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Salidroside prevents tumor necrosis factor-α-induced vascular inflammation by blocking mitogen-activated protein kinase and NF-κB signaling activation

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Abstract. Vascular inflammation is a key factor in the pathogenesis of atherosclerosis. Salidroside is an important active ingredient extracted from the root of the *Rhodiola rosea* plant, which has been reported to have antioxidative, anti-cancer, neuroprotective and cardioprotective effects. However, the effects of salidroside on vascular inflammation have not been clarified. The purpose of the present study was to investigate the protective effects of salidroside against tumor necrosis factor (TNF)-α-induced vascular inflammation in cardiac microvascular endothelial cells (CMECs), a specific cell type derived from coronary micro-vessels. Over a 24-h period, salidroside did not exert any significant cytotoxicity up to a dose of 100 μM. Additionally, salidroside decreased the expression levels of the cell adhesion molecule vascular cell adhesion molecule-1 (VCAM-1) in TNF-α-stimulated CMECs, thus suppressing monocyte-to-CMEC adhesion. Salidroside also decreased the production of inflammatory cytokines such as interleukin (IL)-1β, IL-6 and monocyte chemotactic protein 1 (MCP-1) in TNF-α-induced CMECs, as well as suppressing TNF-α-activated mitogen-activated protein kinase (MAPK) and NF-κB activation. Since MAPKs and NF-κB both serve notable roles in regulating the expression of VCAM-1, IL-1β, IL-6 and MCP-1, the present study provided a preliminary understanding of the mechanism underlying the protective effects of salidroside. Overall, salidroside alleviated vascular inflammation by mediating MAPK and NF-κB activation in TNF-α-induced CMECs. These results indicated that salidroside may have potential applications as a therapeutic agent against vascular inflammation and atherosclerosis.

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Introduction

As a barrier between the vessel lumen and the surrounding tissue, the vascular endothelium is the primary participant in, and regulator of, vascular inflammatory reactions (1). Vascular inflammation has been shown to be involved in the progression of various cardiovascular diseases, including atherosclerosis (2). Tumor necrosis factor (TNF)-α is a pleiotropic, pro-inflammatory cytokine. It serves a critical role in the disruption of vascular function and subsequent inflammatory responses by triggering several intracellular signaling pathways; this may ultimately stimulate the expression of adhesion molecules, particularly vascular cell adhesion molecule-1 (VCAM-1) and cytokines such as interleukin (IL)-1β, IL-6 and monocyte chemotactic protein 1 (MCP-1) (3-5). These molecules recruit monocytes to the endothelial cell surface, resulting in inflammation and, ultimately, atherosclerosis (6). NF-κB and mitogen-activated protein kinases (MAPKs) serve pivotal roles in the progression of vascular inflammation by mediating the expression of adhesion molecules and chemokines in vascular endothelial cells (7,8). Both are essential for the transcriptional regulation of factors induced by TNF-α (9); therefore, compounds that can suppress TNF-α-induced MAPK and NF-κB activation represent promising candidates for the treatment and prevention of vascular endothelial dysfunction and inflammation.

Salidroside, a principal active ingredient isolated from the root of the *Rhodiola rosea* plant, has been reported to exert various physiological and pharmacological effects. A review summarized that salidroside was of benefit to patients with diabetes mellitus, as it was able to regulate 5'-AMP-activated protein kinase pathway-mediated glycolipid metabolism, oxidative stress and inflammatory responses (10). A previous study also demonstrated that salidroside retarded the proliferation of breast cancer cells by inhibiting MAPK pathway activation (11). In terms of cardiac protection, one report confirmed that salidroside alleviates

lipopolysaccharide (LPS)-induced cardiac injury by regulating the PI3K/AKT/mTOR pathway (12). Notably, salidroside has been shown to possess strong anti-inflammatory properties. A previous study proposed that salidroside exerts anti-inflammatory effects on macrophages by blocking MAPK and NF-κB activation, and subsequently reducing the secretion of inflammatory cytokines (13). However, the effect of salidroside against TNF-α-stimulated vascular inflammation in CMECs remains to be elucidated. The present study details an investigation into the protective effects of salidroside on CMECs by regulating the production of pro-inflammatory cytokines and adhesion molecules. The results may provide novel ideas and intervention targets for the recognition and treatment of pathogenesis associated with vascular inflammation.

Materials and methods

Reagents. Salidroside was purchased from Yuanye Biotechnology Co., Ltd. Recombinant rabbit TNF-α was purchased from Novoprotein. High-glucose DMEM, RPMI 1640 medium, FBS and trypsin were purchased from Thermo Fisher Scientific, Inc. The primary antibody against factor VIII (cat. no. sc-14014) was purchased from Santa Cruz Biotechnology, Inc., and anti-VCAM-1 (cat. no. ab134047) was obtained from Abcam. Antibodies against p38 (cat. no. 8690), phospho-p38 (cat. no. 4511), p44/42 (cat. no. 4695), phospho-p44/42 (cat. no. 4370), stress-activated protein kinase (SAPK)/JNK (cat. no. 9252), phospho-SAPK/JNK (cat. no. 9255), inhibitor of NF-κB (IκB) α (cat. no. 4814), phospho-IκBα (cat. no. 2589), NF-κB p65 (cat. no. 8242) and phospho-NF-κB p65 (cat. no. 3033) were obtained from Cell Signaling Technology, Inc. The PrimeScript™ RT Reagent kit and SYBR® Premix Ex Taq™ kit were purchased from Takara Bio, Inc. The ELISA kits were purchased from MultiSciences Biotech Co., Ltd. All other chemicals were of analytical grade.

Isolation and culture of CMECs. The isolation and primary culture of CMECs were performed as previously described (14). All experiments related to animals were conducted in accordance with the Guidelines to Laboratory Animal Research of Fudan University and were approved by the Institutional Animal Care and Use Committee of Fudan University. A total of 50 2-week-old Sprague Dawley rats were purchased from Shanghai Jiesijie Experimental Animal Co., Ltd., and were housed under a 12:12 h light: Dark cycle with controlled temperature (21-25˚C), humidity (50±5%) and free access to food and water in the Department of Laboratory Animal Science at Fudan University. Animal health and behavior were monitored every day. The 2-week-old male Sprague Dawley rats (30-40 g) were sacrificed by cervical dislocation after anesthesia with 5% isoflurane, and the heart was immediately excised and rinsed with PBS pre-cooled to 4˚C. After removal of the atrial tissues, great vessels, epicardium and endocardium, the remaining ventricular tissues were cut into 1-mm^3 pieces and plated in a 10-cm culture dish pre-coated with 1 ml FBS. The tissues were then incubated at 37˚C (5% CO_2) for 4 h, and for an additional 48 h in high-glucose DMEM (10% FBS). After a further 48 h, when the cells had reached a confluence of 80%, the tissue pieces were removed and the cells were passaged using trypsin (0.25%). The second generation of cells was used for subsequent experimentation. THP-1 monocytic cells (American Type Culture Collection) were cultured in RPMI-1640 containing 10% FBS, and maintained in conditions identical to those of the CMECs.

Cell viability assay. The Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology) was used to determine cell viability. Briefly, CMECs ($1x10^4$ cells/well) were seeded into a 96-well plate and incubated with 10, 50 or 100 μM salidroside for 24 h. Following salidroside treatment, CCK-8 reagent was added to each well and the plate was incubated for 2 h at 37˚C. The optical density was measured at 450 nm using a microplate reader (Synergy™ H4; BioTek Instruments, Inc.).

Monocyte adhesion assay. To determine the degree of monocyte THP-1 cell adhesion to CMECs, CMECs ($1x10^5$ cells/well) were seeded into 6-well plates, treated with salidroside (according to the aforementioned protocol) and 10 ng/ml TNF-α for 12 h, and incubated until 100% confluence is achieved. THP-1 cells were labeled with 5 μM 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbo-cyanine perchlorate for 10 min and resuspended in high-glucose DMEM (1% FBS); the cells were then added to the confluent CMECs and incubated for 1 h. The cells were washed in PBS to remove non-adherent THP-1 cells, and the adherent monocytes were visualized using a fluorescence microscope at x200 magnification (Olympus Corporation).

Western blot analysis. Total protein was extracted from the CMECs using RIPA buffer (Beyotime Institute of Biotechnology), and the protein concentration was determined using the Bradford method. Equal amounts (30 μg) of total protein were separated by SDS-PAGE using a 10% gel, and transferred onto PVDF membranes (EMD Millipore). After being blocked with 5% non-fat dry milk for 1 h at room temperature, the membranes were incubated with the following primary antibodies at a 1:1,000 dilution, overnight at 4˚C: VCAM-1, p38, phospho-p38, p44/42, phospho-P44/42, SAPK/JNK, phospho-SAPK/JNK, IκBα, phospho-IκBα, NF-κB p65 and phospho-NF-κB p65. A further incubation with a horseradish peroxidase-conjugated secondary antibody (cat. no. 31460; Invitrogen; Thermo Fisher Scientific, Inc.; dilution, 1:5,000) was then conducted at room temperature for 2 h. The bands were visualized using the Gel Doc™ XR+System (Bio-Rad Laboratories, Inc.), and the band intensity was quantified using ImageJ 1.6.0 software (National Institutes of Health).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the CMECs using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and 1 μg/sample of total RNA was reverse transcribed using the PrimeScript™ RT Reagent kit. The reverse transcribed conditions were as follows: 37˚C for 15 min, 85˚C for 5 sec and 4˚C for 5 min. The resulting cDNA was then amplified using the SYBR® Premix Ex Taq™ kit on a CFX Connect™ Real-Time System (Bio-Rad Laboratories, Inc.). The thermal cycler conditions were as follows: Initial denaturation at 95˚C for 30 sec, followed by 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec. The relative quantification of the mRNA expression levels was normalized to that of the β-actin

endogenous control, and calculated using the PCR system software via the $2^{-\Delta\Delta Cq}$ relative quantification method (15). The primers used for PCR were as follows: Rat VCAM-1 forward, 5'-GCTGCTGTTGGCTGTvGACTCTC-3' and reverse, 5'-GCTCAGCGTCAGTGTGGATGTAG-3'; rat IL-6 forward, 5'-AGACTTCCATCCAGTTGCCTTCTTG-3' and reverse, 5'-CATGTGTAATTAAGCCTCCGACTTGTG-3'; rat IL-1β forward, 5'-AACTGTGAAATAGCAGCTTTCG-3' and reverse, 5'-CTGTGAGATTTGAAGCTGGATG-3'; rat MCP-1 forward, 5'-GCAGGTCTCTGTCACGCTTCTG-3' and reverse, 5'-GAATGAGTAGCAGCAGGTGAGTGG-3'; rat β-actin forward, 5'-TACAACCTTCTTGCAGCTCC-3' and reverse, 5'-ATCTTCATGAGGTAGTCTGTC-3'.

Immunocytochemistry. CMECs were cultured on glass coverslips and exposed to TNF-α (10 ng/ml) and salidroside (10, 50 and 100 μM), before being washed with PBS and fixed in 4% paraformaldehyde for 10 min at room temperature. The cells were incubated in 3% bovine serum albumin (Beyotime Institute of Biotechnology)-PBS blocking solution for 30 min at room temperature, with anti-factor VIII (1:250), anti-VCAM-1 (1:700) and anti-p65 (1:800) antibodies, overnight at 4˚C, and then with a secondary antibody labeled with Alexa Fluor 488 (dilution, 1:200; cat. no. A11034; Invitrogen; Thermo Fisher Scientific, Inc.) for 2 h at room temperature. Cell nuclei were stained with DAPI (Sigma-Aldrich; Merck KGaA) for 10 min at room temperature, and all samples were observed under a fluorescence microscope at x400 magnification (Olympus Corporation).

ELISA. CMECs, seeded into 6-well plates at $1x10^5$ cells/well, were pre-treated with 10, 50 or 100 μM salidroside for 12 h, followed by 10 ng/ml TNF-α for a further 12 h in a 37˚C thermostatic cell incubator. The cell supernatants were collected, and IL-1β (cat. no. KGERC007-1), IL-6 (cat. no. KGERC003-1) and MCP-1 (cat. no. KGERC113-1) secretion was quantified using the corresponding ELISA kits (Nanjing KeyGen Biotech Co., Ltd.), according to the manufacturer's instructions.

Statistical analysis. All data are expressed as the mean ± standard deviation. Each experiment was repeated at least 3 times. The data were analyzed using SPSS 19.0 software (SPSS, Inc.). The differences between groups were determined using one-way ANOVA and Tukey's test, and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Isolation and identification of CMECs. After incubation *in vitro* for 24-48 h, primary rat CMECs of the ventricular tissue were spindle-like or polygonal in shape. After 72-96 h incubation, when the CMECs had reached 95-100% confluence, a 'cobblestone' appearance was observed. Most of the CMECs were positively stained with the microvascular endothelial cell specific anti-factor VIII antibody (Fig. 1).

Salidroside inhibits TNF-α-induced binding of monocytes to CMECs. To ensure that a non-cytotoxic concentration of salidroside was used, a CCK-8 assay was performed at three predetermined concentrations of salidroside (10, 50 and 100 μM) in TNF-α-stimulated CMECs. Since cell viability was not affected by up to 100 μM salidroside (Fig. 2A), CMECs were treated with 10, 50 and 100 μM in the subsequent experiments. Considering that monocyte-to-endothelial cell adhesion is an essential step in the development of atherosclerosis (6), the inhibitory effects of salidroside on the adhesion of THP-1 monocytes to TNF-α-stimulated CMECs were further investigated. As indicated, 100 μM salidroside did not alter the number of THP-1 cells adhering to the CMECs, and 10 ng/ml TNF-α significantly increased these numbers; however, salidroside pretreatment prior to TNF-α administration decreased the degree of monocyte adhesion to TNF-α-treated CMECs in a concentration-dependent manner (Fig. 2B and C).

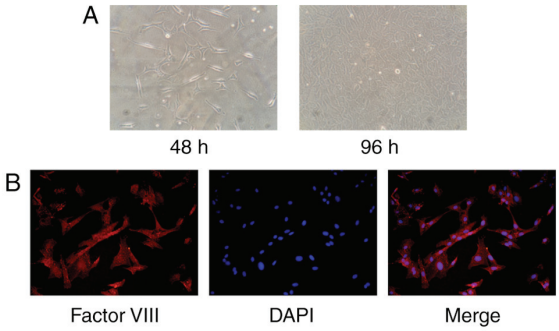

Figure 1. Morphological and immunological identification of CMECs. Cultures were identified by their morphological features and the expression of factor VIII-related antigen. (A) Primary CMECs possessed a spindle or polygonal shape after 24 h incubation, and a 'cobblestone' appearance after 96 h incubation (magnification, x200). (B) Most of the CMECs were positively stained with a microvascular endothelial cell specific anti-factor VIII antibody (magnification, x400). CMECs, cardiac microvascular endothelial cells.

Salidroside suppresses TNF-α-induced VCAM-1 protein expression. To determine whether VCAM-1 was involved in the salidroside-associated inhibition of TNF-α-induced monocyte-to-CMEC binding, the VCAM-1 mRNA expression level was quantified, and protein abundance and immunofluorescence intensity were determined. According to the observed results, treatment of CMECs with 10 ng/ml TNF-α strongly induced VCAM-1 gene expression (Fig. 3A). By contrast, pretreatment with salidroside inhibited the induction of VCAM-1 by TNF-α. In accordance with these results, western blot analysis and immunocytochemistry revealed that salidroside treatment also decreased the protein expression levels of VCAM-1 in CMECs, compared with a single dose of TNF-α (Fig. 3B and C).

Salidroside reduces the production of pro-inflammatory cytokines in CMECs. In order to identify the effect of salidroside on the production of TNF-α-induced pro-inflammatory cytokines, the expression levels of IL-1β, IL-6 and MCP-1 in CMECs were measured. After a 12-h incubation with 10, 50 or 100 μM salidroside, CMECs were treated with 10 ng/ml TNF-α for a further 12 h. RT-qPCR analysis and ELISA revealed that the TNF-α-induced mRNA expression levels and secretion of IL-1β, IL-6 and MCP-1 were reduced by salidroside treatment in CMECs (Fig. 4).

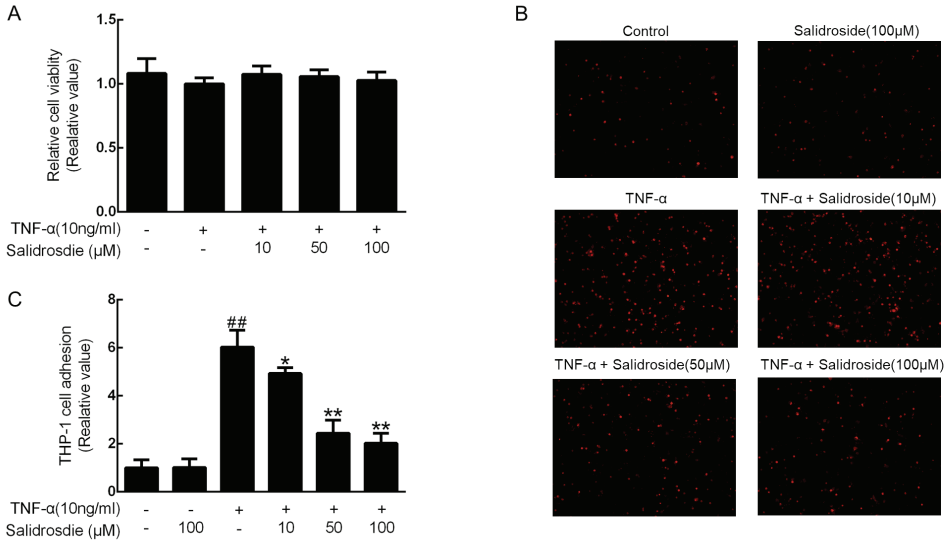

Figure 2. Effect of salidroside on the adhesion of THP-1 monocytes to TNF-α-activated CMECs. (A) The cytotoxic effects of salidroside were determined using the Cell Counting Kit-8 assay. (B) CMECs were pretreated with salidroside and stimulated with 10 ng/ml TNF-α for 12 h each, then co-cultured with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbo-cyanine perchlorate-labeled THP-1 cells for 1 h. (C) Adherent cell numbers were quantified (magnification, x40). Values are shown as the mean ± standard deviation from three independent experiments. $^{\#\#}$P<0.01 vs. the control group; *P<0.05 and **P<0.01 vs. the TNF-α-only group. CMECs, cardiac microvascular endothelial cells; TNF-α, tumor necrosis factor α.

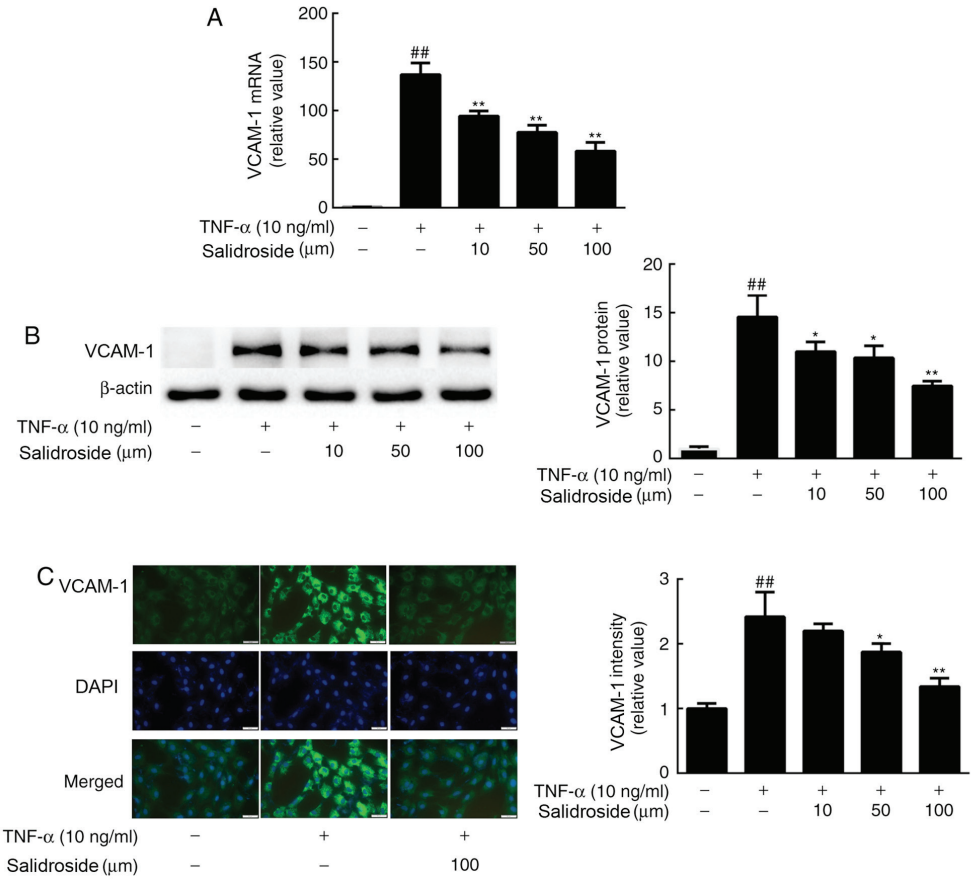

Figure 3. Effect of salidroside on TNF-α-induced VCAM-1 expression in CMECs. (A) Reverse transcription-quantitative PCR analysis of VCAM-1 in CMECs treated with 10 ng/ml TNF-α for 12 h, after treatment with the indicated concentrations of salidroside for 12 h. (B) Western blot analysis of VCAM-1 in CMECs treated with 10 ng/ml TNF-α for 12 h after treatment with the indicated concentrations of salidroside for 12 h. (C) CMECs pretreated with 100 μM salidroside for 12 h were stimulated with TNF-α for a further 12 h. VCAM-1 (green) was detected by immunofluorescence using an anti-VCAM-1 antibody and the nuclei were stained with DAPI (magnification, x200). Values are shown as the mean ± standard from three independent experiments. $^{\#\#}$P<0.01 vs. the control group; *P<0.05 and **P<0.01 vs. the TNF-α-only group. CMECs, cardiac microvascular endothelial cells; VCAM-1, vascular cell adhesion molecule-1; TNF-α, tumor necrosis factor α.

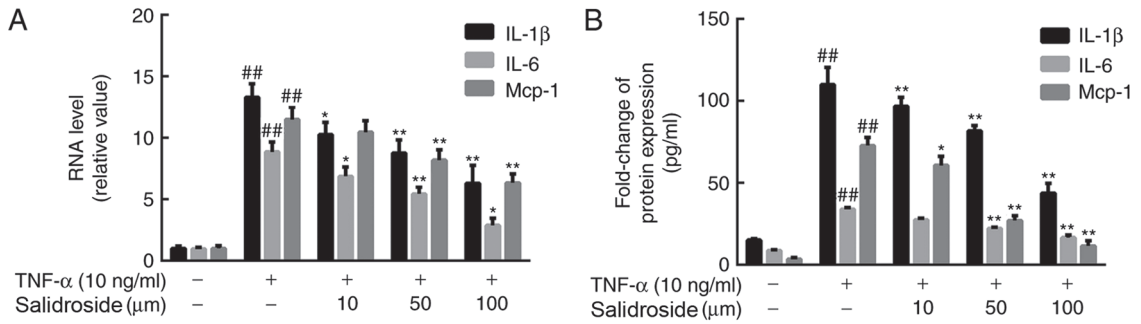

Figure 4. Effect of salidroside on TNF-α-induced proinflammatory cytokine expression in CMECs. (A) Reverse transcription-quantitative PCR analysis and (B) ELISA of IL-1β, IL-6 and MCP-1 in CMECs treated with 10 ng/ml TNF-α for 12 h, following treatment with the indicated concentrations of salidroside for 12 h. Values are shown as the mean ± standard deviation from three independent experiments. ##P<0.01 vs. respective control group; *P<0.05 and **P<0.01 vs. respective TNF-α-only group. TNF-α, tumor necrosis factor α; CMECs, cardiac microvascular endothelial cells; IL, interleukin; MCP-1, monocyte chemotactic protein 1.

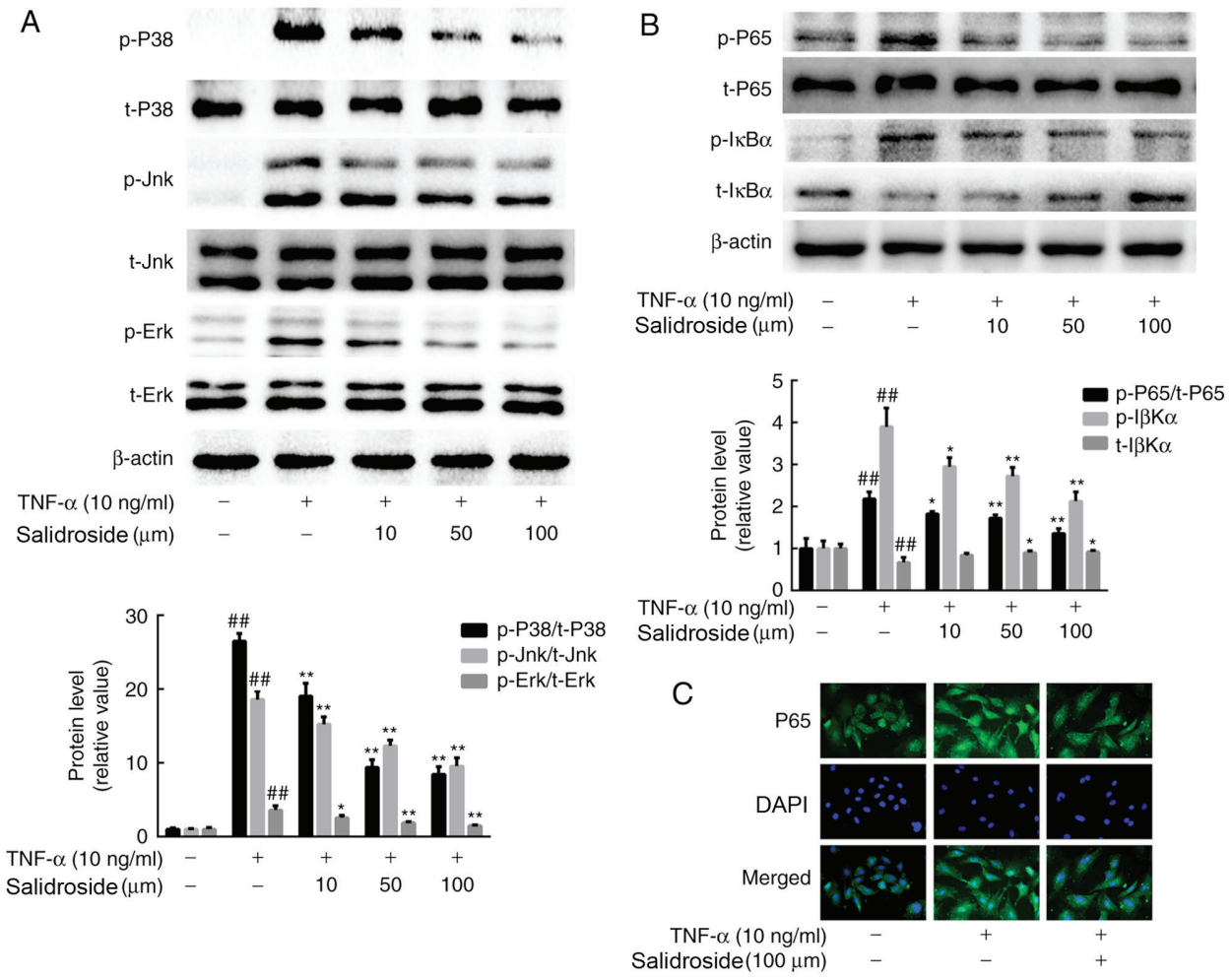

Figure 5. Effect of salidroside on TNF-α-induced MAPK and NF-κB activation in CMECs. (A) Western blot analysis of the phosphorylation of p38, Jnk, Erk and total p38, Jnk and Erk in CMECs treated with 10 ng/ml TNF-α for 15 min, after treatment with the indicated concentrations of salidroside for 12 h. (B) Western blot analysis of the phosphorylation of NF-κB p65, IκBα and total NF-κB p65 and IκBα in CMECs treated with 10 ng/ml TNF-α for 15 min, after treatment with the indicated concentrations of salidroside for 12 h. (C) CMECs were pre-incubated with 100 μM salidroside for 12 h and TNF-α (10 ng/ml) for 30 min. NF-κB p65 (green) was detected by immunofluorescence using an anti-NF-κB p65 antibody, and the nuclei were stained with DAPI (magnification, x400). The values are shown as the mean ± standard deviation from three independent experiments. ##P<0.01 vs. respective control group; *P<0.05 and **P<0.01 vs. respective TNF-α-only group. TNF-α, tumor necrosis factor α; CMECs, cardiac microvascular endothelial cells; IκBα, inhibitor of NF-κB; p, phosphorylated; t, total.

Salidroside regulates MAPK and NF-κB signaling in CMECs. In order to verify the possible downstream changes stimulated by salidroside, CMECs were pretreated with salidroside for 12 h and then incubated with TNF-α for an additional 30 min. The salidroside concentration was regulated, and MAPK and NF-κB activation were investigated. The levels of p38, Jnk and

Erk phosphorylation were markedly upregulated following TNF-α treatment. However, salidroside effectively suppressed the TNF-α-induced activation of p38, Jnk and Erk, as indicated by a reduction in the phosphorylation of these proteins (Fig. 5A). Also, the effect of salidroside on NF-κB nuclear accumulation was detected by p65 immunostaining. Salidroside was demonstrated to strongly suppress the degradation and phosphorylation of IκBα, and the phosphorylation of NF-κB p65, in a concentration-dependent manner (Fig. 5B and C).

Discussion

Endothelial dysfunction is the cause of a number of cardiovascular diseases (16). Oxidative and endoplasmic reticulum stress, unfolded endothelial cell protein responses and various other factors may lead to endothelial dysfunction (17), and vascular inflammation serves a key role in the pathogenesis of endothelial dysfunction and associated cardiovascular diseases (18,19). Attenuating endothelial inflammatory responses alleviates endothelial dysfunction and reduces the incidence of cardiovascular disease. Previous studies have demonstrated that activation of the endothelium at sites of inflammation results in the expression of a series of adhesion molecules, including VCAM-1, and cytokines such as IL-1β, IL-6 and MCP-1 (20,21). The present study demonstrated that salidroside significantly reduced TNF-α-induced monocyte adherence to CMECs by downregulating the expression of VCAM-1, as well as IL-1β, IL-6 and MCP-1. Furthermore, it was indicated that these pharmacological properties of salidroside were associated with its inhibitory effects on MAPK and NF-κB activation.

VCAM-1 is an immunoglobulin-like adhesion molecule expressed on activated endothelial cells. It is able to recruit leukocytes and promote their infiltration into injured arteries; this initiates atherosclerotic plaque development via its interaction with α4β1 integrin, which is constitutively expressed on lymphocytes, monocytes and eosinophils (6). Under normal physiological conditions, VCAM-1 is not usually expressed, but is rapidly induced by stimuli such as cytokine secretion and TLR activation (22). TNF-α is a pleiotropic pro-inflammatory cytokines (5) and was used to induce vascular inflammation in the present study. The results demonstrated that salidroside attenuated the TNF-α-induced expression of VCAM-1 in a concentration-dependent manner at both the mRNA and protein levels. Additionally, the secretion of pro-inflammatory cytokines (IL-1β, IL-6 and MCP-1) by CMECs was decreased in a concentration-dependent manner following salidroside treatment. Furthermore, salidroside significantly inhibited THP-1-to-CMEC adhesion. Previous studies have demonstrated that a reduction in the overexpression of VCAM-1 and other inflammatory cytokines serves a preventative role in the development of inflammatory diseases, resulting in improved patient prognosis (23-26). Thus, the present study revealed that salidroside modulates vascular inflammation by suppressing inflammatory responses in TNF-α-activated CMECs.

The MAPK and NF-κB pathways are indispensable for the activation of TNF-α-stimulated endothelial cells (27). Specifically, NF-κB is a major transcription factor which participates in the inflammatory regulation of endothelial cells by responding to pro-inflammatory stimuli (28). During endothelial inflammation, NF-κB regulates the expression of VCAM-1 and numerous inflammatory cytokines (29-31). In the cytoplasm, NF-κB exists in its inactive form associated with the inhibitory protein IκB via its IκBα subunit. Once stimulated by cytokines, IκBα becomes phosphorylated and degraded, which creates the optimal conditions for NF-κB translocation into the nucleus, triggering gene transcription (32). Also, the MAPK signaling pathway is known to crucially regulate endothelial inflammation, and serves an important role in the induction of pro-inflammatory mediators (p38, Jnk and Erk) in endothelial cells after stimulation with TNF-α. The MAPKs primarily constitute a highly-conserved serine/threonine protein kinase family, which is important for signal transduction from the cell surface to the nucleus. MAPKs regulate cell growth, differentiation, environmental stress adaptation, inflammatory responses, and other important physiological and pathological processes within the cell (33). It has been shown that MAPKs also regulate the expression of VCAM-1 and inflammatory cytokines (8,34,35). A previous study indicated that salidroside reduces cell mobility by regulating NF-κB and MAPK signaling in LPS-treated-microglial cells (36). The present study revealed that salidroside significantly suppressed the TNF-α-induced phosphorylation and degradation of IκBα, and the subsequent nuclear translocation of NF-κB in CMECs. Simultaneously, salidroside was observed to inhibit TNF-α-induced p38, Erk and Jnk1/2 phosphorylation in CMECs. These data suggest that salidroside may suppress TNF-α-induced endothelial cell inflammation by inhibiting NF-κB and MAPK activation.

In conclusion, the present study revealed the suppressive effects of salidroside on TNF-α-induced CMEC activation, which effectively inhibited TNF-α-induced monocyte/CMEC interactions and the release of proinflammatory mediators, including VCAM-1, IL-1β IL-6 and MCP-1. Such suppressive effects are likely to have resulted from MAPK and NF-κB restriction. These results provide novel insights into the therapeutic potential of salidroside in preventing vascular inflammatory diseases, including atherosclerosis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

HS and XG contributed to the conception of the study. RSL, XZ and RCL contributed significantly to analysis and manuscript preparation. RSL and ZD also performed data analyses and wrote the manuscript, and FY and YC helped perform the

analysis with constructive discussions. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics committee of Fudan University, Shanghai, China.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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