo Scientific SuperSignal West Pico PLUS Chemiluminescent Substrate; cat. no. 34577; Thermo Fisher Scientific, Inc.) and semi-quantified by ImageJ 1.48j software (National Institutes of Health).

1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanideperchlorate (Dil)-LDL uptake assay. LDL labeling with Dil-LDL represents the cellular cholesterol uptake ability (35). A total of 5x10^5 HepG2 cells were cultured, inoculated into confocal dishes and subjected to different treatments with both LPS (5 μg/ml) and different concentrations of DNLA (0.035, 0.35 and 3.5 μg/ml) for 48 h at 37˚C. Following the removal of culture medium and replacement with serum-free medium, the cells were incubated with 10 μg/ml Dil-LDL (Beijing Solarbio Science & Technology Co., Ltd.) for 4-5 h at 37˚C in the dark. Subsequently, the cells were washed with PBS and fixed in 4% paraformaldehyde (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) at room temperature for 15 min. The nuclei of the cells were then stained with DAPI (Beyotime Institute of Biotechnology) for 10 min. The cells were examined by confocal microscope (Leica SP8 laser-scanning confocal microscope) and subsequently analyzed using ImageJ 1.48j software (National Institutes of Health). In each group, six fields of view were used per well for quantification.

LDLR immunofluorescence. A total of 5x10^5 HepG2 cells were seeded into 12-well plates lined with cell slides and treated as aforementioned. Following three washes with PBS, HepG2 cells were fixed with 4% paraformaldehyde for 15 min at room temperature and 0.5% Triton X-100 in PBS was applied for 10 min. Subsequently, the cells were blocked in 10% sheep serum blocking solution (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) for 1 h at room temperature and incubated with primary anti-LDLR rabbit (1:1,000; cat. no. SA00001-2; Proteintech Group, Inc.) antibody at 4˚C overnight, followed by incubation with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:1,000; cat. no. ZF-0511; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) at room temperature for 1 h. Excess antibody was removed by washing with PBS. The cells were subsequently stained with DAPI (5 μg/ml; Beyotime Institute of Biotechnology) at room temperature for 10 min before being imaged using a laser-scanning confocal microscope (Leica SP8). The cells were analyzed using the ImageJ 1.48j software (National Institutes of Health). In each group, six fields of view used per well for quantification.

Statistical analysis. All experiments were repeated at least three times. The data are presented as the mean ± standard deviation. The significance of the differences was evaluated using one-way ANOVA followed by the Bonferroni post hoc test. Statistical analysis was performed using SPSS 19.0 (IBM Corp.) and GraphPad Prism 8.0 software (Dotmatics). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of different concentrations of DNLA and LPS on HepG2 cell viability. To assess the cytotoxic effects of DNLA and LPS, HepG2 cells were treated with various concentrations of DNLA (0.035, 0.35 and 3.5 μg/ml) for 24 or 48 h, followed by evaluation of cell viability with the CCK-8 assay. None of the concentrations of DNLA tested exerted significant cytotoxic effects on HepG2 cells at the 24 and 48 h time points (Fig. 1A). Examination of the viability of HepG2 cells treated with different concentrations of LPS (1, 2, 5 and 10 μg/ml) for

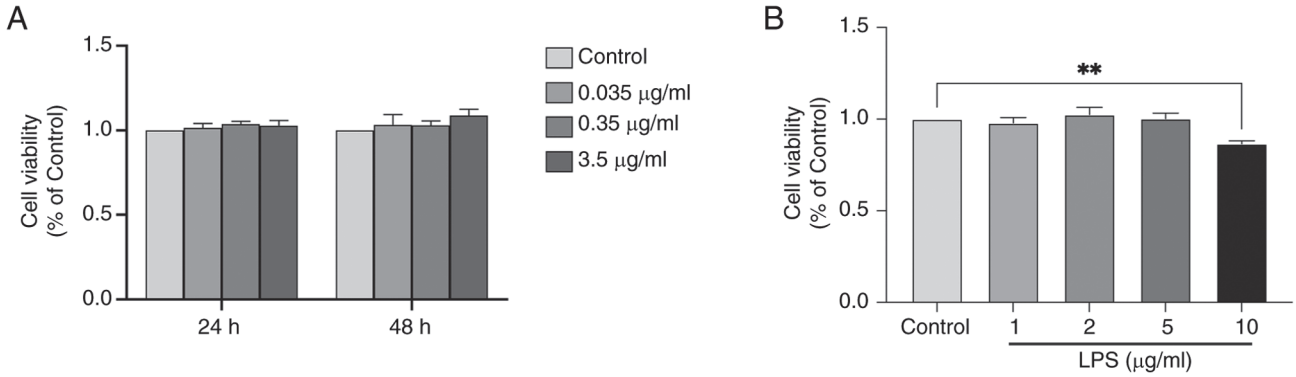

Figure 1. Effects of DNLA and LPS on cell viability. HepG2 cells were treated with (A) the indicated concentrations of DNLA for 24 and 48 h, or (B) LPS (1, 2, 5 and 10 μg/ml) for 48 h. Results are representative of three independent experiments (n=3). **P<0.01. DNLA, *Dendrobium nobile* Lindl. alkaloids; LPS, lipopolysaccharides.

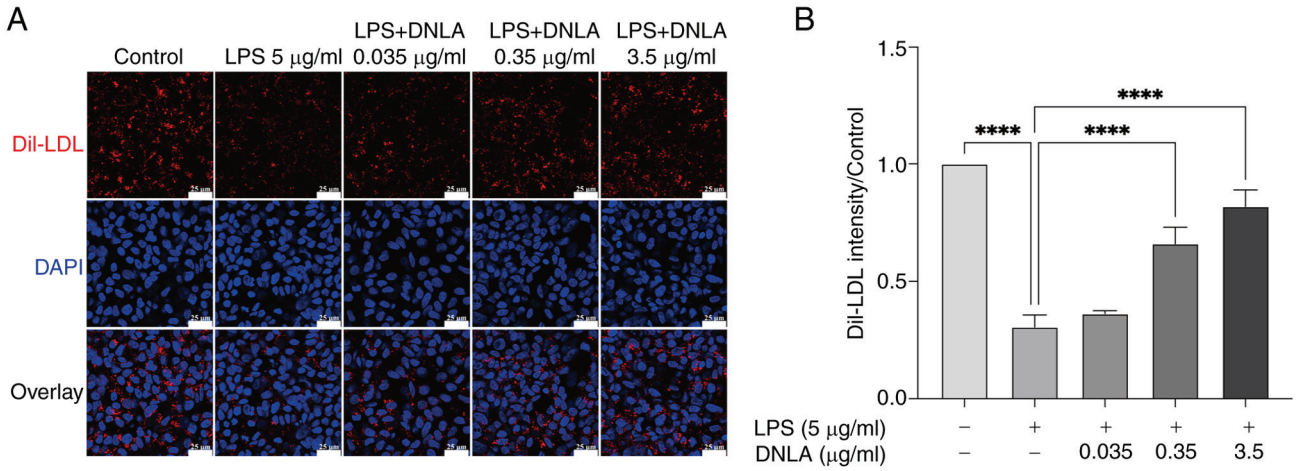

Figure 2. DNLA increases LDL uptake inhibited by LPS in HepG2 cells as determined using confocal microscopy. (A) Dil-LDL staining (red), DAPI staining (blue) and overlay. Magnification, x400. Scale bar, 25 μm. (B) Semi-quantification of (A). Data are presented as the mean ± SD of three independent experiments. ****P<0.0001. DNLA, *Dendrobium nobile* Lindl. alkaloids; LPS, lipopolysaccharides; LDL, low-density lipoprotein; Dil, 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanideperchlorate.

48 h revealed that 10 μg/ml LPS exerted a significant cytotoxic effect at this time point compared with the control group (Fig. 1B). Based on these findings, 5 μg/ml LPS and DNLA (0.035, 0.35 and 3.5 μg/ml) were selected for 48 h treatment under basal conditions for subsequent experiments.

DNLA restores LDL uptake by HepG2 cells under LPS stimulation. To investigate the effect of DNLA on the uptake of LDL, treated HepG2 cells were incubated with Dil-LDL particles for 4-5 h, before LDL uptake was visualized by confocal microscope. As shown in Fig. 2A and B, LPS significantly reduced LDL uptake. However, treatment with DNLA (at concentrations of 0.35 and 3.5 μg/ml) significantly restored the uptake of LDL by HepG2 cells in the presence of LPS (Fig. 2).

DNLA causes an upregulation in the protein and mRNA expression levels of LDLR in HepG2 cells. LDL uptake is associated with the amount of LDLR on the cell surface (20). Immunofluorescence and western blotting results revealed a significant decrease in LDLR protein expression following LPS stimulation compared with that in the control group (Fig. 3A-D). DNLA treatment (at concentrations of 0.35 and 3.5 μg/ml) led to a marked increase in the LDLR protein expression in HepG2 cells in the presence of LPS (Fig. 3A-D). To investigate whether LDLR expression could be regulated by DNLA at the transcriptional level, LDLR mRNA expression was then examined. The results of RT-qPCR demonstrated that DNLA significantly increased the mRNA expression levels of LDLR in HepG2 cells in the presence of LPS (Fig. 3E). Therefore, these findings suggested that DNLA could increase the expression levels of LDLR at both transcriptional and post-translation levels in the presence of LPS.

DNLA downregulates IDOL and LXRα protein expression in LPS-stimulated HepG2 cells. IDOL is an important factor involved in post-transcriptional regulation of LDLR, promoting its ubiquitination and lysosomal degradation, and IDOL expression is regulated by LXRα (36). Western blot analysis was used to examine IDOL and LXRα protein expression in treated cells. As shown in Fig. 4, LPS stimulation significantly enhanced the protein expression levels of IDOL and LXRα compared with those in the control group. By contrast, treatment with DNLA induced a significant reversal of the increase in IDOL and LXRα protein expression

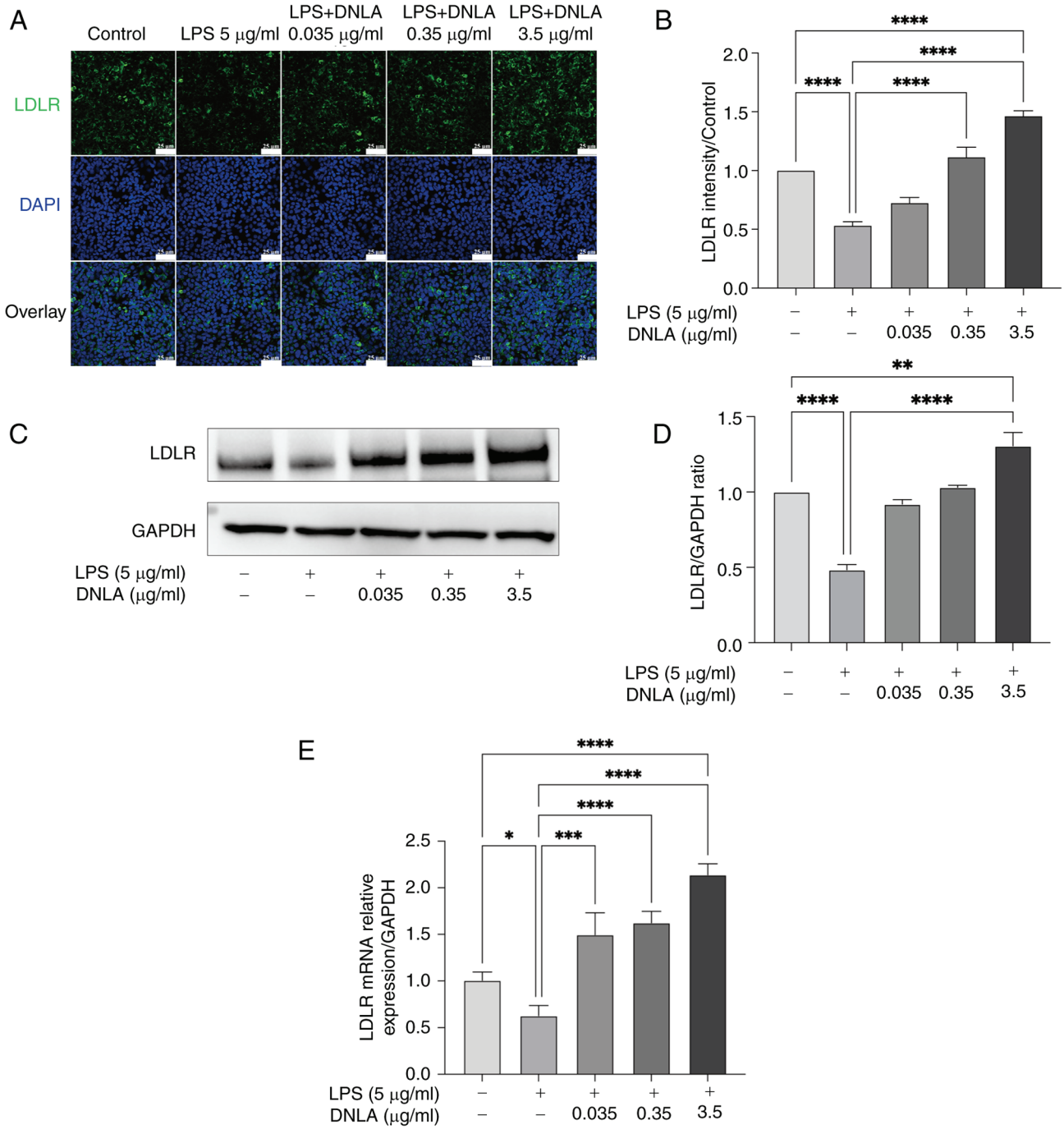

Figure 3. DNLA promotes LDLR expression in HepG2 cells. Cells were treated with a range of concentrations of DNLA (0.035, 0.35 and 3.5 μg/ml) and/or LPS (5 μg/ml) for 48 h. (A) Immunofluorescence detection of LDLR in HepG2 cells. Magnification, x400. Scale bar, 25 μm. (B) Semi-quantification of (A). (C) Protein expression of LDLR in HepG2 cells examined using western blotting. (D) Semi-quantification of (C). (E) mRNA expression levels of LDLR in HepG2 cells analyzed by reverse transcription-quantitative PCR. Data are represented as the mean ± SD of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. DNLA, *Dendrobium nobile* Lindl. alkaloids; LPS, lipopolysaccharides; LDLR, low-density lipoprotein receptor.

caused by LPS (Fig. 4). These results suggested that DNLA could serve a role in ameliorating lipid metabolism disorder by activating the LXRα/IDOL/LDLR signal pathway.

DNLA increases LDLR content in LPS-stimulated HepG2 cells through regulation of the LXRα/IDOL axis. LXRs are cholesterol-sensitive transcription factors activated in response to excess intracellular cholesterol to induce the expression of key genes involved in the regulation of cholesterol homeostasis, including IDOL (37). To further investigate the potential mechanism underlying the therapeutic effects of DNLA on lipid metabolism disorders, the LXR agonist T0901317 was next utilized. Before the protein expression levels of LDLR, IDOL and LXRα were detected by western blot analysis. Treatment with T0901317 significantly reversed the DNLA-mediated reduction of IDOL and LXRα protein expression, whilst significantly decreasing LDLR expression in HepG2 cells (Fig. 5). Based on these findings, it was proposed that DNLA increased LDLR expression by regulating the LXRα/IDOL signaling pathway in HepG2 cells.

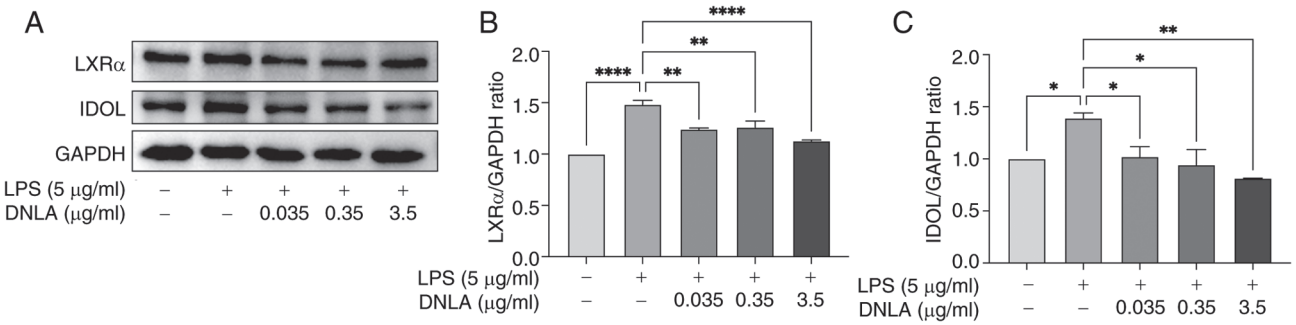

Figure 4. DNLA suppresses LXRα and IDOL protein expression in HepG2 cells. (A) Protein expression of LXRα and IDOL in HepG2 cells was assessed by western blotting. Normalized intensity of (B) LXRα and (C) IDOL vs. GAPDH, presented as the mean ± SD of three independent experiments. *P<0.05, **P<0.01 and ****P<0.0001. DNLA, *Dendrobium nobile* Lindl. alkaloids; LPS, lipopolysaccharides; LXRα, liver X receptor α; IDOL, inducible degrader of the low-density lipoprotein receptor.

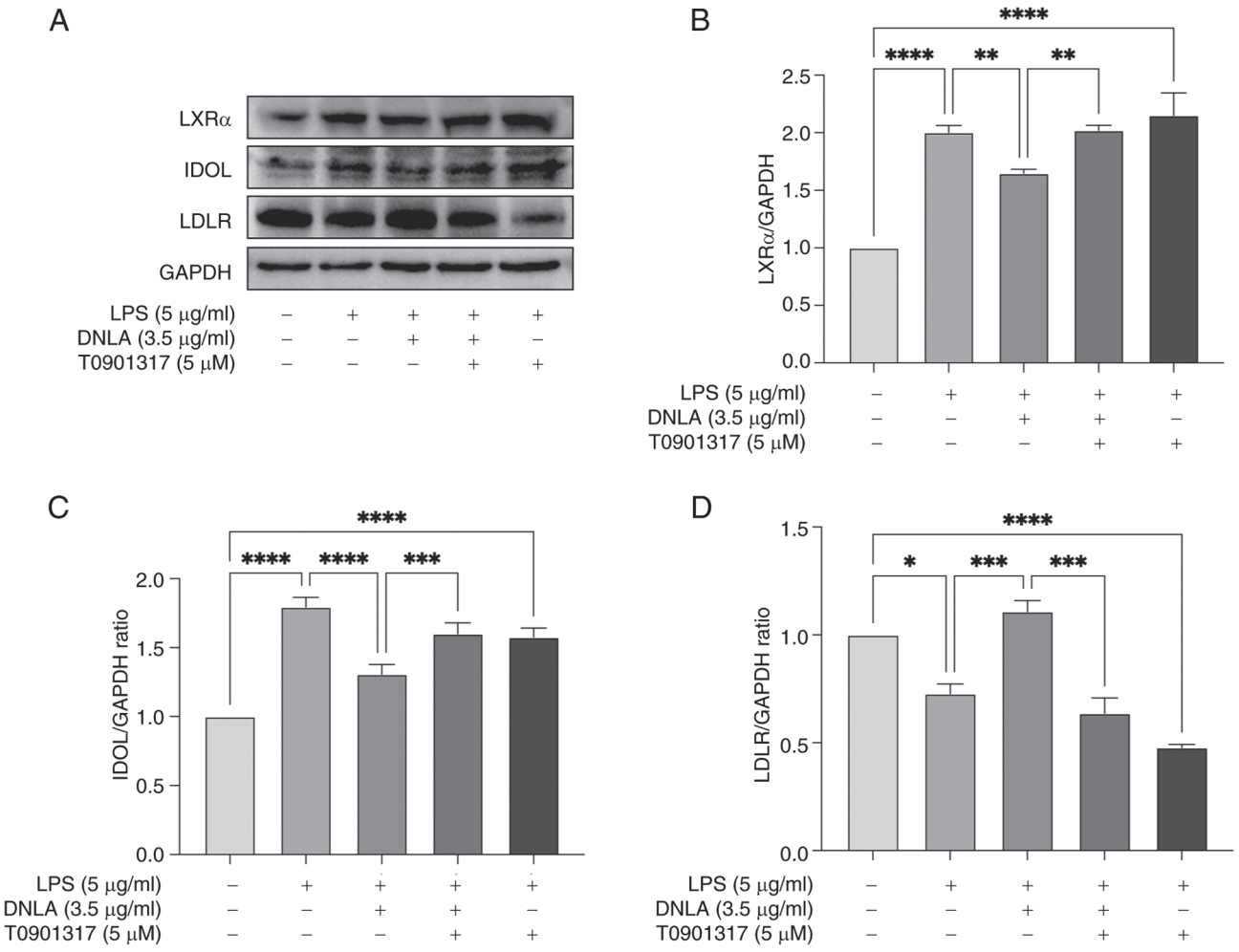

Figure 5. T0901317, an LXRα agonist, reverses the decrease in IDOL and LXRα expression induced by DNLA and reduces the LDLR content in HepG2 cells. (A) Protein expression of LXRα, IDOL and LDLR in HepG2 cells assessed by western blotting. Normalized intensity of (B) LXRα, (C) IDOL and (D) LDLR vs. GAPDH, presented as the mean ± SD of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. DNLA, *Dendrobium nobile* Lindl. alkaloids; LPS, lipopolysaccharides; LXRα, liver X receptor α; IDOL, inducible degrader of the low-density lipoprotein receptor; LDLR, low-density lipoprotein receptor.

DNLA suppresses PCSK9, SREBP2 and HNF1α protein expression in LPS-stimulated HepG2 cells. PCSK9 is a post-transcriptional regulator that can promote the degradation of LDLR and reduce its recycling to the cell membrane (38). As shown in Fig. 6D, LPS stimulation led to significant upregulation of PCSK9 protein expression, which was significantly reversed following DNLA intervention. PCSK9 expression is further regulated by SREBP2 and HNF1α (39). LPS was found to induce a significant increase in SREBP2 and HNF1α expression, which was markedly reversed by DNLA treatment (Fig. 6A and B).

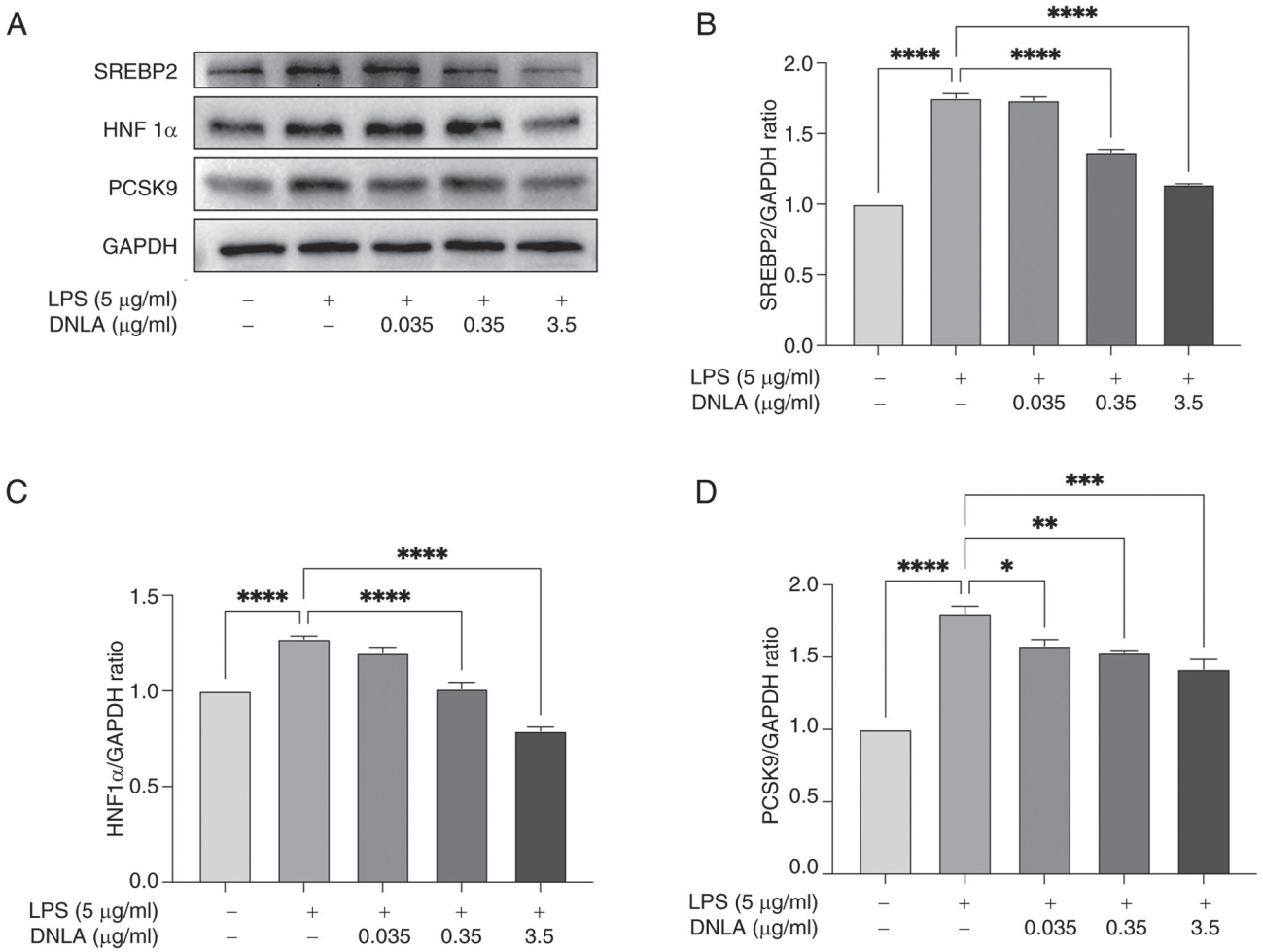

Figure 6. DNLA suppresses PCSK9, SREBP2 and HNF1α protein expression in HepG2 cells. HepG2 cells were treated with varying concentrations of DNLA (0.035, 0.35 and 3.5 μg/ml) and/or LPS (5 μg/ml) for 48 h. (A) Protein expression of SREBP2, HNF1α and PCSK9 in HepG2 cells assessed through western blotting. Normalized intensity of (B) SREBP2, (C) HNF1α and (D) PCSK9 vs. GAPDH, presented as the mean ± SD of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. DNLA, *Dendrobium nobile* Lindl. alkaloids; LPS, lipopolysaccharides; PCSK9, proprotein convertase subtilisin/Kexin type 9; SREBP2, sterol regulatory element-binding protein 2; HNF1α, hepatocyte nuclear factor 1α.

Taken together, these data suggested that DNLA may improve lipid metabolism disorders by regulating the expression levels of PCSK9, SREBP2 and HNF1α.

DNLA enhances CYP7A1 expression and suppresses HMGCR expression in LPS-stimulated HepG2 cells. DNLA appeared to enhance the cellular uptake of LDL. To determine whether DNLA serves a role in maintaining intracellular cholesterol homeostasis, its effects on the expression levels of HMGCR, which is the rate-limiting enzyme in the cholesterol synthesis pathway, were examined (40). In addition, the effects of DNLA on the expression levels of CYP7A1, which is the rate-limiting enzyme in the classical pathway of cholesterol conversion to bile acids, were examined (41). DNLA effectively decreased HMGCR and increased CYP7A1 expression at the protein level in HepG2 cells following LPS stimulation (Fig. 7).

DNLA reduces PCSK9 expression in rosuvastatin calcium-treated HepG2 cells. Statins are widely used to lower plasma LDL-C levels due to their ability to upregulate hepatic LDLR expression whilst enhancing the subsequent uptake of LDL-C from the blood, in addition to their inhibitory effects on HMGCR activity (42). In the present study, the effects of DNLA in combination with statins on the expression levels of LDLR and PCSK9 were examined. LDLR and PCSK9 protein levels were determined by western blot analysis. As shown in Fig. 8, rosuvastatin calcium induced the upregulation of PCSK9 expression compared with the LPS stimulation group. Administration of DNLA led to a significant decrease in rosuvastatin calcium-induced PCSK9 expression (Fig. 8). The combined treatment with DNLA and rosuvastatin calcium resulted in an overall increase in LDLR expression compared with treatment with rosuvastatin in HepG2 cells under LPS stimulation. These findings support the hypothesis that DNLA could inhibit statin-induced PCSK9 expression and increase the intracellular LDLR content, thereby potentially improving the efficacy of statin therapy.

Discussion

The present study focused on the effects of DNLA on LPS-induced lipid metabolism disorders in HepG2 cells. The results suggest that DNLA effectively increased the expression levels of LDLR, enhanced the uptake of Dil-LDL, and

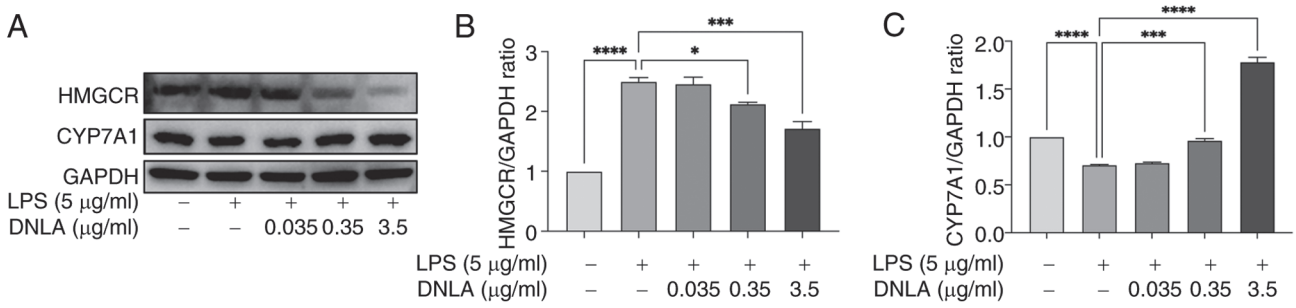

Figure 7. DNLA decreases HMGCR and increases CYP7A1 protein expression in LPS-stimulated HepG2 cells. (A) Protein expression of HMGCR and CYP7A1 in HepG2 cells assessed via western blotting. Normalized intensity of (B) HMGCR and (C) CYP7A1 vs. GAPDH, presented as the mean ± SD of three independent experiments. *P<0.05, ***P<0.001 and ****P<0.0001. DNLA, *Dendrobium nobile* Lindl. alkaloids; LPS, lipopolysaccharides; HMGCR, 3-hydroxy-3-methyl glutaryl-coenzyme A reductase; CYP7A1, cytochrome P450 7A1.

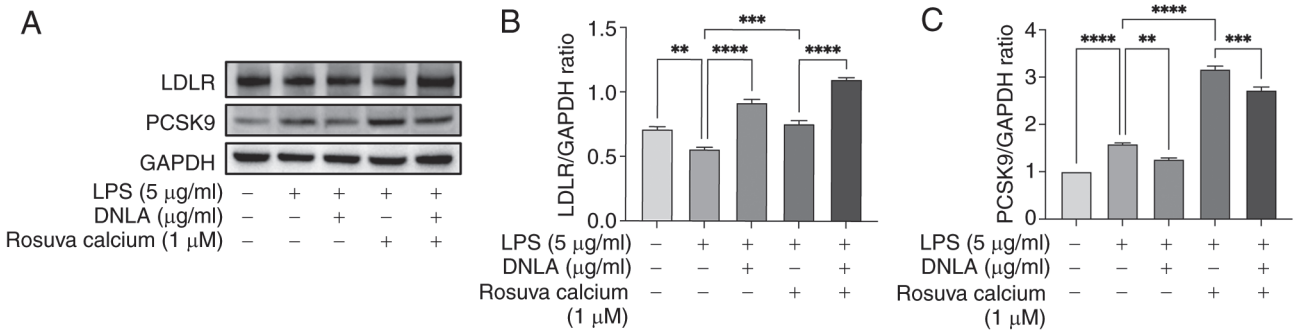

Figure 8. Combination of DNLA with rosuva calcium treatment reduces statin-induced PCSK9 expression, increasing the LDLR content in HepG2 cells. (A) Protein expression of PCSK9 and LDLR in HepG2 cells was assessed by western blotting. Normalized intensity of (B) LDLR and (C) PCSK9 vs. GAPDH, presented as the mean ± SD of three independent experiments. **P<0.01, ***P<0.001 and ****P<0.0001. DNLA, *Dendrobium nobile* Lindl. alkaloids; LPS, lipopolysaccharides; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/Kexin type 9; rosuva, rosuvastatin.

inhibited IDOL, LXRα, PCSK9, SREBP2 and HNF1α protein expression in HepG2 cells. Simultaneously, DNLA inhibited the expression of HMGCR, the rate-limiting enzyme in the cholesterol synthesis pathway whilst promoting that of CYP7A1, the rate-limiting enzyme in the classical metabolic pathway of cholesterol to bile acids. In addition, DNLA in combination with statins was found to enhance LDLR expression in HepG2 cells, and DNLA inhibited the statin-induced increase in PCSK9 expression. These findings suggested that DNLA alleviated lipid metabolism disorders by regulating the LXRα/IDOL/LDLR pathway in HepG2 cells.

Elevated plasma LDL-C is an important risk factor for atherosclerosis, where inflammation serves a key role (43). In the absence of stimulation by other inflammatory factors, excessive free cholesterol can induce an inflammatory response, which then further promotes the uptake and accumulation of lipids by cells to inhibit the outflow of cellular lipids, increasing the risk of lipid metabolism disorders to accelerate the process of atherosclerosis (44). Disorders in lipid metabolism eventually trigger an inflammatory response (45). Atherosclerosis is a complex pathological process, where abnormal lipid metabolism is typically accompanied by an inflammatory response (46,47). Previous studies have shown that LPS stimulation could suppress LDLR expression, and increase SREBP2 and PCSK9 expression, whilst decreasing Dil-LDL uptake by HepG2 cells (48,49). In mice, LPS-induced systemic inflammation was observed to increase PCSK9 mRNA expression, decrease hepatic LDLR expression and increase plasma LDL-C levels (50). In the present study, stimulation with LPS consistently suppressed the protein levels of LDLR and the uptake of LDL in HepG2 cells.

Dendrobium species, which have been documented to confer beneficial therapeutic effects, have been widely used as a traditional Chinese medicine (23). The use of *Dendrobium nobile* Lindl. as a herbal medicine is particularly common in Guizhou, China (25,26), which is included in the Chinese Pharmacopoeia. To date, ≥82 active ingredients have been isolated from *Dendrobium nobile* Lindl., including alkaloids, glycosides, polysaccharides, phenanthrene and dibenzyl compounds (51,52). DNLA appears to be the main active compound, and its pharmacological effects are relatively complex (53). To the best of our knowledge, its effects on LDL have not been previously studied. In total, >70% of plasma LDL-C is cleared by cell surface LDLR-mediated endocytosis (8). LDLR is a receptor that is mainly expressed in the liver. Therefore, the liver is an important site for cholesterol metabolism (4,5). Increasing hepatic LDLR expression or its activity will likely accelerate the clearance of circulating LDL particles, serving as a potential strategy for regulating cholesterol metabolism. Changes in LDLR expression are driven by a combination of transcriptional and post-translational regulation processes (54-56). At the transcriptional level, LDLR expression is mainly regulated by SREBP, which binds to the SRE region of the LDLR promoter to promote

transcription, thereby increasing the expression of LDLR on the cell membrane (54). IDOL and PCSK9 are the two main regulators of LDLR stability during the post-translational phase (14). Both of these aforementioned proteins can induce the lysosomal degradation of LDLR through different pathways. Extracellular mature PCSK9 interacts with cell surface LDLR to trigger receptor-mediated endocytosis, leading to lysosomal LDLR degradation and reducing its localization to the cell membrane (19). By contrast, intracellular IDOL binds to the intracellular structural domain of LDLR, promoting ubiquitination of this region and protein localization to lysosomes for degradation (51,57). In the present study, DNLA was found to increase LDLR protein expression and enhance the uptake of LDL in LPS-stimulated HepG2 cells.

IDOL expression is mainly regulated by the LXR, which is activated by a number of LXR ligands, such as oxysterols and synthetic agonists (18,58). Previous genome-wide association studies have identified genetic variants at the IDOL locus that can affect serum LDL-C levels (59-61). Furthermore, knockdown of IDOL expression with short interference RNA has been previously associated with elevated LDLR levels in HepG2 cells (62,63). Therefore, inhibition of IDOL-mediated LDLR degradation may provide a therapeutic direction to improve hepatic clearance of LDL-C. In the present study, DNLA suppressed IDOL and LXRα protein expression in LPS-stimulated HepG2 cells. To further investigate the mechanisms by which DNLA can improve lipid metabolism disorders, the LXR synthetic agonist T0901317 was used. T0901317 was able to reverse the inhibitory effects of DNLA on IDOL and LXRα protein expression to reduce the expression of LDLR in HepG2 cells. These findings suggested that DNLA could promote LDLR expression in HepG2 cells in association with the inhibition of IDOL and LXRα expression. Specifically, DNLA reduced LXRα expression, which in turn reduced IDOL expression. Therefore, it can be proposed that DNLA functioned as an inhibitor of LXRα rather than antagonizing the activity of LXRα. Accordingly, DNLA may exert beneficial effects on lipid metabolism in HepG2 cells by regulating the LXRα/IDOL/LDLR pathway.

PCSK9 is a plasma protein regulated mainly by SREBP2 and HNF1α (64). Deficiency of PCSK9 has been previously associated with an increase in cell surface LDLR expression and a decrease in the plasma cholesterol concentration (65). PCSK9 has been identified to be a therapeutic target for cardiovascular diseases, where its inhibitors compete with LDLR to interact with PCSK9. This suppresses endocytosis and degradation of LDLR to increase the LDLR content on the hepatocyte membrane, thereby promoting the metabolism of LDL-C by the liver and reducing plasma LDL-C levels (66). The results of the present study indicated that DNLA downregulated PCSK9, SREBP2 and HNF1α protein expression in LPS-stimulated HepG2 cells, suggesting that its activity against lipid metabolism disorders may also be associated with regulation of the PCSK9-related pathway. However, the present study only focused on the LXRα/IDOL/LDLR pathway using the agonist of LXR, and the results demonstrated that DNLA regulated LDLR expression. Further studies are necessary to establish the underlying mechanisms.

Statins are the most widely used cholesterol-lowering drugs. They act as potent inhibitors of HMGCR, the rate-limiting enzyme for *ab initio* cholesterol synthesis (67,68). Statins upregulate LDLR and PCSK9 expression whilst increasing LDL-C uptake by activating the SREBP2 pathway (69). Concomitant PCSK9 expression attenuates LDLR protein activity and is considered to limit the efficacy of statins in lowering cholesterol levels (70). In the present study, DNLA intervention suppressed rosuvastatin calcium-induced PCSK9 expression and further increased LDLR levels compared with those mediated by rosuvastatin calcium alone. However, the molecular mechanism of action underlying the effects of this DNLA-statin combination remain to be fully established. The present study showed that DNLA reduced statin-induced PCSK9 expression and increased LDLR protein stability. Therefore, combinations of these drugs with different mechanisms of action may exert a synergistic effect, thereby improving the efficacy of statin therapy.

Intracellular cholesterol homeostasis is maintained by cholesterol biosynthesis and export in addition to relying on uptake (3). In total, ~30 reaction steps are involved in cholesterol biosynthesis. HMGCR, the rate-limiting enzyme involved in this pathway, is regulated by SREBP2 (71). Conversion of cholesterol to bile acids and biliary excretion of cholesterol are critical mechanisms for cholesterol removal *in vivo* (72). CYP7A1 is involved in the initial and rate-limiting steps in the classical pathway of cholesterol conversion to bile acids (73). In the present study, DNLA treatment was found to inhibit HMGCR expression and enhance CYP7A1 expression in LPS-stimulated HepG2 cells. One suggested theory to explain these findings is that DNLA maintains intracellular cholesterol homeostasis with regard to synthesis and metabolism whilst increasing LDL uptake by HepG2 cells.

In conclusion, the results of the present study suggested that DNLA could serve a role in ameliorating lipid metabolism disorders by regulating the LXRα/IDOL/LDLR pathway to increase LDLR expression in HepG2 cells. In addition, the effects of DNLA on LDLR are potentially associated with inhibition of SREBP2, HNF1α and PCSK9 protein expression, and the detailed mechanism of the effects of the SREBP2/HNF1α/PCSK9 pathway on LDLR expression should be examined in future studies. In combination with statins, DNLA could increase LDLR expression whilst inhibiting the statin-induced upregulation of PCSK9 protein expression in HepG2 cells. Simultaneously, DNLA could regulate the expression of HMGCR and CYP7A1 proteins, which are rate-limiting enzymes in cholesterol synthesis and metabolism. The results of the present study provided insights into the pathways regulated by DNLA in terms of lipid metabolism. However, further *in vivo* studies are necessary to comprehensively establish the pharmacological actions of DNLA and evaluate its utility as a combination drug for the prevention and treatment of ASCVD.

Acknowledgements

Not applicable.

Funding

The present study was supported by Peking Union Medical College Youth Fund (grant no. 3332018200), National Natural Science Foundation of China (grant no. 82060750) and

Guizhou Provincial Department of Education 125 Major Special Projects (grant no. 2012-012).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JS, HRL and YXZ completed the project, analyzed the data and wrote the manuscript. QW and RXX established the study, interpreted the data, and reviewed and edited the manuscript. WZ contributed to analyzing the data. JSS contributed to establishing the study, interpreting the data and reviewing the manuscript. QW and RXX confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kobiyama K and Ley K: Atherosclerosis. Circ Res 123: 1118-1120, 2018.
2. Ridker PM: LDL cholesterol: Controversies and future therapeutic directions. Lancet 384: 607-617, 2014.
3. Luo J, Yang H and Song BL: Mechanisms and regulation of cholesterol homeostasis. Nat Rev Mol Cell Biol 21: 225-245, 2020.
4. Barale C, Melchionda E, Morotti A and Russo I: PCSK9 biology and its role in atherothrombosis. Int J Mol Sci 22: 5880, 2021.
5. Li H, Yu XH, Ou X, Ouyang XP and Tang CK: Hepatic cholesterol transport and its role in non-alcoholic fatty liver disease and atherosclerosis. Prog Lipid Res 83: 101109, 2021.
6. Brown MS and Goldstein JL: A receptor-mediated pathway for cholesterol homeostasis. Science 232: 34-47, 1986.
7. Burnett JR and Hooper AJ: MK-0616: An oral PCSK9 inhibitor for hypercholesterolemia treatment. Expert Opin Investig Drugs 32: 873-878, 2023
8. Raschi E, Casula M, Cicero AFG, Corsini A, Borghi C and Catapano A: Beyond statins: New pharmacological targets to decrease LDL-cholesterol and cardiovascular events. Pharmacol Ther 250: 108507, 2023.
9. O'Donoghue ML, Fazio S, Giugliano RP, Stroes ESG, Kanevsky E, Gouni-Berthold I, Im K, Pineda AL, Wasserman SM, Češka R, *et al*: Lipoprotein(a), PCSK9 inhibition, and cardiovascular risk. Circulation 139: 1483-1492, 2019.
10. Rosenson RS, Hegele RA, Fazio S and Cannon CP: The evolving future of PCSK9 inhibitors. J Am Coll Cardiol 72: 314-329, 2018.
11. Goldstein JL, DeBose-Boyd RA and Brown MS: Protein sensors for membrane sterols. Cell 124: 35-46, 2006.
12. Hua X, Yokoyama C, Wu J, Briggs MR, Brown MS, Goldstein JL and Wang X: SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. Proc Natl Acad Sci USA 90: 11603-11607, 1993.
13. Shimano H, Shimomura I, Hammer RE, Herz J, Goldstein JL, Brown MS and Horton JD: Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. J Clin Invest 100: 2115-2124, 1997.
14. Yang HX, Zhang M, Long SY, Tuo QH, Tian Y, Chen JX, Zhang CP and Liao DF: Cholesterol in LDL receptor recycling and degradation. Clin Chim Acta 500: 81-86, 2020.
15. Bartolomei M, Bollati C, Li J, Arnoldi A and Lammi C: Assessment of the cholesterol-lowering effect of MOMAST®: Biochemical and cellular studies. Nutrients 14: 493, 2022.
16. Ding Z, Pothineni NVK, Goel A, Lüscher TF and Mehta JL: PCSK9 and inflammation: Role of shear stress, pro-inflammatory cytokines, and LOX-1. Cardiovasc Res 116: 908-915, 2020.
17. Jia Q, Cao H, Shen D, Li S, Yan L, Chen C, Xing S and Dou F: Quercetin protects against atherosclerosis by regulating the expression of PCSK9, CD36, PPARγ, LXRα and ABCA1. Int J Mol Med 44: 893-902, 2019.
18. Olsson PA, Korhonen L, Mercer EA and Lindholm D: MIR is a novel ERM-like protein that interacts with myosin regulatory light chain and inhibits neurite outgrowth. J Biol Chem 274: 36288-36292, 1999.
19. Lagace TA: PCSK9 and LDLR degradation: Regulatory mechanisms in circulation and in cells. Curr Opin Lipidol 25: 387-393, 2014.
20. van Loon NM, van Wouw SAE, Ottenhoff R, Nelson JK, Kingma J, Scheij S, Moeton M and Zelcer N: Regulation of intestinal LDLR by the LXR-IDOL axis. Atherosclerosis 315: 1-9, 2020.
21. Zelcer N, Hong C, Boyadjian R and Tontonoz P: LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. Science 325: 100-104, 2009.
22. Zhang L, Reue K, Fong LG, Young SG and Tontonoz P: Feedback regulation of cholesterol uptake by the LXR-IDOL-LDLR axis. Arterioscler Thromb Vasc Biol 32: 2541-2546, 2012.
23. da Silva JA and Ng TB: The medicinal and pharmaceutical importance of Dendrobium species. Appl Microbiol Biotechnol 101: 2227-2239, 2017.
24. Zhou J, Zhang Y, Li S, Zhou Q, Lu Y, Shi J, Liu J, Wu Q and Zhou S: Dendrobium nobile Lindl. alkaloids-mediated protection against CCl4-induced liver mitochondrial oxidative damage is dependent on the activation of Nrf2 signaling pathway. Biomed Pharmacother 129: 110351, 2020.
25. Li DD, Fan HX, Yang R, Li YY, Zhang F and Shi JS: Dendrobium Nobile Lindl. Alkaloid suppresses NLRP3-mediated pyroptosis to alleviate LPS-induced neurotoxicity. Front Pharmacol 13: 846541, 2022.
26. Huang Q, Liao X, Wu Q, Li F, Wang LY and Shi JS: Effects of Dendrobium nobile Lindl. alkaloids on blood glucose and liver fatty degeneration in diabetic rats. Chin J New Drugs Clin Rem 32: 490-493,2013.
27. Xu YY, Xu YS, Wang Y, Wu Q, Lu YF, Liu J and Shi JS: Dendrobium nobile Lindl. alkaloids regulate metabolism gene expression in livers of mice. J Pharm Pharmacol 69: 1409-1417, 2017.
28. Huang S, Wu Q, Liu H, Ling H, He Y, Wang C, Wang Z, Lu Y and Lu Y: Alkaloids of dendrobium nobile lindl. Altered hepatic lipid homeostasis via regulation of bile acids. J Ethnopharmacol 241: 111976, 2019.
29. Zhou J, Zhang Y, Li S, Zhou Q, Lu Y, Shi J, Liu J, Wu Q and Zhou S: Dendrobium nobile Lindl. alkaloids-mediated protection against CCl4-induced liver mitochondrial oxidative damage is dependent on the activation of Nrf2 signaling pathway. Biomed Pharmacother 129: 110351, 2020.
30. Xian S, Yang Y, Nan N, Fu X, Shi J, Wu Q and Zhou S: Inhibition of mitochondrial ROS-mediated necroptosis by Dendrobium nobile Lindl. alkaloids in carbon tetrachloride induced acute liver injury. J Ethnopharmacol 330: 118253, 2024.
31. Nie J, Tian Y, Zhang Y, Lu YL, Li LS and Shi JS: Dendrobium alkaloids prevent Aβ25-35-induced neuronal and synaptic loss via promoting neurotrophic factors expression in mice. PeerJ 4: e2739, 2016.
32. Li LS, Lu YL, Nie J, Xu YY, Zhang W, Yang WJ, Gong QH, Lu YF, Lu Y and Shi JS: Dendrobium nobile Lindl alkaloid, a novel autophagy inducer, protects against axonal degeneration induced by Aβ25-35 in hippocampus neurons in vitro. CNS Neurosci Ther 23: 329-340, 2017.
33. Huang J, Huang N, Zhang M, Nie J, Xu YY, Wu Q and Shi J: Dendrobium alkaloids decrease Aβ by regulating α- and β-secretases in hippocampal neurons of SD rats. PeerJ 7: e7627, 2019.
34. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.

35. Emmanuel UO: TNFα-Induced LDL cholesterol accumulation involve elevated LDLR cell surface levels and SR-B1 downregulation in human arterial endothelial cells. Int J Mol Sci 22: 6236, 2021.
36. Scotti E, Calamai M, Goulbourne CN, Zhang L, Hong C, Lin RR, Choi J, Pilch PF, Fong LG, Zou P, *et al*: IDOL stimulates clathrin-independent endocytosis and multivesicular body-mediated lysosomal degradation of the low-density lipoprotein receptor. Mol Cell Biol 33: 1503-1514, 2013.
37. Nazih H and Bard JM: Cholesterol, oxysterols and LXRs in breast cancer pathophysiology. Int J Mol Sci 21: 1356, 2020.
38. Seidah NG and Prat A: The multifaceted biology of PCSK9. Endocr Rev 43: 558-582, 2020.
39. Nourse JL, Leung VM, Abuwarda H, Evans EL, Izquierdo-Ortiz E, Ly AT, Truong N, Smith S, Bhavsar H, Bertaccini G, *et al*: Piezo1 regulates cholesterol biosynthesis to influence neural stem cell fate during brain development. J Gen Physiol 154: e202213084, 2022.
40. Yang T, Wang Y, Cao X, Peng Y, Huang J, Chen L, Pang J, Jiang Z, Qian S, Liu Y, *et al*: Targeting mTOR/YY1 signaling pathway by quercetin through CYP7A1-mediated cholesterol-to-bile acids conversion alleviated type 2 diabetes mellitus induced hepatic lipid accumulation. Phytomedicine 113: 154703, 2023.
41. Li W, Li H, Zha C, Che B, Yu Y, Yang J and Li T: Lipids, lipid-modified drug target genes, and the risk of male infertility: A Mendelian randomization study. Front Endocrinol (Lausanne) 15: 1392533, 2024.
42. Gormley M, Yarmolinsky J, Dudding T, Burrows K, Martin RM, Thomas S, Tyrrell J, Brennan P, Pring M, Boccia S, *et al*: Using genetic variants to evaluate the causal effect of cholesterol lowering on head and neck cancer risk: A Mendelian randomization study. PLoS Genet 17: e1009525, 2021.
43. Navarese EP, Robinson JG, Kowalewski M, Kolodziejczak M, Andreotti F, Bliden K, Tantry U, Kubica J, Raggi P and Gurbel PA: Association between baseline LDL-C level and total and cardiovascular mortality after LDL-C lowering: A systematic review and meta-analysis. JAMA 319: 1566-1579, 2018.
44. Lan R, Luo H, Wu F, Wang Y and Zhao Z: Chitosan oligosaccharides alleviate heat-stress-induced lipid metabolism disorders by suppressing the oxidative stress and inflammatory response in the liver of broilers. Antioxidants (Basel) 12: 1497, 2023.
45. Sun Y, Ishibashi M, Seimon T, Lee M, Sharma SM, Fitzgerald KA, Samokhin AO, Wang Y, Sayers S, Aikawa M, *et al*: Free cholesterol accumulation in macrophage membranes activates Toll-like receptors and p38 mitogen-activated protein kinase and induces cathepsin K. Circ Res 104: 455-465, 2009.
46. Wadström BN, Pedersen KM, Wulff AB and Nordestgaard BG: Inflammation compared to low-density lipoprotein cholesterol: Two different causes of atherosclerotic cardiovascular disease. Curr Opin Lipidol 34: 96-104, 2023.
47. Hussain A and Ballantyne CM: New approaches for the prevention and treatment of cardiovascular disease: Focus on lipoproteins and inflammation. Annu Rev Med 72: 431-446, 2021.
48. Wu YR, Li L, Sun XC, Wang J, Ma CY, Zhang Y, Qu HL, Xu RX and Li JJ: Diallyl disulfide improves lipid metabolism by inhibiting PCSK9 expression and increasing LDL uptake via PI3K/Akt-SREBP2 pathway in HepG2 cells. Nutr Metab Cardiovasc Dis 31: 322-332, 2021.
49. Gu B, Jiang Y, Huang Z, Li H, Yu W, Li T, Liu C, Wang P, Chen J, Sun L, *et al*: MRG15 aggravates sepsis-related liver injury by promoting PCSK9 synthesis and secretion. Int Immunopharmacol 140: 112898, 2024.
50. Feingold KR, Moser AH, Shigenaga JK, Patzek SM and Grunfeld C: Inflammation stimulates the expression of PCSK9. Biochem Biophys Res Commun 374: 341-344, 2008.
51. Nie X, Chen Y, Li W and Lu Y: Anti-aging properties of Dendrobium nobile Lindl.: From molecular mechanisms to potential treatments. J Ethnopharmacol 257: 112839, 2020.
52. Fan C, Sun X, Wang X and Yu H: Therapeutic potential of the chemical composition of Dendrobium nobile Lindl. Front Pharmacol 14: 1163830, 2023.
53. Mou Z, Zhao Y, Ye F, Shi Y, Kennelly EJ, Chen S and Zhao D: Identification, Biological activities and biosynthetic pathway of dendrobium alkaloids. Front Pharmacol 12: 605994, 2021.
54. Horton JD, Goldstein JL and Brown MS: SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109: 1125-1131, 2002.
55. Sorrentino V and Zelcer N: Post-transcriptional regulation of lipoprotein receptors by the E3-ubiquitin ligase inducible degrader of the low-density lipoprotein receptor. Curr Opin Lipidol 23: 213-219, 2012.
56. Lin XL, Xiao LL, Tang ZH, Jiang ZS and Liu MH: Role of PCSK9 in lipid metabolism and atherosclerosis. Biomed Pharmacother 104: 36-44, 2018.
57. Yu Q, Zheng H and Zhang Y: Inducible degrader of LDLR: A potential novel therapeutic target and emerging treatment for hyperlipidemia. Vascul Pharmacol 140: 106878, 2021.
58. Scotti E, Hong C, Yoshinaga Y, Tu Y, Hu Y, Zelcer N, Boyadjian R, de Jong PJ, Young SG, Fong LG and Tontonoz P: Targeted disruption of the idol gene alters cellular regulation of the low-density lipoprotein receptor by sterols and liver x receptor agonists. Mol Cell Biol 31: 1885-1893, 2011.
59. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ, *et al*: Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466: 707-713, 2010.
60. Chasman DI, Paré G, Mora S, Hopewell JC, Peloso G, Clarke R, Cupples LA, Hamsten A, Kathiresan S, Mälarstig A, *et al*: Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. PLoS Genet 5: e1000730, 2009.
61. Isaacs A, Willems SM, Bos D, Dehghan A, Hofman A, Ikram MA, Uitterlinden AG, Oostra BA, Franco OH, Witteman JC and van Duijn CM: Risk scores of common genetic variants for lipid levels influence atherosclerosis and incident coronary heart disease. Arterioscler Thromb Vasc Biol 33: 2233-2239, 2013.
62. Dong B, Wu M, Cao A, Li H and Liu J: Suppression of Idol expression is an additional mechanism underlying statin-induced up-regulation of hepatic LDL receptor expression. Int J Mol Med 27: 103-110, 2011.
63. Hong C, Marshall SM, McDaniel AL, Graham M, Layne JD, Cai L, Scotti E, Boyadjian R, Kim J, Chamberlain BT, *et al*: The LXR-Idol axis differentially regulates plasma LDL levels in primates and mice. Cell Metab 20: 910-918, 2014.
64. Lin YK, Yeh CT, Kuo KT, Yadav VK, Fong IH, Kounis NG, Hu P and Hung MY: Pterostilbene increases LDL metabolism in HL-1 cardiomyocytes by modulating the PCSK9/HNF1α/SREBP2/LDLR signaling cascade, upregulating epigenetic hsa-miR-335 and hsa-miR-6825, and LDL receptor expression. Antioxidants (Basel) 10: 1280, 2021.
65. Lebeau PF, Byun JH, Platko K, Saliba P, Sguazzin M, MacDonald ME, Paré G, Steinberg GR, Janssen LJ, Igdoura SA, *et al*: Caffeine blocks SREBP2-induced hepatic PCSK9 expression to enhance LDLR-mediated cholesterol clearance. Nat Commun 13: 770, 2022.
66. Sabatine MS: PCSK9 inhibitors: Clinical evidence and implementation. Nat Rev Cardiol 16: 155-165, 2019.
67. Jeong HJ, Lee HS, Kim KS, Kim YK, Yoon D and Park SW: Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. J Lipid Res 49: 399-409, 2008.
68. Rashid S, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, Ho YK, Hammer RE, Moon YA and Horton JD: Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. Proc Natl Acad Sci USA 102: 5374-5379, 2005.
69. Liang J, Li W, Liu H, Li X, Yuan C, Zou W and Qu L: Di'ao Xinxuekang capsule improves the anti-atherosclerotic effect of atorvastatin by downregulating the SREBP2/PCSK9 signalling pathway. Front Pharmacol 13: 857092, 2022.
70. Careskey HE, Davis RA, Alborn WE, Troutt JS, Cao G and Konrad RJ: Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. J Lipid Res 49: 394-398, 2008.
71. Shi Q, Chen J, Zou X and Tang X: Intracellular cholesterol synthesis and transport. Front Cell Dev Biol 10: 819281, 2022.
72. Roca-Agujetas V, Barbero-Camps E, de Dios C, Podlesniy P, Abadin X, Morales A, Marí M, Trullàs R and Colell A: Cholesterol alters mitophagy by impairing optineurin recruitment and lysosomal clearance in Alzheimer's disease. Mol Neurodegener 16: 15, 2021.
73. Rizzolo D, Buckley K, Kong B, Zhan L, Shen J, Stofan M, Brinker A, Goedken M, Buckley B and Guo GL: Bile acid homeostasis in a cholesterol 7α-Hydroxylase and sterol 27-Hydroxylase double knockout mouse model. Hepatology 70: 389-402, 2019.