

Matrine ameliorates the inflammatory response and lipid metabolism in vascular smooth muscle cells through the NF- κ B pathway

GUANGLEI WANG^{1*}, CHENGYU JI^{2*}, CHUNLING WANG³,
ZHONGHUI LIU¹, AIZHONG QU⁴ and HUAIXIN WANG¹

¹Department of Emergency Medicine, Yidu Central Hospital of Weifang, Weifang, Shandong 262500;
Departments of ²Cardiology and ³Stomatology, Linqu People's Hospital, Weifang, Shandong 262600;
⁴Hematology Department, Weifang Yidu Central Hospital, Weifang, Shandong 262500, P.R. China

Received June 4, 2020; Accepted April 19, 2021

DOI: 10.3892/etm.2021.10744

Abstract. Atherosclerosis is a chronic inflammatory disease associated with inflammatory responses and the uncontrolled proliferation and excessive apoptosis of vascular smooth muscle cells. However, the effects of matrine on the inflammatory response, abnormal lipid metabolism and cell proliferation and apoptosis marker proteins in human aortic vascular smooth muscle cells (HAVSMCs) have not been elucidated. Therefore, the present study aimed to investigate the effect of matrine on an *in vitro* model of atherosclerosis using HAVSMCs. The HAVSMCs were divided into normal, model and matrine groups. The model group was treated with oxidized low-density lipoprotein (oxLDL), the matrine group was treated with oxLDL and matrine and the normal group was treated with physiological saline. Total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE) levels were measured in the cell supernatant. In addition, the relative mRNA levels of inflammatory factors were quantified using reverse transcription-quantitative PCR, and the cell proliferation and apoptosis rates were evaluated using Cell Counting Kit-8 and flow cytometry assays, respectively. The expression levels of proteins associated with proliferation and apoptosis were also determined using western blotting. The levels of TC, FC and CE and the mRNA levels of IL-1 β , IL-6, and TNF- α in the matrine group were lower than those in the model group, but higher than those in the normal group. After 48 and 96 h of treatment, the cell proliferation and apoptotic

rates were lower in the matrine group compared with the model group. The relative expression levels of Ki-67, proliferating cell nuclear antigen and Bax were decreased, while that of Bcl-2 was increased in the matrine group compared with the model group. In addition, the relative protein expression of nuclear factor κ B (NF- κ B) in the matrine group was lower than that in the model group, but higher than that in the normal group. In summary, matrine inhibited activation of the NF- κ B pathway and reduced cell proliferation and apoptosis in the oxLDL-induced atherosclerosis model, and exhibited anti-inflammatory effects. These results suggest that matrine attenuated abnormal biological reactions in HAVSMCs through the NF- κ B pathway.

Introduction

Atherosclerosis is the most common type of arteriosclerosis, which occurs in the subendothelial layer of the large and medium arteries, resulting in the blockage of blood flow triggered by endothelial dysfunction and the subendothelial retention of lipoproteins (1). Atherosclerosis is a key cause of mortality worldwide. In Western societies, it is the underlying cause of ~50% of all mortality (2), and results in several medical complications, including myocardial infarction, stroke and peripheral arterial disease (3). A number of inflammatory biomarkers (including IL-6, IL-1 β , IL-10, TNF- α , E-selectin, vascular cell adhesion molecule-1, adiponectin, high-sensitivity C-reactive protein and pentraxin 3) have been identified as independent risk factors for cardiovascular diseases, and studies have provided evidence for low-density lipoprotein (LDL)-induced immune activation in human atherosclerotic lesions (4). Numerous studies have been conducted with the aim of developing improved treatment strategies for atherosclerosis (5,6).

Matrine is a key substance used in traditional Chinese medicine (7). It exerts anti-allergic, anti-inflammatory, antiviral and antifibrotic effects and is considered to be helpful for protecting against cardiovascular disease (8). The anti-inflammatory mechanism of matrine in microvascular endothelial cells has been shown to involve the increase of nitric

Correspondence to: Dr Huaixin Wang, Department of Emergency Medicine, Yidu Central Hospital of Weifang, 4138 Linglongshan South Road, Weifang, Shandong 262500, P.R. China
E-mail: whxinwan@163.com

*Contributed equally

Key words: matrine, NF- κ B pathway, human aortic vascular smooth muscle cells, inflammatory response

oxide-dependent vasodilatation and the inhibition of lipopolysaccharide-induced inflammatory cytokines, indicating that matrine acts as a protective agent against inflammatory tissue damage (9). A previous study demonstrated that matrine was effective in treating liver cancer by inhibiting the expression of matrix metalloproteinase-9 and the invasion of human liver cancer cells, and further showed that the inhibitory effect was partly associated with downregulation of the nuclear factor- κ B (NF- κ B) signaling pathway (10). In another study, matrine was shown to inhibit the invasion and metastasis of melanoma cells *in vitro*, and the induction of apoptosis was associated with the downregulation of heparinase mRNA and protein expression (11).

Based on these previous findings, we hypothesized that matrine may affect the inflammatory response and abnormal lipid metabolism of vascular smooth muscle cells, and aimed to investigate this and to elucidate the underlying mechanism in the present study.

Materials and methods

Cell culture and drug treatment. Human aortic vascular smooth muscle cells (HAVSMCs; Shenzhen Haodi Huatuo Biotechnology Co. Ltd.) were plated in 6-well plates and routinely cultured in high-glucose Dulbecco's modified Eagle's medium (Qingdao Jiesikang Biotechnology Co., Ltd.) containing 10% fetal bovine serum (Shanghai Xinfan Biotechnology Co., Ltd.) without antibiotics at 37°C in a 5% CO₂ incubator until they reached 60-70% confluency.

The proliferation of HAVSMCs treated with various concentrations (0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml of matrine (Shanghai Yuanye Biotechnology Co., Ltd.) for 24 and 48 h was analyzed at 37°C. In subsequent experiments, the cells were further assigned to normal, model and matrine groups. The model group was treated with 50 mg/ml oxidized LDL (oxLDL; Shanghai Lianmai Biological Engineering Co., Ltd.) to establish the atherosclerosis model. The matrine group was treated with 50 mg/ml oxLDL and 1.0 mg/ml matrine. The normal group was treated with the same volume of normal saline. In the western blotting experiment, model + Bay11-7082 (NF- κ B inhibitor, MedChemExpress, cat. no. HY-13453) and matrine + Bay11-7082 groups were also established by treatment with 2.5 μ mol/l Bay11-7082 for 2 h at 37°C. A flowchart of the study protocol is shown in Fig. 1.

Cell growth analysis. Cell proliferation in the normal, model and matrine groups was evaluated using a Cell Counting Kit-8 (CCK-8) assay kit (cat. no. ab228554; Abcam). A suspension of HAVSMCs was prepared and the 1x10⁶ cell suspension (100 μ l) was added to each well of a 96-well plate. Three replicates were prepared for each group and time point. Each well was treated with 20 μ l CCK-8 reagent after incubation for 24, 48, 72 and 96 h at 37°C in a 5% CO₂ incubator. After incubation for 2 h with the CCK-8 reagent, the absorbance at 490 nm was measured using an automated microplate reader. The experiment was repeated three times.

Analysis of lipid metabolism markers. Cell supernatants were collected from the normal, model and matrine groups

and the levels of total cholesterol (TC; cat. no. JL19339), free cholesterol (FC; cat. no. JL20022) and cholesterol ester (CE; cat. no. JL19339) in the cell supernatants were determined using ELISA kits (Shanghai Jianglai Biological Technology Co., Ltd.) in accordance with the manufacturer's instructions. Experiments were repeated three times.

Detection of apoptotic rates. An apoptosis assay was performed on cells from the normal, model and matrine using an apoptosis kit according to the manufacturer's instructions (Apoptotic DNA-Ladder kit; Hangzhou Xinjing Biological Reagent Development Co., Ltd.). Flow cytometry (BD FACSCalibur; BD Pharmingen) was used to analyze the cells. The experiment was repeated three times.

Western blot analysis. Cells from the various treatment groups were lysed using radio-immunoprecipitation assay buffer (Beyotime Institute of Biotechnology). Protein determination was performed via the BCA method and total protein was isolated. Equal amounts (25 μ g) total protein were separated on a 10% gel via sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride (PVDF) membrane. The PVDF membrane was blocked with 5% milk for 1 h at room temperature. The PVDF membrane was then washed with TBST (0.1% Tween-20) three times. The PVDF membrane was incubated with primary antibodies against Ki-67 (1:1,000; cat. no. ab15580; Abcam), proliferating cell nuclear antigen (PCNA; 1:1,000; cat. no. ab280088; Abcam), Bcl-2 (1:1,000; cat. no. ab32124; Abcam) and Bax (1:1,000; cat. no. ab182734; Abcam) at 4°C overnight. Secondary horseradish peroxidase (HRP)-rabbit antibody (1:5,000; cat. no. ab6858; Abcam) was then added to the membrane, after which it was incubated at room temperature for 2 h. The PVDF membrane was then washed with TBST three times and developed using 5 ml enhanced chemiluminescence substrate (Roche Diagnostics; cat. no. 11684817910) for 3 times and ImageJ software (version k 1.45; National Institutes of Health) was used for analysis.

Analysis of the mRNA expression of inflammatory factors. Total RNA was obtained from the cells using TRIzol[®] reagent (Thermo Fisher Scientific, Inc.), following the manufacturer's instructions. The concentration and purity of RNA were quantified using a UV spectrophotometer (Shanghai Qinxiang Scientific Instrument Co., Ltd.) by measuring the ratio of optical densities at 260 and 280 nm, which were 1.8 and 2.0, respectively. cDNA was synthesized from the RNA by reverse transcription (RT) using reverse transcriptase (Shenzhen Zike Biotechnology Co., Ltd.) and oligonucleotides. The reaction mix contained (in 20 μ l volume): 1 dNTPs, 1 primers, 4 buffer, 2 reverse transcriptase, 2 total RNA and 12 μ l RNase-free water. The reaction conditions comprised incubation at 42°C in a water bath for 1 h, followed by incubation at 95°C in a water bath for 5 min. This was followed by quantitative PCR (qPCR) amplification using a Real-Time qPCR kit (Guangzhou Huafeng Biological Technology Co., Ltd.). Specific primers were used to detect the expression of interleukin (IL)-1 β , IL-6, tumor necrosis factor- α (TNF- α) and β -actin, which served as an internal

Table I. Primer sequences.

Name	Forward (5'-3')	Reverse (5'-3')
IL-1 β	CTTCAAATCTCACAGCAGCAGCATC	GCTGTCTAATGGGAACATCACA
IL-6	GTTTGACCAGAGGACCCAGA	TCCTTTGTTACGGCTTCCAG
TNF- α	GTGCCTCAGCCTCTTCTCATT	CTCTGCTTGGTGGTTTGCTAC
β -actin	CACCCGCGAGTACAACCTTC	CCCATACCCACCATCACAAA

IL, interleukin; TNF, tumor necrosis factor.

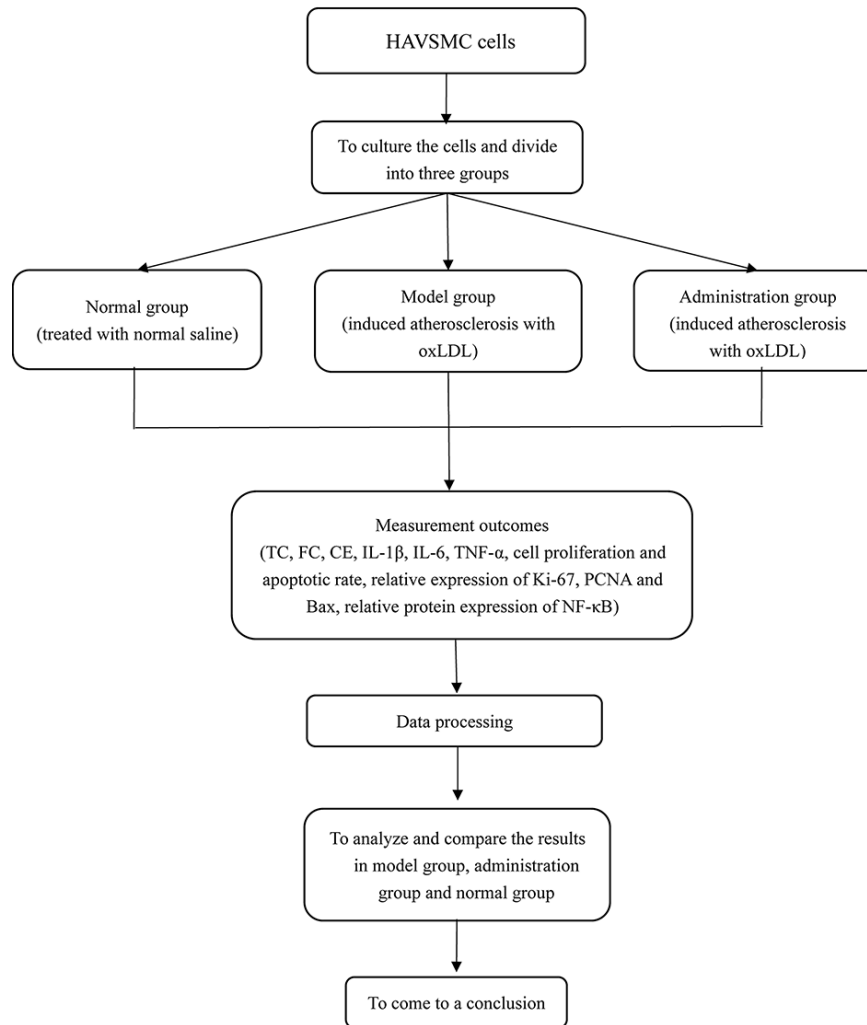


Figure 1. Flowchart of the study. HAVSMCs, human aortic vascular smooth muscle cells; oxLDL, oxidized low-density lipoprotein; TC, total cholesterol; FC, free cholesterol; CE, cholesterol ester; IL, interleukin; TNF, tumor necrosis factor; PCNA, proliferating cell nuclear antigen; NF- κ B, nuclear factor κ B.

control. The qPCR reaction mixture (20 μ l) consisted of 0.4 μ l each of the upstream and downstream primers and 0.5 μ l Taq DNA polymerase diluted in ddH₂O. The conditions of the qPCR were as follows: 94°C for 10 sec, followed by 40 cycles of 94°C for 5 sec, 52°C for 30 sec and 72°C for 15 sec. All experiments were performed in triplicate and repeated three times. The results were analyzed using a relative quantitation method to internal controls; specifically, the expression levels of IL-1 β , IL-6 and TNF- α were calculated using the 2^{- $\Delta\Delta$ C_q} method (12). The sequences of the primers

used for the qPCR analysis of inflammatory factors and the internal controls (Suzhou Hongxun Biological Technology Co., Ltd.) are listed in Table I.

Statistical analysis. Statistical analysis was performed using SPSS 20.0 (IBM Corp.) statistical software. Data are expressed as the mean \pm standard deviation. One-way ANOVA with Tukey's post hoc test was used for the comparison of multiple groups. P<0.05 was considered to indicate a statistically significant result.

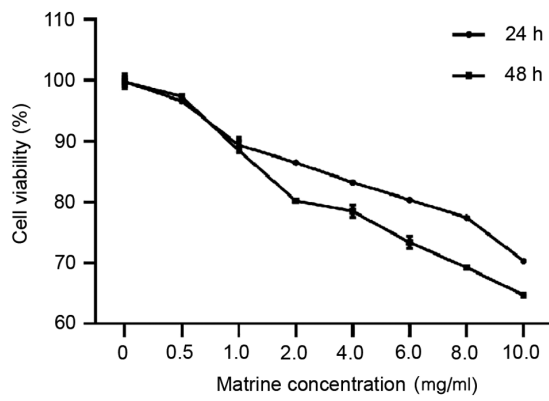


Figure 2. Selection of the optimum matrine concentration. Human aortic vascular smooth muscle cells were treated with 0.5-10 mg/ml matrine for 24 and 48 h and cell viability was then monitored using a Cell Counting Kit-8 assay. Cell viability was >90% at matrine concentrations <1 mg/ml and was inversely proportional to the matrine concentration at concentrations >1 mg/ml.

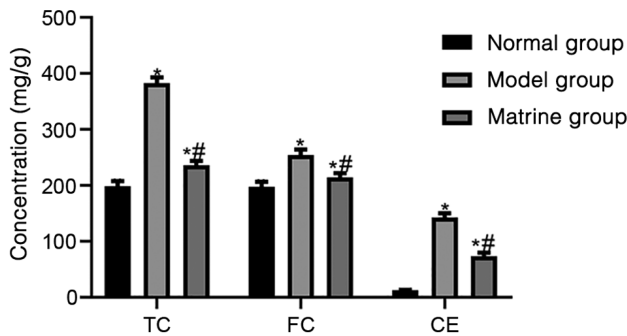


Figure 3. Effect of matrine on lipid metabolism in human aortic vascular smooth muscle cells treated with oxidized low-density lipoprotein. The levels of the lipid metabolism markers TC, FC and CE were analyzed in the normal, model and matrine groups. * $P < 0.05$ vs. the normal group; # $P < 0.05$ vs. the model group. TC, total cholesterol; FC, free cholesterol; CE, cholesterol ester.

Results

Selection of the optimum concentration of matrine. The optimal matrine concentration was determined by treating HAVSMCs with 0.5-10 mg/ml matrine for 24 and 48 h, and then analyzing the cell viability. The results of the CCK-8 assay show that cell viability was >90% for cells treated with <1 mg/ml matrine, and for cells treated with matrine at concentrations >1 mg/ml, the cell viability was inversely proportional to the concentration. Thus, 1 mg/ml matrine was selected for subsequent experiments (Fig. 2).

Effect of matrine on lipid metabolism. The lipid metabolism markers TC, FC and CE were detected in the HAVSMCs in the normal, model and matrine groups. The levels of TC, FC and CE were 198.23 ± 9.47 , 197.37 ± 9.23 and 12.48 ± 0.58 mg/g, respectively, in the normal group; 382.58 ± 10.4 , 254.23 ± 10.32 and 142.67 ± 7.35 mg/g, respectively, in the model group; and 235.54 ± 8.4 , 214.24 ± 7.6 and 73.24 ± 6.54 mg/g, respectively, in the matrine group. The results showed that the levels TC, FC and CE were significantly higher in the oxLDL-treated model group compared with the normal group ($P < 0.05$). Furthermore,

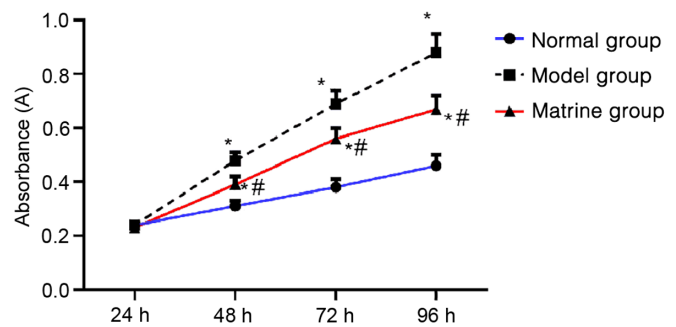


Figure 4. Effect of matrine on the growth of human aortic vascular smooth muscle cells treated with oxidized low-density lipoprotein. Cell proliferation in the normal, model and matrine groups was analyzed using a Cell Counting Kit-8 assay. * $P < 0.05$ vs. the normal group; # $P < 0.05$ vs. the model group.

the levels of TC, FC and CE in the matrine group were lower than those in the model group, although they remained higher than those in the normal group ($P < 0.05$; Fig. 3)

Effect of matrine on cell proliferation, apoptosis and associated proteins. From 48-96 h, cell growth was higher in the model and matrine groups compared with the normal group, and the cell growth in the matrine group was reduced compared with that in the model group ($P < 0.05$). The cell growth in each group presented an upward trend over time (Fig. 4).

The apoptotic rates in the normal, model and matrine groups as determined using flow cytometry were 4.28 ± 0.43 , 17.57 ± 3.24 and $7.59 \pm 2.52\%$, respectively. These results indicate that the model group had an increased apoptosis rate compared with that of the normal group ($P < 0.05$). Furthermore, the apoptosis rate of the matrine group was lower compared with that of the model, but higher than that of the normal group ($P < 0.05$; Fig. 5).

The expression of proliferation- and apoptosis-associated proteins was evaluated using western blotting. The relative expression levels of Ki-67, PCNA, Bcl-2 and Bax were 0.19 ± 0.02 , 0.23 ± 0.03 , 0.87 ± 0.04 and 0.16 ± 0.01 , respectively, in the normal group; 0.86 ± 0.05 , 0.82 ± 0.04 , 0.27 ± 0.02 and 0.79 ± 0.03 , respectively, in the model group; and 0.37 ± 0.02 , 0.38 ± 0.03 , 0.45 ± 0.04 and 0.33 ± 0.03 , respectively, in the matrine group. In the model group, the expression levels of Ki-67, PCNA and Bax were increased while those of Bcl-2 were decreased compared with those in the normal group ($P < 0.05$). In addition, the matrine group showed decreased expression levels of Ki-67, PCNA and Bax and increased expression levels of Bcl-2 compared with those in the model group ($P < 0.05$; Fig. 6).

Matrine regulates cell proliferation and apoptosis through the NF- κ B pathway. To further investigate the underlying mechanism of matrine, the expression of NF- κ B was evaluated using western blotting. The relative protein expression levels of NF- κ B in the normal, model and matrine groups were 0.27 ± 0.04 , 0.71 ± 0.05 and 0.40 ± 0.03 , respectively, indicating that the relative protein expression level of NF- κ B in the model group was higher compared with that in the normal group ($P < 0.05$). In the matrine group, the

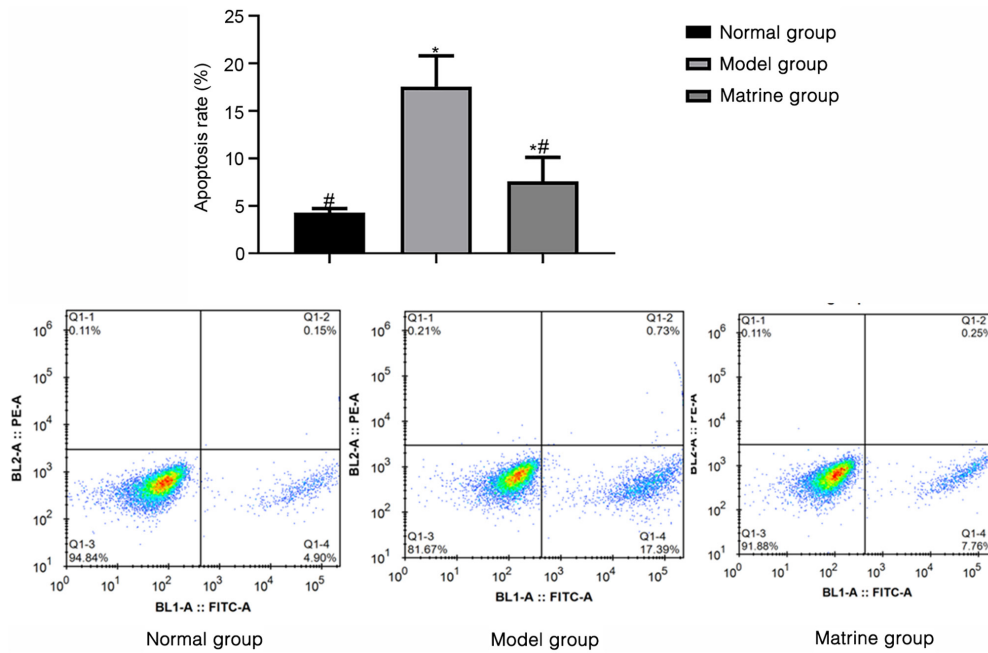


Figure 5. Effect of matrine on the apoptosis rate of human aortic vascular smooth muscle cells treated with oxidized low-density lipoprotein. The apoptotic rates in the normal, model and matrine groups were analyzed using flow cytometry. *P<0.05 vs. the normal group; #P<0.05 vs. the model group.

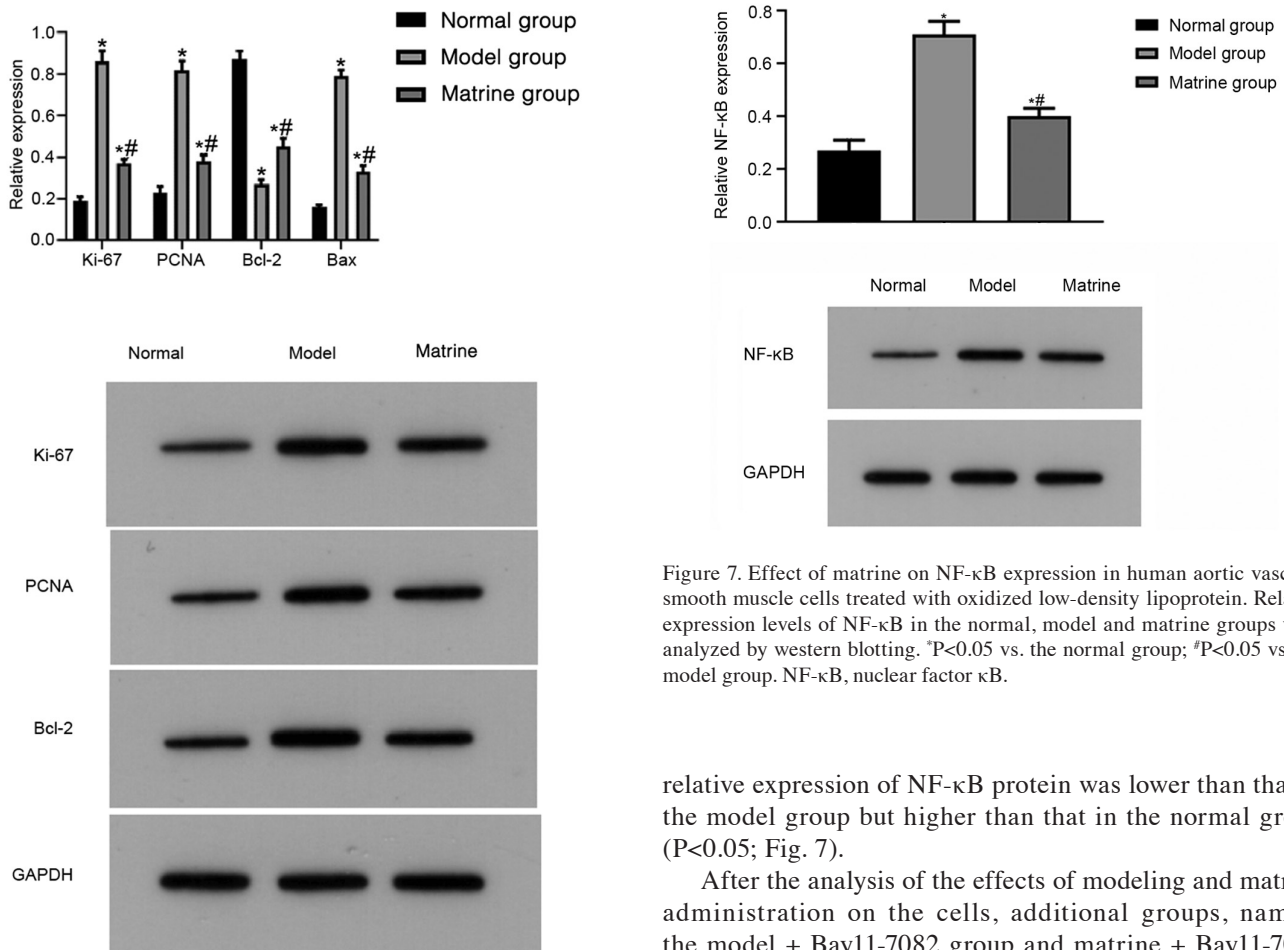


Figure 6. Effect of matrine on markers of cell proliferation and apoptosis in human aortic vascular smooth muscle cells treated with oxidized low-density lipoprotein. Relative expression levels of Ki-67, PCNA, Bcl-2 and Bax were analyzed in the normal, model and matrine groups by western blotting. *P<0.05 vs. the normal group; #P<0.05 vs. the model group. PCNA, proliferating cell nuclear antigen.

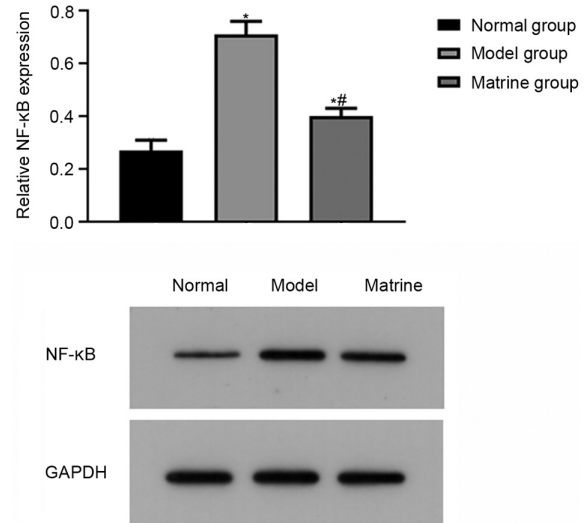


Figure 7. Effect of matrine on NF-κB expression in human aortic vascular smooth muscle cells treated with oxidized low-density lipoprotein. Relative expression levels of NF-κB in the normal, model and matrine groups were analyzed by western blotting. *P<0.05 vs. the normal group; #P<0.05 vs. the model group. NF-κB, nuclear factor κB.

relative expression of NF-κB protein was lower than that in the model group but higher than that in the normal group (P<0.05; Fig. 7).

After the analysis of the effects of modeling and matrine administration on the cells, additional groups, namely the model + Bay11-7082 group and matrine + Bay11-7082 group were established, and the effects of Bay11-7082 on the expression of proliferation- and apoptosis-associated proteins were assessed using western blotting. Consistent with the aforementioned results, compared with the normal group, the relative expression of Ki-67, PCNA and Bax

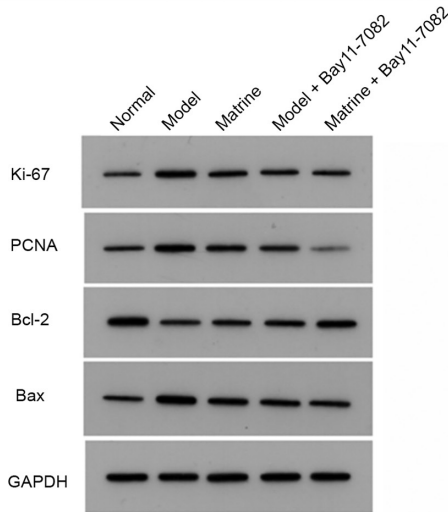
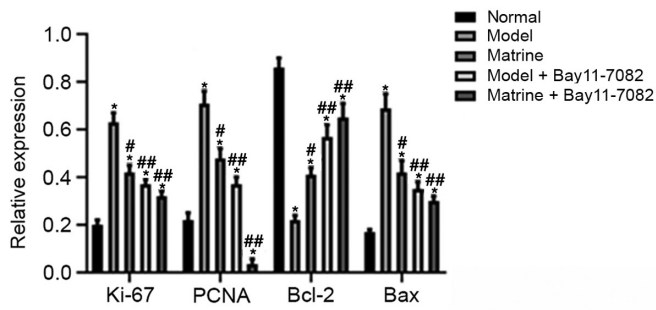


Figure 8. Matrine affects markers of cell proliferation and apoptosis through the NF- κ B pathway. * $P < 0.05$ vs. the normal group; # $P < 0.05$ and ## $P < 0.01$ vs. the model group. NF- κ B, nuclear factor κ B.

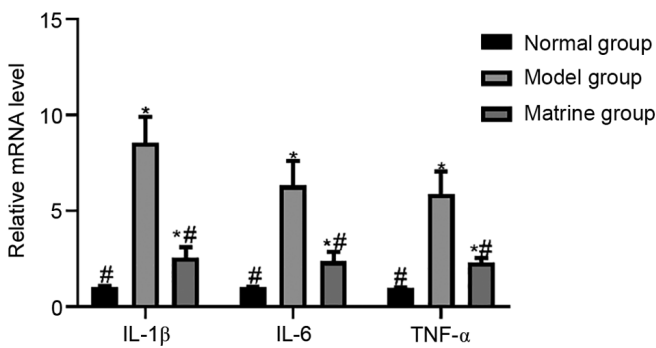


Figure 9. Effect of matrine on inflammatory factors in human aortic vascular smooth muscle cells treated with oxidized low-density lipoprotein. The relative mRNA levels of the inflammatory factors IL-1 β , IL-6 and TNF- α were analyzed in the normal, model and matrine groups. * $P < 0.05$ vs. the normal group; # $P < 0.05$ vs. the model group.

increased in the model group, while that of Bcl-2 decreased ($P < 0.05$). However, the matrine, model + Bay11-7082 and matrine + Bay11-7082 groups exhibited lower relative expression levels of Ki-67, PCNA and Bax and higher relative expression levels of Bcl-2 compared with those in the model group ($P < 0.05$; Fig. 8).

Matrine reduces inflammatory factors through the NF- κ B pathway. The effect of matrine on oxLDL-induced inflammatory factors was assessed using RT-qPCR. The relative mRNA levels of IL-1 β , IL-6 and TNF- α were 1.03 \pm 0.02, 1.02 \pm 0.01

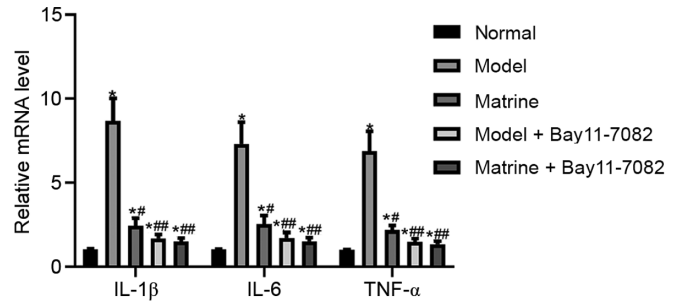


Figure 10. Matrine regulates the expression of inflammatory factors through the NF- κ B pathway. * $P < 0.05$ vs. the normal group; # $P < 0.05$ and ## $P < 0.01$ vs. the model group.

and 0.99 \pm 0.01, respectively, in the normal group; 8.57 \pm 1.34, 6.32 \pm 1.29 and 5.87 \pm 1.18, respectively, in model group; and 2.57 \pm 0.54, 2.38 \pm 0.48 and 2.29 \pm 0.25, respectively, in the matrine group. The results indicated that exposure to oxLDL in the model group significantly increased the relative mRNA levels of IL-1 β , IL-6 and TNF- α compared with those in the normal group ($P < 0.05$). The relative mRNA levels of IL-1 β , IL-6 and TNF- α in the matrine group were lower than those in the model group, but higher than those in the normal group ($P < 0.05$; Fig. 9).

The effect of Bay11-7082 on these inflammatory factors was also evaluated. As in the aforementioned results, the model group showed significantly upregulated mRNA levels of IL-1 β , IL-6 and TNF- α compared with those in the normal group ($P < 0.05$). However, the matrine, model + Bay11-7082 and matrine + Bay11-7082 groups exhibited lower mRNA levels of 1 β , IL-6 and TNF- α compared with those in the model group ($P < 0.05$; Fig. 10).

Discussion

Atherosclerosis is considered to be a chronic inflammatory disease of the vascular walls, while matrine has anti-inflammatory effects and also affects the cardiovascular system. Therefore, we hypothesized that matrine may have potential therapeutic use for preventing the progression of atherosclerotic lesions. Furthermore, exploration of the potential role and mechanism of matrine as an anti-atherosclerotic treatment may support research into the properties of traditional Chinese medicine and pharmacology. Inflammation is associated with the pathogenesis of atherosclerosis (13), and acts as a key regulatory process that links the risk factors for atherosclerosis (14). IL-1 β is a key mediator of the host response to infection and inflammation (15). Although it helps in resisting pathogens, it also exacerbates damage during chronic diseases and acute tissue injury (16). A study suggested that IL-1 β may play a local role in the formation and stability of atherosclerosis by inducing macrophages, endothelial cells and smooth muscle cells to produce cytokines and proteolytic enzymes (17). IL-6 is an inflammatory factor that plays a central role in the inflammatory response. It exists in cells and in extracellular deposits of connective tissue matrix in the human atherosclerotic wall and may be a crucial pro-atherosclerotic cytokine (18,19). IL-6 has also been shown to be involved in the development of human atherosclerosis and is highly expressed in atherosclerosis (20).

As a pro-inflammatory cytokine, TNF- α is associated with metabolic disorders and may have a significant effect on the development of cardiovascular disease (21). The expression of TNF- α increases in atherosclerotic cardiovascular diseases (22). A previous study demonstrated that the administration of matrine to oxLDL-exposed macrophages reduced the protein and mRNA expression of inflammatory cytokines in a concentration-dependent manner (23). In the present study, the relative mRNA levels of IL-1 β , IL-6 and TNF- α in the oxLDL-treated cell model were significantly higher than those in the normal group, but after treatment with matrine, the relative mRNA levels of IL-1 β , IL-6 and TNF- α were significantly reduced. These results indicate that the expression levels of IL-1 β , IL-6 and TNF- α were increased under conditions simulating those of atherosclerosis, and matrine inhibited the expression of inflammatory cytokines.

As an acidic nuclear protein, PCNA is considered a histological marker of the G₁/S phase in the cell cycle (24). Ki-67 and PCNA are two nuclear markers commonly used to signal the proliferation phase (25). Bcl-2 family proteins are the main regulators of cell cycle, and among them, Bax is pro-apoptotic, whereas Bcl-2 inhibits apoptosis (26). A previous study revealed the ability of matrine to inhibit and induce the differentiation of K-562 cells (27). In the present study, the results demonstrated that in the matrine group, the expression levels of Ki-67, PCNA and Bax were significantly decreased while those of Bcl-2 was increased compared with the respective levels in the model group. These results indicate that matrine inhibited the proliferation and apoptosis of vascular smooth muscle cells in this atherosclerotic model.

The NF- κ B pathway is well known as a typical pro-inflammatory signaling pathway (28), and NF- κ B has been shown to regulate the expression of proteins that inhibit apoptosis and promote proliferation (29). In a previous study, matrine inhibited vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 expression in TNF- α -stimulated human aortic smooth muscle cells by inhibiting the production of reactive oxygen species and activating the NF- κ B and MAPK pathways, which suggests its potential for the prevention of atherosclerosis (30). In the present study, whether matrine affected inflammatory factors and pro-apoptotic proteins through the NF- κ B pathway was investigated. The results suggest that matrine may exert anti-inflammatory effects and inhibit cell proliferation by inhibiting activation of the NF- κ B pathway. However, only cell experiments were performed, which limits the translational clinical value of the results.

In summary, the present study demonstrates that matrine attenuated the inflammatory response, abnormal lipid metabolism and proliferation of vascular smooth muscle cells exposed to oxLDL, and suggests that these effects were mediated via the NF- κ B pathway.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

GW and HW designed the experiments, CJ and CW carried out the experiments and ZL and AQ analyzed the experimental results. GW wrote the manuscript and HW revised the manuscript. GW and HW confirm the authenticity of all the raw data. All authors read and approved the final 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. Tabas I, García-Cardena G and Owens GK: Recent insights into the cellular biology of atherosclerosis. *J Cell Biol* 209: 13-22, 2015.
2. Gisterå A and Hansson GK: The immunology of atherosclerosis. *Nat Rev Nephrol* 13: 368-380, 2017.
3. Feinberg MW and Moore KJ: MicroRNA regulation of atherosclerosis. *Circ Res* 118: 703-720, 2016.
4. Bäck M and Hansson GK: Anti-inflammatory therapies for atherosclerosis. *Nat Rev Cardiol* 12: 199-211, 2015.
5. Libby P and Everett BM: Novel antiatherosclerotic therapies. *Arterioscler Thromb Vasc Biol* 39: 538-545, 2019.
6. Zhu Y, Xian X, Wang Z, Bi Y, Chen Q, Han X, Tang D and Chen R: Research progress on the relationship between atherosclerosis and inflammation. *Biomolecules* 8: 80, 2018.
7. Zhang MJ and Huang J: Recent research progress of anti-tumor mechanism matrine. *Zhongguo Zhong Yao Za Zhi* 29: 115-118, 2004 (In Chinese).
8. Liu Y, Xu Y, Ji W, Li X, Sun B, Gao Q and Su C: Anti-tumor activities of matrine and oxymatrine: Literature review. *Tumour Biol* 35: 5111-5119, 2014.
9. Suo Z, Liu Y, Ferreri M, Zhang T, Liu Z, Mu X and Han B: Impact of matrine on inflammation related factors in rat intestinal microvascular endothelial cells. *J Ethnopharmacol* 125: 404-409, 2009.
10. Yu HB, Zhang HF, Li DY, Zhang X, Xue HZ and Zhao SH: Matrine inhibits matrix metalloproteinase-9 expression and invasion of human hepatocellular carcinoma cells. *J Asian Nat Prod Res* 13: 242-250, 2011.
11. Liu XY, Fang H, Yang ZG, Wang XY, Ruan LM, Fang DR, Ding YG, Wang YN, Zhang Y, Jiang XL and Chen HC: Matrine inhibits invasiveness and metastasis of human malignant melanoma cell line A375 in vitro. *Int J Dermatol* 47: 448-456, 2008.
12. 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.
13. de Boer OJ, van der Wal AC and Becker AE: Atherosclerosis, inflammation, and infection. *J Pathol* 190: 237-243, 2000.
14. Libby P, Ridker PM, Hansson GK and Leducq Transatlantic Network on Atherothrombosis: Inflammation in atherosclerosis: From pathophysiology to practice. *J Am Coll Cardiol* 54: 2129-2138, 2009.

15. Faggioni R, Fantuzzi G, Fuller J, Dinarello CA, Feingold KR and Grunfeld C: IL-1 β mediates leptin induction during inflammation. *Am J Physiol* 274: R204-R208, 1998.
16. Lopez-Castejon G and Brough D: Understanding the mechanism of IL-1 β secretion. *Cytokine Growth Factor Rev* 22: 189-195, 2011.
17. Bhaskar V, Yin J, Mirza AM, Phan D, Vanegas S, Issafras H, Michelson K, Hunter JJ and Kantak SS: Monoclonal antibodies targeting IL-1 beta reduce biomarkers of atherosclerosis in vitro and inhibit atherosclerotic plaque formation in Apolipoprotein E-deficient mice. *Atherosclerosis* 216: 313-320, 2011.
18. Rus HG, Vlaicu R and Niculescu F: Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. *Atherosclerosis* 127: 263-271, 1996.
19. Stenvinkel P, Barany P, Heimbürger O, Pecoits-Filho R and Lindholm B: Mortality, malnutrition, and atherosclerosis in ESRD: What is the role of interleukin-6? *Kidney Int Suppl*: 103-108, 2002.
20. Seino Y, Ikeda U, Ikeda M, Yamamoto K, Misawa Y, Hasegawa T, Kano S and Shimada K: Interleukin 6 gene transcripts are expressed in human atherosclerotic lesions. *Cytokine* 6: 87-91, 1994.
21. Skoog T, Dichl W, Boquist S, Skoglund-Andersson C, Karpe F, Tang R, Bond MG, de Faire U, Nilsson J, Eriksson P and Hamsten A: Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *Eur Heart J* 23: 376-383, 2002.
22. Suarez EC, Lewis JG and Kuhn C: The relation of aggression, hostility, and anger to lipopolysaccharide-stimulated tumor necrosis factor (TNF)-alpha by blood monocytes from normal men. *Brain Behav Immun* 16: 675-684, 2002.
23. Zhou J, Ma W, Wang X, Liu H, Miao Y, Wang J, Du P, Chen Y, Zhang Y and Liu Z: Matrine suppresses reactive oxygen species (ROS)-mediated MKKs/p38-induced inflammation in oxidized low-density lipoprotein (ox-LDL)-stimulated macrophages. *Med Sci Monit* 25: 4130-4136, 2019.
24. Zhong W, Peng J, He H, Wu D, Han Z, Bi X and Dai Q: Ki-67 and PCNA expression in prostate cancer and benign prostatic hyperplasia. *Clin Invest Med* 31: E8-E15, 2008.
25. Kayaselçuk F, Zorludemir S, Gümürdühü D, Zeren H and Erman T: PCNA and Ki-67 in central nervous system tumors: Correlation with the histological type and grade. *J Neurooncol* 57: 115-121, 2002.
26. Reed JC: Proapoptotic multidomain Bcl-2/Bax-family proteins: Mechanisms, physiological roles, and therapeutic opportunities. *Cell Death Differ* 13: 1378-1386, 2006.
27. Zhang LP, Jiang JK, Tam JW, Zhang Y, Liu XS, Xu XR, Liu BZ and He YJ: Effects of matrine on proliferation and differentiation in K-562 cells. *Leuk Res* 25: 793-800, 2001.
28. Lawrence T: The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol* 1: a001651, 2009.
29. Moynagh PN: The NF-kappaB pathway. *J Cell Sci* 118: 4589-4592, 2005.
30. Liu J, Zhang L, Ren Y, Gao Y, Kang L and Lu S: Matrine inhibits the expression of adhesion molecules in activated vascular smooth muscle cells. *Mol Med Rep* 13: 2313-2319, 2016.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.