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Original Research

Decursin inhibits the oxidation of low-density lipoprotein and protects human aortic endothelial cells against oxidative damage

Xueting Wang[1], Jing Yu[1], Binlong Ji[2], Yuling Yang[1,*]

[1] Department of International medical, The First Affiliated Hospital of Harbin Medical University, Harbin, PR China
[2] Department of Emergency Medicine, the 401st Hospital of PLA, Qingdao, PR China

*Correspondence to: tkgv14@163.com

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Abstract: Atherosclerosis is the pathological basis of cardiovascular diseases (CVDs) which are the leading cause of death worldwide. The pathogenesis of atherosclerosis itself is complex. Low-density lipoprotein (LDL) oxidation has been shown to increase lipid peroxide levels in arterial wall of atherosclerosis lesion site. Decursin is a coumarin with a range of pharmacological effects. The present study investigated the inhibitory effect of decursin on LDL oxidation, and its protective effect against oxidative damage in human aortic endothelial cells (HAECs). Two models of oxidative damage were used in this study: Cu^{2+}-induced LDL oxidative damage and 2,2'-azobis-2-methyl-propanimidyl, dihydrochloride (AAPH)-induced oxidative damage of HAECs. The inhibitory effect of decursin on LDL oxidation, and its protective effect on oxidative damage of HAECs were determined. The results showed that the level of thiobarbituric acid reactive substances (TBARS) was significantly increased by Cu^{2+}, but was significantly and concentration-dependently reduced after treatment with decursin ($p < 0.05$). There were only a few viable cells in AAPH-treated group, but treatment with decursin led to significant, time- and concentration-dependent increases in their viability ($p < 0.05$). The AAPH-induced oxidative damage significantly increased the activity of lactate dehydrogenase (LDH) and level of reactive oxygen species (ROS), but significantly reduced the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) ($p < 0.05$). However, treatment of HAECs with decursin significantly and concentration-dependently reduced the LDH activity and level of ROS, but significantly increased the activities of SOD and GPx, concentration-dependently ($p < 0.05$). It also significantly and concentration-dependently reduced apoptosis induced by AAPH ($p < 0.05$). These results show that decursin effectively inhibits LDL oxidation induced by Cu^{2+}, and protects HAECs from oxidative damage caused by AAPH *in vitro* via a mechanism involving activation of SOD and GPx.

Key words: Decursin; Oxidative damage; Low-density lipoprotein; Aortic endothelial cells; Antioxidant enzyme.

Introduction

Cardiovascular diseases (CVDs) are characterized by high incidence of mortality, when compared with tumors (1). They affect the quality of life of sufferers. Atherosclerosis is the pathological basis of cardiovascular and cerebrovascular diseases (2). The pathogenesis and treatment of these diseases have been exhaustively worked on. Injury to vascular endothelial cells is responsible for the initiation and progression of atherosclerosis (3). Oxidized low density lipoprotein (ox-LDL) has been implicated in the pathogenesis of atherosclerosis, and it links atherosclerosis with endothelial cell injury (4). On formation, ox-LDL directly damages vascular endothelial cells and promotes monocytes infiltration; it increases the migration and adhesion of monocytes, and promotes the synthesis of sticky cell surface glycoprotein. These lead to increases in lipid peroxide levels in the arterial wall of the atherosclerosis lesion site. A large number of mononuclear macrophages accumulate lipids and convert them to foam cells which promote the formation of atherosclerosis.

Decursin, a coumarin isolated from *Angelica sinensis* possesses a range of pharmacological properities (5). It has been shown to be effective against leukemia, diabetes mellitus, hypertension, nephrotoxicity and some tumors (6-8). It also possesses antioxidant effect. Superoxide anion and hydroxyl radical have strong oxidative abilities, and readily oxidize biological macromolecules such as carbohydrates, amino acids, proteins, nucleic acids and lipids, resulting in cell apoptosis (9, 10). Reports on the inhibitory effect of decursin on LDL oxidation and atherosclerosis formation are scanty. The present study investigated the inhibitory effect of decursin on LDL oxidation, and its protective effect against oxidative damage in HAECs.

Materials and Methods

Cell lines, drugs and reagents

Human aortic endothelial cells (HAECs) were obtained from Shanghai Cell Library of Chinese Academy of Sciences; LDL was purchased from Shanghai JingKeHuaXue Science and Technology Co., Ltd., while dimethyl sulfoxide (DMSO) and propidium iodide (PI) were products of Sigma-Aldrich, USA). Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco BRL Co. (USA). 3-(4,5-Dimethylthiazol-

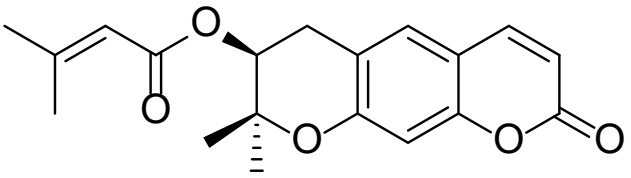

Figure 1. Chemical structure of decursin.

2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit, and trypsin were purchased from Amresco (USA). Annexin V-fluorescein isothiocyanate (FITC)/PI kit was a product of Blender Medsystems Co. (Australia). Bicinchoninic acid (BCA) protein kit was purchased from Pierce (USA). Lactate dehydrogenase (LDH), SOD, and GPx kits were products of Nanjing Jiancheng Bioengineering Institute. All the reagents used were of analytical grade.

Copper II ion (Cu^{2+})- induced LDL oxidation

The LDL oxidation reaction system consisted of 100 µg/mL LDL and 10 µM, $CuSO_4$. The mixture was reacted at 37°C in the dark. The oxidation reaction was terminated on addition of mixed solutions of 1 mmol/L EDTA and 1 mmol/L BHT in equal volumes (11). Then, graded concentrations of decursin (10 - 100 µmol/L) were added to the reaction mixture to assess its inhibitory effect on LDL oxidation. A reaction mixture without $CuSO_4$ and decursin served as control. In the model group, only $CuSO_4$ was used. The level of LDL oxide was determined using TBARS method.

Determination of level of TBARS

The levels of LDL oxide were determined using TBARS method (12). A 1-mL solution of LDL (100 mg/mL) was thoroughly mixed with 10 µM $CuSO_4$, and oxidized at 37°C for 4 h in the dark with or without the addition of 20 µL of graded concentration of decursin (10 - 100 µmol/L). The reaction was terminated through sequential addition of 1 mmol/L EDTA, 1 mL 20 % acetic acid and 1 mL 0.67 % TBA, followed by heating at 95°C for 30 min. The reaction mixture was thereafter cooled to room temperature and centrifuged at 1,500 rpm for 10 min to obtain the supernatant. The absorbance of the supernatant was read at 532 nm. The standard used was 10 µmol/L 1,1,3,3-tetraethoxysilane (TEP) which was treated following the same procedure. The level of TBARS was then calculated.

Cell culture

The HAECs were cultured in DMEM medium supplemented with 15 % fetal bovine serum (FBS), 20 U/mL fibroblast growth factor (FGF) and 1 % penicillin/streptomycin solution at 37°C for 24 h in a humidified atmosphere of 5 % CO_2 and 95 % air. After attaining 60-70 % confluency, the cells were treated with serum-free medium and graded concentrations of decursin (10-100 µmol/L) for 24 h. Normal cell culture without decursin served as control group. Cells in logarithmic growth phase were used in this study.

MTT assay

The viability of HAECs in the presence of decursin was assessed using MTT assay. The cells were seeded at a density of 1 x 10^6 cells/well in 96-well plates and cultured in DMEM for 24 h. Then, decursin (10-100 µmol/L) was added to the cells and incubated for 72 h. At the end of the third day, 20 µL of MTT solution (5 mg/mL) was added to the wells, followed by incubation at 37°C for 2 h. The medium was finally replaced with 150 µL of 0.1 % DMSO to completely dissolve the formazan crystals formed. The model stimulation group was treated with AAPH (2 mmol/L) only. The cells were then cultured at different time points (24, 36 and 48 h). The absorbance of the samples was read in a microplate reader at 570 nm. The assay was performed in triplicate. Cell viability was calculated as shown in Equation 1:

$$Cell\ viability\ (\%) = \frac{Absorbance\ of\ the\ experimental\ group \times 100}{Absorbance\ of\ the\ control\ group} \quad (1)$$

Determination of ROS level

The levels of ROS in HAECs were determined using DCFH-DA assay. The cells at a density of 2 × 10^5 cells/mL were treated with decursin at doses of 2 and 10 µmol/L, and were then washed with phosphate-buffered saline (PBS) after an initial incubation for 72 h. Then, 10 µM solution of DCFH-DA was added to the plates and incubated for another 30 min at 37°C. Thereafter, the cells were washed with PBS and injected into the flow cytometer for analysis.

Determination of LDH activity

The cells were trypsinized with 0.25 % trypsin and the resultant cell suspension was centrifuged at 3000 rpm for 10 min to obtain a supernatant. The activity of LDH in supernatant was determined using LDH assay kit.

Determination of activities of SOD and GPx

The cells were seeded in 6-well culture plates at a density of 2 × 10^5 cells/mL and lysed with ultrasonic wave in an ice bath and the protein concentration was determined using BCA method. The activities of SOD and GPx in the lysate were determined using their respective assay kits.

Apoptosis assay

The cells were seeded at a density of 1 x 10^5 cells/L into 6-well plates and cultured for 24 h. Then, decursin at doses of 2 and 10 µmol/L, was added to the medium and incubated for another 72 h, and thereafter washed with PBS, and thoroughly mixed with 300 µL binding buffer. The cells were then stained with 5 µL each of annexin V-fluorescein isothiocyanate and propidium iodide within 25 min at room temperature in the dark. Cell apoptosis was assessed using a flow cytometer fitted with argon laser operated at 485 nm.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using Graphpad Prism (7.0). Groups were compared using Student *t*-test. Values of $p < 0.05$ were considered statistically significant.

Results

Effect of decursin on Cu^{2+}-induced LDL oxidation

As shown in Figure 2, the level of TBARS was si-

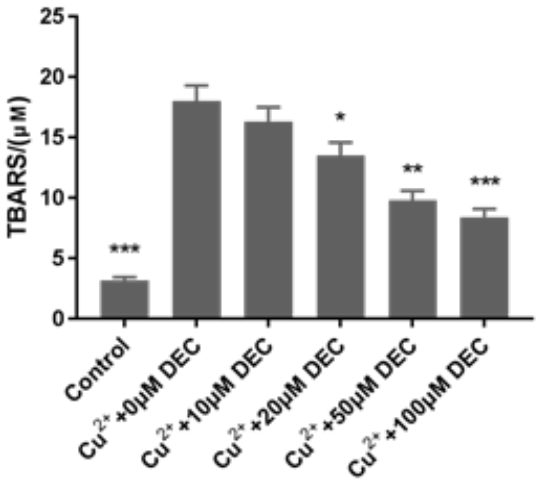

Figure 2. Inhibitory effect of decursin on Cu^{2+}- induced LDL oxidation *in vitro*. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, when compared with Cu^{2+} control group.

gnificantly increased by Cu^{2+}, but was significantly and concentration-dependently reduced after treatment with decursin ($p < 0.05$).

Effect of decursin on AAPH-induced oxidative damage in HAECs

There were only a few viable cells in AAPH-treated group, but treatment with decursin led to significant , and time- and concentration-dependent increases in their viability (Figure 3).

Effects of decursin on ROS level, and activities of LDH, SOD and GPx in AAPH oxidized cells

The oxidative damage induced by AAPH significantly increased LDH activity ROS level, but significantly reduced the activities of SOD and GPx ($p < 0.05$). However, treatment of HAECs with decursin significantly and concentration-dependently reduced LDH activity and ROS level, but increased the activities of SOD and GPx, significantly and concentration-dependently ($p < 0.05$). These results are shown in Figure 4.

Effect of decursin on HAEC apoptosis

Treatment of HAECs with decursin significantly and concentration-dependently reduced apoptosis induced by AAPH ($p < 0.05$; Figure 5).

Discussion

Atherosclerosis is characterized by the deposition of lipid and other blood components in the endarterium, proliferation of smooth muscle cells and increased synthesis of collagen fibers, as well as formation of porridge-like, fat-bearing necrotic lesions and vascular wall sclerosis. It is the pathological basis of CVDs, which has become the leading cause of death globally (13). The oxidation of LDL is the most important factor in arteriosclerosis (14, 15). Therefore, the prevention and control of arteriosclerosis can effectively reduce the incidence and mortality associated with CVDs. At present, the strategies employed in the treatment of CVDs only serve as mere palliatives. The cellular damage induced by LDL oxidation is due to the generation of

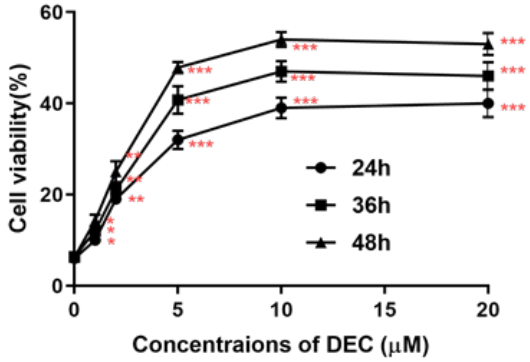

Figure 3. Effect of decursin on the viability of HAECs in the presence of AAPH. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs HAECs with out decursin.

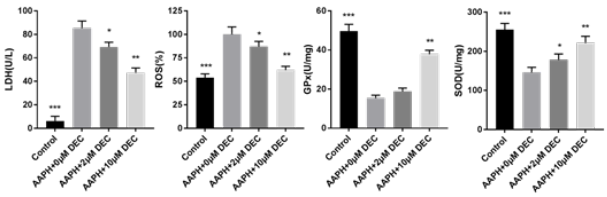

Figure 4. Effects of decursin on activities of LDH, SOD and GPx, and ROS level in HAECs $^{*}p < 0.05$; $^{*}p < 0.01$; $^{***}p < 0.001$, when compared with AAPH control group.

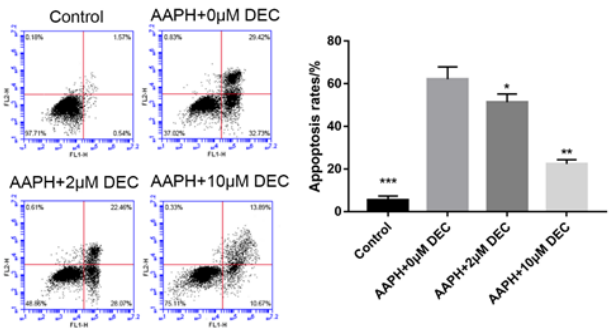

Figure 5. Effect of decursin on apoptosis induced by AAPH. $^{*}p < 0.05$, $^{**}p < 0.01$; $^{***}p < 0.001$, when compared with AAPH control group.

ROS, and is one of the key factors leading to vascular endothelial cell injury (16). The product of LDL oxidation (ox-LDL) promotes the secretion of adhesion molecules by endothelial cells and monocytes, and readily forms foam cells (17). Low-density lipoprotein (LDL) is readily oxidized by transition metal ions *in vitro*. In this study, Cu^{2+} was used to induce LDL oxidation *in vitro* to simulate the oxidative process. Being a water-soluble free radical initiator, AAPH decomposes under physiological conditions releasing free radicals, thereby inducing oxidative damage in cells. However, the rate at which free radicals are produced is regulated. Therefore, AAPH is considered an ideal model for inducing oxidative stress *in vitro*. In this study, Cu^{2+}-induced LDL oxidation and AAPH-induced free radical generation were used as models of oxidative damage.

Decursin is a coumarin isolated from Chinese *Angelica*, and possesses a range of physiological and pharmacological properties (18). The results of this study

suggest that decursin may effectively reduce the level of lipid peroxidation caused by Cu^{2+}. It is likely that decursin effectively inhibits LDL oxidation induced by Cu^{2+} *in vitro*.

Under normal physiological conditions, cells are in a state of oxidation-antioxidation equilibrium. When excessively stimulated by external oxidation factors, this equilibrium is distorted, thereby promoting oxidative stress and serious damage to the antioxidant system. The level of cellular ROS reflects the degree of oxidative stress. Superoxide dismutase (SOD) and GPx are important antioxidant enzymes, which play key roles in the maintenance of redox balance in cells (19). Lactate dehydrogenase (LDH) is a relatively stable protein which exists in the cytosol of most cells and cannot be secreted out of the cell. However, once the cell membrane is damaged, LDH is released to the outside of the cell. By determining the activity of LDH in cell culture, it is possible to determine the degree of cell damage (20). The results of this study suggest that decursin may effectively repair cell damage induced by AAPH by reducing LDH leakage in HAECs. It is possible that decursin maintains cell membrane integrity within a certain dose range. The results also suggest that decursin may enhance the activities of SOD and GPx in damaged cells, thereby increasing the ability of endothelial cells to resist oxidative damage and apoptosis induced by AAPH.

The results of this study show that decursin effectively inhibits LDL oxidation induced by Cu^{2+}, and protects HAECs from oxidative damage caused by AAPH *in vitro* via a mechanism involving activation of SOD and GPx.

Acknowledgements
None.

Conflict of Interest
There are no conflicts of interest in this study.

Author's contribution
All work was done by the author s named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. The study was conceived and designed by YulingYang; Xueting Wang, Jing Yu, Binlong Ji, YulingYang collected and analysed the data; Xueting Wang wrote the text and all authors have read and approved the text prior to publication.

References

1. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. N Engl J Med 2019; 380: 33-44.
2. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. N Engl J Med 2019; 380: 23-32.
3. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362: 801.
4. Kita T, Ishii K, Yokode M, Kume N, Nagano Y, Arai H, et al. The role of oxidized low density lipoprotein in the pathogenesis of atherosclerosis. Eur Heart J 1990; 11: 122-127.
5. Ahn KS, Sim WS, Kim IH. Decursin: a cytotoxic agent and protein kinase C activator from the root of Angelica gigas. Planta Med 1996; 62: 7-9.
6. Kang S, Lee K, Sung S, Kim Y. Coumarins isolated from *Angelica gigas* inhibit acetylcholinesterase: structure-activity relationships. J Nat Prod 2000; 49: 226-227.
7. Herath W, Reddy N, Khan I. Microbial metabolism. Part 8. The pyranocoumarin, decursin. Chem Pharm Bull (Tokyo) 2007; 55: 1512-1513.
8. Lee S, Lee YS, Jung SH, Shin KH, Kim BK, Kang SS. Anti-tumor activities of decursinol angelate and decursin from *Angelica gigas*. Arch Pharm Res 2003; 26: 727-730.
9. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 2010; 15: 7313-7352.
10. Hwang JT, Kim SH, Hur HJ, Kim HJ, Park JH, Sung MJ, et al. Decursin, an active compound isolated from *Angelica gigas*, inhibits fat accumulation, reduces adipocytokine secretion and improves glucose tolerance in mice fed a high-fat diet. Phytother Res 2012; 26: 633-638.
11. Thilakarathna SH, Rupasinghe HV, Needs PW. Apple peel bioactive rich extracts effectively inhibit in vitro human LDL cholesterol oxidation. Food Chem 2013; 138: 463-470.
12. Liu F, Ng T. Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Sci 2000; 66: 725-735.
13. Libby PJ. Changing concepts of atherogenesis. J Intern Med 2000; 247: 349-358.
14. Chait A, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am J Med 1993; 94: 350-356.
15. World Health Organization (WHO). World health statistics 2016: monitoring health for the SDGs sustainable development goals. World Health Organization 2016; 41: 293-328.
16. Pan Y, Guo C, Ma X, Wang J, Liu X, Sun M, et al. The estrogen-like protective effect of ginsenoside Rb3 on oxidative stress and dysfunction of endothelial cells induced by oxidized low-density lipoprotein. Yao Xue Xue Bao 2014; 49: 1406-1412.
17. Yang TL, Lin FY, Chen YH, Chiu JJ, Shiao MS, Tsai CS, et al. Salvianolic acid B inhibits low-density lipoprotein oxidation and neointimal hyperplasia in endothelium-denuded hypercholesterolaemic rabbits. J Sci Food Agric 2011; 91: 134-141.
18. Kim BS, Seo H, Kim HJ, Bae SM, Son HN, Lee YJ, et al. Decursin from *Angelica gigas* Nakai inhibits B16F10 melanoma growth through induction of apoptosis. J Med Food 2015; 18: 1121-1127.
19. Ighodaro O, Akinloye OJ. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med 2018; 54: 287-293.
20. Fotakis G, Timbrell JA. *In vitro* cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett 2006; 160: 171-177.