DANSHENSU MITIGATES OXIDIZED LOW-DENSITY LIPOPROTEIN-INDUCED HUMAN VASCULAR ENDOTHELIAL CELL INJURY THROUGH ACTIVATION OF P38/JNK SIGNALING

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ABSTRACT

Introduction*: This study investigated the effect of Danshensu (DSS) on oxidized low-density lipoprotein (ox-LDL) induced injury of cultured human umbilical vein endothelial cells (HUVEC).*

Materials and methods*: The results showed that ox-LDL suppressed HUVEC viability, induced apoptosis and upregulated phosphorylation of p38 MAPK and c-Jun N-terminal kinase (JNK). DSS showed a protective effect against ox-LDL induced loss in cell viability and an increase in apoptosis. Furthermore, the suppressed cell viability and increased apoptosis induced by ox-LDL could be rescued by inhibitor of p38 (SB203580) and JNK (SP600125), which can effectively inhibit the protective effect of DSS on cell viability loss and apoptosis induced by ox-LDL. In vivo experiment with genetically deficient apolipoprotein E (ApoE) male mice of C57BL/6J strain (Apo E-/-) was also conducted which received DSS.*

Results*: The aorta was harvested for hematoxylin and eosin staining and the result showed that DSS treatment ameliorated atherosclerotic lesions. Moreover, Western blot results revealed that DSS inhibits injury to HUVEC induced by ox-LDL, and the expression of p38 and JNK in apo E-/- mice was also deregulated.*

Conclusion*: Collectively, we suggested that the protective effect of DSS against ox-LDL induced HUVEC apoptosis might, at least in part, be obtained via inhibition of the p38/JNK signaling pathway.*

Keywords: *Danshensu, human umbilical vein endothelial cells, oxidized low-density lipoprotein.*

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Introduction

Dysfunction of vascular endothelial cells (EC) is now believed to play an important role in the pathogenesis of atherosclerosis (AS)[(1)]. A number of studies have shown that EC apoptosis functions as an initiating step for AS by inducing atherosclerotic lesion formation and plaque shedding[(2,3)]. Oxidized low-density lipoprotein (ox-LDL), an essential atherosclerotic risk factor, has been found to play a crucial role in multiple functional alternations occurring during the pathogenesis of AS, including enhancing EC apoptosis[(4, 5)].

Therefore, inhibition of ox-LDL induced EC apoptosis may have therapeutic significance in the prevention and treatment of AS.

The mechanism of ox-LDL induced apoptosis was thought to be related to ox-LDL induced phosphorylation of mitogen-activated protein kinase (MAPK) family members p38 and JNK in endothelial cells[(7-10)]. Danshensu(3-(3, 4-dihydroxyphenyl)-2-hydroxy-propanoic acid, DSS) is a main water-soluble bioactive substance isolated from Danshen (Salvia miltiorrhiza), a famous Chinese herb which has been widely used in both Asia and western countries in treating atherosclerosis and its

related disorders such as cerebrovascular diseases, hyperlipidemia, and coronary artery disease[11, 12]. Previously, studies revealed that DSS suppresses the progression of atherosclerosis by inhibiting the apoptosis of vascular smooth muscle cells and the proliferation and migration of macrophages induced by ox-LDL[13-15,16,17]. However, the protective effect of DSS against ox-LDL induced HUVEC cytotoxicity is poorly understood.

In this study, we used human umbilical vein endothelial cells (HUVECs) to investigate the effects and the relevant underlying mechanisms of DSS on ox-LDL induced cytotoxicity in HUVECs.

Materials and methods

Cell Culture

Human umbilical vein endothelial cells (HUVEC) were purchased from the Chinese Academy of Sciences, Shanghai, China. Cells were cultured in RPMI 1640 medium supplemented with 10% FBS and maintained a t37 °C, 5% CO_2. The culture medium was replaced every 2-3 days. Cultured cells were randomly chosen for the various experimental treatments in the culture medium with 10% FBS and assays.

Animals

Male ApoE deficient mice (ApoE-/-) were originally from Cavens Animal co. (Changzhou, China, SCXK (su): 2011-0003). ApoE-/- mice were used to explore the potential mechanism of DSS in the recent study because they spontaneously develop hypercholesterolemia and atherosclerotic lesions which were similar to humans. This study was approved by the Committee for Ethics in Animal Experimentation of the School of Traditional Chinese Medicine, Southern Medical University. All mice were (male, 6 weeks old) were kept in micro isolator cages on a 12-hour day/night cycle. The feed was purchased from the Southern Medical University Laboratory Animal Center, N 0:0005868, in the SPF room of Guangzhou Southern Medical University Laboratory, the room temperature of the ApoE gene knockout (ApoE-/-) mice was 18 °C to 26 °C, the flight duration was 12 h, the relative humidity was about 55% , the feeding cages and water bottles were disinfected regularly during the experiment. The mice were randomly divided into 3 groups, DSS group, ox-LDL group, and control group. The high-fat diet consists with standard diet + 0.15% cholesterol+21% lard. The ApoE-/- mice were all fed a high-fat diet and then randomly divided into 5 groups (6 mice/group), model group, ox-LDL 4mg/kg group, DSS2.5mg/kg group, DSS 5mg/kg group and DSS7.5 mg/kg group. After 12 weeks of ingesting a high-fat diet, ox-LDL and DSS group were intraperitoneal injected under tongue with 4mg/kg ox-LDL while model group not. The mice in the control group received daily intraperitoneal saline injection, and mice in the three DSS groups were treated with intraperitoneal2.5 mg/kg, 5 mg/kg, and 7.5mg/kg DSS injection each day, respectively.

After the blood samples were obtained by Enucleation, the blood was allowed to coagulate naturally at room temperature for 30 min, then centrifuged for 10 min at 8000 R/min, the supernatant was removed and then stored in-20 °C refrigerator, after the ApoE-/-mice were killed, the heart and blood vessels were perfused with PBS buffer, 4% paraformaldehyde, for about 5 minutes until the blood was washed clean and the liver became Pale and unelastic, then the thoracic aorta was isolated by opening the chest cavity of the mice. In the experiment, the Thoracic Aorta was cut about 0.5 cm near the root of the Aorta. Finally, a portion of the tissue was stored in a refrigerator at 4 °C with a timely preparation of 4% paraformaldehyde fixative solution. On the following day, the tissue was first treated with a replacement buffer solution, followed by dehydration, translucency, and wax immersion followed by conventional methods to prepare pathological sections. Note: When Making a serial section, the section thickness is about 5 m and placed under the microscope for selective section, each mouse section is about 50 pieces.

Lipid Accumulation Assessment

ELISA kits (Yingsi, Guangzhou, China) were employed to determine the level of HDL-c, LDL-c, TG, and serum TC. The results were analyzed by an automated analyzer. The upper segment of the aorta of the mouse livers were sequentially fixed, dehydrated, infiltrated first, and then slices were cut intermittently and uniformly with a slice thickness of 5mm followed by staining with hematoxylin-eosin (HE) (18), this was used to observe changes in the histopathology of the Aorta in mice. A portion of the fresh tissue was kept in a refrigerator at-80 °C for further testing by Western blotting.

Hematoxylin and Eosin Staining and Pathological Morphology

Immunohistochemical staining is based on the

principle of a specific combination of antigen and antibody. Through chemical reaction, the developer of labeled antibody, Fluorescein, enzyme and so on can be used to determine the antigen and protein in tissue cells.

One slice was taken from an interval of 5 slices and stained with HE. Three sections from each mouse sample were randomly chosen. Three fields in each slice were randomly selected for atherosclerotic lesion measurement using Image J software (National Institutes of Health, Bethesda, Md).

Western Blotting Analysis

Briefly, the aorta was cut into pieces on ice, then the protein extracts were collected after centrifugation at 8000 rpm at 4 ºC. Protein concentrations were measured by the Lowry protein assay (Bio-Rad, Hercules, CA, USA). Equal concentrations of the protein extracts were subjected to sodium dodecyl sulfate-polyacrylamide (12%) gel electrophoresis and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA) by electroblotting. The membrane was blocked with 1% BSA and subsequently incubated with anti-p38/anti-JNK antibodies(Abcam) (The antibodies come from Cell Signaling Technology, an American company) at 4°C overnight. The visualization and densitometric analysis of bands were performed by ECL Western blotting detection reagents (Amersham) and Image J software (National Institutes of Health, Bethesda, Md). β-actin was used as the internal control.

Statistical analysis

The experimental results were analyzed with SPSS 13.0 software. One-way ANOVA was used to analyze the variance or student t-test, and Post Hoc Bonferroni or Turkey test was used to compare the two groups. The data were expressed as mean ± SD ($p < 0.05$).

Results

Effects of ox-LDL induced cytotoxicity in HUVECs

We first examined the cytotoxicity of ox-LDL on HUVECs. Our results showed that incremental doses of ox-LDL treatment for 24 h significant affect HUVECs cell viability in a dose dependent manner at the concentration of 0.25 mg/L, 50 mg/L, 100 mg/L ($p < 0.05$ vs. control) (Figure 1A). Meanwhile, we found ox-LDL at 50 mg/L lowered the ox-LDL-caused decrease in viability in a time dependent manner at 3 h, 6 h, 12 h, 24 h(Figure 1B).

Apoptosis of HUVECs was analyzed by flow cytometry. As shown in Figure 1C, treatment of HUVECs with different concentration of ox-LDL for 24 h increased the apoptotic cell population up to 24.7%. Furthermore, ox-LDL at 50 mg/L significantly lowered the apoptosis of HUVECs at 3 h, 6 h, 12 h, 24 h, respectively (Figure 1D).

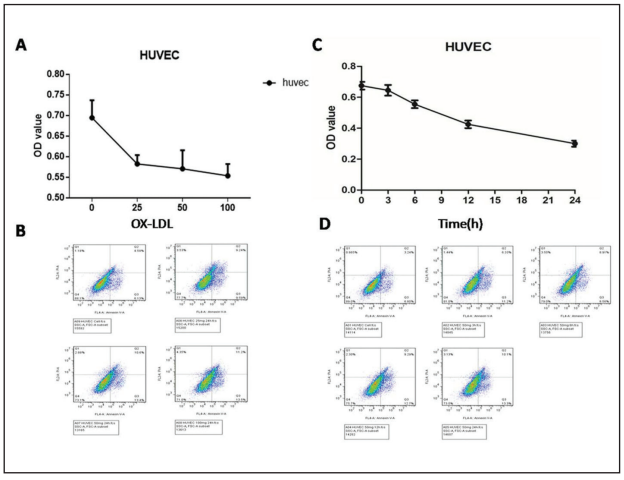

Figure 1: The cytotoxicity of ox-LDL on HUVECs (**A**) Effect of ox-LDL on cell viability of HUVEC is in a dose dependent manner; (**B**) Different concentration of ox-LDL increased the apoptotic of HUVECs (24h, FACS assay); (**C**) The decreased viability induced by ox-LDL is in a time dependent manner (50 mg/L); (**D**) Treatment of ox-LDL (50 mg/L) inhibited the viability in a time dependent manner (FACS assay).

Effect of ox-LDL induced phosphorylation in HUVECs

It has been described that phosphorylation of p38 and JNK was strongly related to ox-LDL induced apoptosis in endothelial cells[7-10]. Hence, we wanted to explore the effect of ox-LDL on the expression level of p38 and JNK. We observed that incremental level of ox-LDL induced upregulation of p-p38 and p-JNK. Moreover, the results also showed ox-LDL treatment at 50 mg/L significantly increased the level of p-p38 and p-JNK in a time dependent manner (Figure 2B).

Effect of DSS on ox-LDL induced cell viability and apoptosis in HUVECs

We performed MTT and FACS assay to investigate whether DSS play a vital role against the reduced viability and promoted apoptosis induced by ox-LDL. The results showed that ox-LDL reduced cell viability was slightly rescued by the treatment with DSS (Figure 3A), while ox-LDL induced

apoptosis was obviously reduced by the treatment with DSS in a dose dependent manner (Figure 3A).

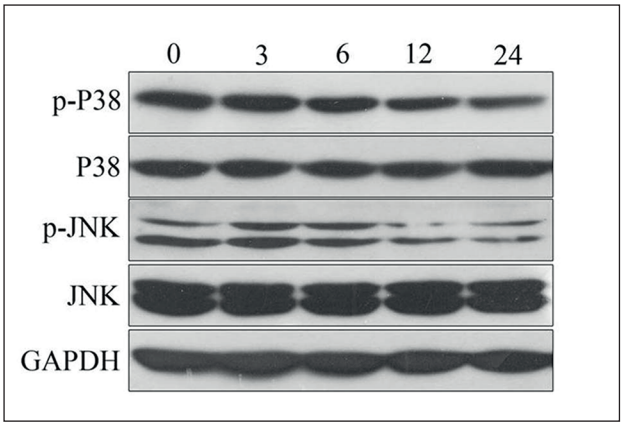

Figure 2: Ox-LDL induced phosphorylation of HUVECs. Ox-LDL treatment at 50 mg/L significantly increased the level of p-p38 and p-JNK in a time dependent manner. .

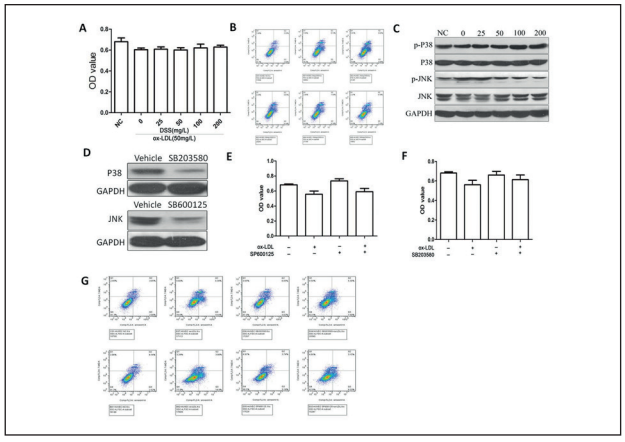

Figure 3: Protective effect of DSS against ox-LDL-induced apoptosis (**A**) Ox-LDL reduced cell viability was rescued by DSS in HUVECs which is in a dose dependent manner; (**B**) Protective effect of DSS against ox-LDL-induced apoptosis is in a dose dependent manner (FACS assay); (**C**) Western blot result of different concentration DSS against ox-LDL (50mg/L); (**D**) SB253080/SB600125 represses the expression of P38/JNK; (**E,F**) Inhibitor of p38 and JNK rescued increased apoptotic induced by ox-LDL (MTT); (**G**) Inhibitor of p38 and JNK rescued the ox-LDL induced apoptotic (FACS assay).

p38 MAPK inhibitor (SB-203580) and a JNK inhibitor (SP-600125) were used to determine whether the inhibitory effect of DSS on cell viability and apoptosis induced by ox-LDL is regulated through p38 and JNK-dependent signaling pathways. Cells were preincubated with or without ox-LDL for 24 h, and then treated with or without SB-203580 or SP-600125 for 15 min. Interestingly, in the presence of p38 and JNK inhibitor, ox-LDL induced cell viability decrease and apoptosis increase was attenuated (Figure 3C, D).

These findings indicate that the p38 and JNK-dependent signaling pathway is involved in ox-LDL induced apoptosis in endothelial cells.

Effect of DSS on Atherosclerotic Lesions in the Aorta

Serum analysis was performed to further ascertain the effect of DSS on atherosclerotic lesions. First, serum analysis of ApoE-/- mice with LDL showed increased level of TC (23.18±3.27 vs. 2.61±0.31, $p<0.01$), TG (2.43±0.65 vs. 1.67±0.56, $p<0.05$), LDL-C (5.07±0.85 vs. 0.77±0.37, $p<0.01$), while level of HDL-C (1.05±0.26 vs. 1.85±0.56, $p<0.05$) were decreased, compared with vehicle group. Compared with LDL group, administration of 2.5 mg/kg DSS showed slightly decrease of TC, TG, LDL-C and HDL-C, but not with statistical significance, while the administration of DSS (5 mg/kg, 7.5 mg/kg) resulted in a significant decrease of serum TC, TG and LDL-C levels and increase of HDL-C in a dose-dependent manner.

Hematoxylin and eosin staining showed that mice in LDL group showed rendered aorta atherosclerosis changes with aorta atherosclerotic plaque formation, thickened intima and smooth muscle cell hypertrophy. Compared with the vehicle group, the size of the atherosclerosis was slightly reduced after treatment with 2.5 mg/kg DSS and significantly repressed with 5 mg/kg and 7.5 mg/kg DSS which suggested the inhibition of DSS was in a concentration-dependent manner (Figure 4A).

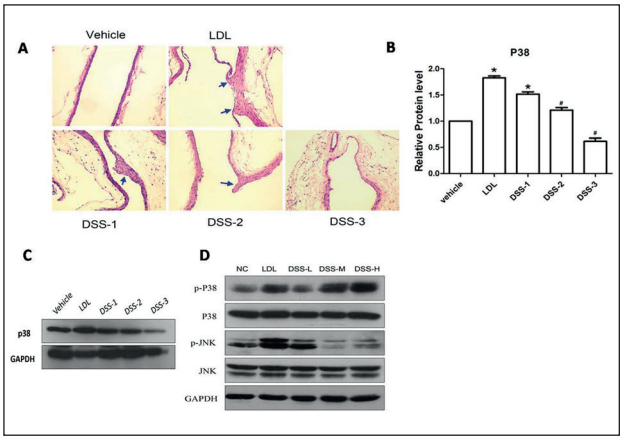

Figure 4: Effect of DSS on Atherosclerotic Lesions of ApoE-/- mice (**A**) Hematoxylin and eosin staining of aorta atherosclerosis; (**B,C**) DSS rescued the increased expression of p38 induced by ox-LDL; (D) p-p38 and p-JNK expression exhibited a downward trend with treatment of DSS.

Aortic p38 and JNK protein expression was further detected by Western blot. The results showed

that the level of p-p38 and p-JNK was significantly greater in LDL group than that of the vehicle group (Figure 4B). After DSS treatment, p-p38 and p-JNK expression exhibited a downward trend in a dose dependent manner with statistical significance (Figure 4C).

Discussion

Atherosclerosis is a chronic, progressive cardiovascular disease, characterized by endothelial cell injury and dysfunction as an early marker. Specifically, MAPK phosphorylation and endothelial cell apoptosis are reported to be a key event in the pathogenesis of arteriosclerosis[19-21]. Accumulating evidence demonstrated that ox-LDL, a potent oxidative stress factor-induced endothelial cell apoptosis, plays vital roles in the pathogenesis of atherosclerosis via repressing vascular integrity, promoting deposition of lipids, migration of monocytes, and formatting atherosclerotic plaque[22,23]. Therefore, suppressing ox-LDL induced endothelial cell apoptosis may provide a new therapeutic option for the prevention and treatment of atherogenesis. In this study, we employed human umbilical vein endothelial cells (HUVECs) as an in vitro study model.

Previous studies reported that ox-LDL could induce apoptosis in endothelial cells through activating caspase-9 and caspase-3[20,24,25]. In this study, we also confirmed that ox-LDL could induce apoptosis in a dose-dependent manner. JNK and p38 activation are thought to be involved in pro-apoptotic pathways. And studies have reported that ox-LDLcould induce rapid p38 phosphorylation in human coronary artery endothelial cells and significantly upregulate JNK phosphorylation in endothelial cells[9]. In the present study, we found that ox-LDL not only induced JNK phosphorylation but also led to p38 activation in a dose-dependent manner. In our study, the exposure of HUVEC to a specific p38 and JNK inhibitor prevented the increase in ox-LDL induced apoptosis, which suggests that ox-LDL induced p38 and JNK activation was a major target for apoptosis.

The extract of S. miltiorrhiza (ESM), which contains DSS has been proven clinically effective for the prevention and treatment of atherosclerosis. Previous study has demonstrated that DSS can inhibit tumor necrosis factor (TNF-a) induced endothelial permeability. In this study, we found that the ox-LDL was able to induce expression of phosphorylated p38 and JNK. Furthermore, ox-LDL showed a marked effect on HUVECs apoptosis. Interestingly, we found that DSS was able to modulate the expression of p-p38 and p-JNK, which led to its protective effect against ox-LDL induced apoptosis in HUVECs. Reduction of serum cholesterol level has been proven as an effective way to prevent atherosclerosis development. In vivo study further demonstrated that in serum of ApoE-/- mice TC, TG and LDL-C levels were reduced by DSS in a dose-dependent manner, which also ameliorated the lension of Atherosclerosis. DSS also reduced the level of the expression of phosphorylated p38 and JNK ox-LDL in LDL mice.

Conclusion

In summary, our study provides a new insight into the anti-atherogenic properties of DSS reducing lipid accumulation via inactivating p38 and JNK signaling pathway. DSS might also affect atherosclerosis through other mechanisms. The findings of this study provide a novel explanation for DSS's anti-atherogenic action and suggest that DSS could be a potential therapeutic intervention in anti-atherosclerosis therapy.

References

1) Libby P. Inflammation in atherosclerosis. Nature. 2002; 420: 868-874.
2) Durand E, Scoazec A, Lafont A, Boddaert J, Al Hajzen A, Addad F, Mirshahi M, Desnos M, Tedgui A, Mallat Z. In vivo induction of endothelial apoptosis leads to vessel thrombosis and endothelial denudation: a clue to the understanding of the mechanisms of thrombotic plaque erosion. Circulation. 2004; 109: 2503-2506.
3) Wang N, Han Y, Tao J, Huang M, You Y, Zhang H, Liu S, Zhang X, Yan C. Overexpression of CREG attenuates atherosclerotic endothelium apoptosis via VEGF/PI3K/AKT pathway. Atherosclerosis. 2011; 218: 543-551.
4) Chang HC, Chen TG, Tai YT, Chen TL, Chiu WT, Chen RM. Resveratrol attenuates oxidized LDL-evoked Lox-1 signaling and consequently protects against apoptotic insults to cerebrovascular endothelial cells. J Cereb Blood Flow Metab. 2011; 31: 842-854.
5) Mitra S, Deshmukh A, Sachdeva R, Lu J, Mehta JL. Oxidized low-density lipoprotein and atherosclerosis implications in antioxidant therapy. Am J Med Sci. 2011; 342: 135-142.
6) Wu CY, Tang ZH, Jiang L, Li XF, Jiang ZS, Liu LS. PCSK9 siRNA inhibits HUVEC apoptosis induced by ox-LDL via Bcl/Bax-caspase9-caspase3 pathway. Mol Cell Biochem. 2012; 359: 347-358.
7) Takahashi M, Okazaki H, Ogata Y, Takeuchi K, Ikeda U, Shimada K. Lysophosphatidylcholine induces apoptosis in human endothelial cells through a p38-mitogen-

activated protein kinase-dependent mechanism. Atherosclerosis 2002; 161: 387-394.
8) Choi JS, Choi YJ, Shin SY, Li J, Kang SW, Bae JY, Kim DS, Ji GE, Kang JS, Kang YH. Dietary flavonoids differentially reduce oxidized LDL-induced apoptosis in human endothelial cells: role of MAPK- and JAK/STAT-signaling. J Nutr 2008; 138: 983-990.
9) Yin Y, Liu W, Ji G, Dai Y. The essential role of p38 MAPK in mediating the interplay of oxLDL and IL-10 in regulating endothelial cell apoptosis. Eur J Cell Biol 2013; 92: 150-159.
10) Yu J, Wang L, Akinyi M, Li Y, Duan Z, Zhu Y, Fan G. Danshensu protects isolated heart against ischemia reperfusion injury through activation of Akt/ERK1/2/Nrf2 signaling. Int J Clin Exp Med. 2015; 8: 14793-14804.
11) Zhou L, Zuo Z, Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol. 2005; 45: 1345-1359.
12) Cighetti G, Debiasi S, Ciuffreda P, Allevi P. Beta-ethoxyacrolein contamination increases malondialdehyde inhibition of milk xanthine oxidase activity. Free Radic Biol Med. 1998; 25: 818-825.
13) Yang GD, Zhang H, Lin R, Wang WR, Shi XL, Liu Y, Ji QL. Down-regulation of CD40 gene expression and inhibition of apoptosis with Danshensu in endothelial cells. Basic Clin Pharmacol Toxicol. 2009; 104: 87-92.
14) Guan Y, Yin Y, Zhu YR, Guo C, Wei G, Duan JL, Wang YH, Zhou D, Quan W, Weng Y, Xi MM, Wen AD. Dissection of mechanisms of a chinese medicinal formula: danhong injection therapy for myocardial ischemia/reperfusion injury in vivo and in vitro. Evid Based Complement Alternat Med. 2013; 2013: 972370.
15) Yang RX, Huang SY, Yan FF, Lu XT, Xing YF, Liu Y, Zhao YX. Danshensu protects vascular endothelia in a rat model of hyperhomocysteinemia. Acta Pharmacol Sin. 2010; 31: 1395-1400.
16) Wang L, Bao Y, Yang Y, Wu Y, Chen X, Si S, Hong B. Discovery of antagonists for human scavenger receptor CD36 via an ELISA-like high-throughput screening assay. J Biomol Screen. 2010; 15: 239-250.
17) Ma KL, Ruan XZ, Powis SH, Chen Y, Moorhead JF, Varghese Z. Inflammatory stress exacerbates lipid accumulation in hepatic cells and fatty livers of apolipoprotein E knockout mice. Hepatology. 2008; 48: 770-781.
18) Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004; 109: III27-32.
19) Rahaman SO, Lennon DJ, Febbraio M, Podrez EA, Hazen SL, Silverstein RL. A CD36-dependent signaling cascade is necessary for macrophage foam cell formation. Cell Metab. 2006; 4: 211-221.
20) Jeong YJ, Choi YJ, Kwon HM, Kang SW, Park HS, Lee M, Kang YH. Differential inhibition of oxidized LDL-induced apoptosis in human endothelial cells treated with different flavonoids. Br J Nutr. 2005; 93: 581-591.
21) Boronkai A, Bellyei S, Szigeti A, Pozsgai E, Bognar Z, Sumegi B, Gallyas F Jr. Potentiation of paclitaxel-induced apoptosis by galectin-13 overexpression via activation of Ask-1-p38-MAP kinase and JNK/SAPK pathways and suppression of Akt and ERK1/2 activation in U-937 human macrophage cells. Eur J Cell Biol. 2009; 88: 753-763.
22) Su X, Ao L, Shi Y, Johnson TR, Fullerton DA, Meng X. Oxidized low density lipoprotein induces bone morphogenetic protein-2 in coronary artery endothelial cells via Toll-like receptors 2 and 4. J Biol Chem. 2011; 286: 12213-12220.
23) Shi YH. Comparative study of composite danshen droplet pills and sordi in treatment of patients with chronic stable angina. Zhongguo Zhong Xi Yi Jie He Za Zhi. 1997; 17: 23-25.
24) Ding M, Ye TX, Zhao GR, Yuan YJ, Guo ZX. Aqueous extract of Salvia miltiorrhiza attenuates increased endothelial permeability induced by tumor necrosis factor-alpha. Int Immunopharmacol. 2005; 5: 1641-1651.
25) Grundy SM. Statin trials and goals of cholesterol-lowering therapy. Circulation. 1998; 97: 1436-1439.

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