Delphinidin attenuates stress injury induced by oxidized low-density lipoprotein in human umbilical vein endothelial cells

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1. Introduction

Atherosclerosis is a complicated vascular disorder, which mainly contributes to the pathogenesis of myocardial and cerebral infarction. Apoptosis is a key factor for the pathogenesis of many disorders, including atherosclerosis and its complications [1]. In recent years, the apoptosis-related signaling pathways have been intensely studied. One central player is the Bcl-2 family of proteins, which can either promote cell survival (Bcl-2, Bcl-XL, A1, Mcl-1 and Bcl-W) or cell death (Bax, Bak, Bcl-1/X, Bad, Bid, Bik, Bim, Hrk and Bok) [2]. Besides, generation of reactive oxygen species (ROS) in vascular endothelial cells is involved in several clinical conditions associated with hypercholesteremia, atherosclerosis and disseminated intravascular coagulation [3,4]. The oxidative damage can be partially repaired by some free-radical scavengers such as flavonoids, Vitamin E and superoxide dismutase (SOD) [5–7].

Anthocyanins are known as a subclass of polyphenols that are widely distributed in food of vegetal origin. They are of great nutritional interest because of the significant daily intake, which is much higher than the intake estimated for other flavonoids [8]. It has been reported that anthocyanins have the properties against cardiovascular diseases and cancer due to their multitude biological activities [9]. The exact mechanism(s) by which they act has not been elucidated, but may be related to their peculiar chemical structure [10]. These molecules are very reactive towards ROS because their electron deficiency inhibits oxidation of low-density lipoproteins and interact with the NO-generating pathway [11].

Delphinidin is a polyphenol belonging to the group of anthocyanins (Fig. 1). Previous studies have shown that independent from the antioxidant property, this compound can induce an endothelium-dependent relaxant effect, which is related to its ability to stimulate NO production through an increase in cytosolic calcium [12]. Thus, delphinidin may have protective effects on oxidative stress-induced vascular endothelium dysfunction that significantly contributes to the development of cardiovascular diseases. While delphinidin has not been used as a medicine, research on its potential protective effects against cardiovascular diseases would be highly valuable since it is enriched in many kinds of daily food.

Human umbilical vein endothelial cells (HUVECs) are frequently used as a model to explore the mechanisms involved in the pathogenesis of cardiovascular diseases [13]. Oxidized low-density lipoprotein (oxLDL) is present in atherosclerotic areas [14], and it plays a crucial role in atherogenesis by inducing inflammation, inhibiting endothelial cell proliferation, stimulating the expression of adhesion molecules, and inducing endothelial cell apoptosis [15–17]. Recently, it has been widely employed in the studies about atherosclerosis [18]. In this study, we used HUVECs to test whether delphinidin can attenuate stress injury induced by oxLDL. For this
purpose, we measured the cell viability, the release of lactate dehydrogenase (LDH), the production of intracellular ROS, the activities of SOD, and malondialdehyde (MDA) and the NO level. Furthermore, we assessed the changes of apoptotic index and by flow cytometric analysis and the expression of Bcl-2 and Bax protein by Western blot. Our results showed that delphinidin could effectively protect HUVECs against oxidative stress induced by oxLDL.

2. Materials and methods

2.1. Chemicals and reagents

Cell culture media HyQ M199/EBSS (M199) and fetal bovine serum (FBS) were both purchased from Hyclone Laboratories (Logan, UT, USA). Dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), phosphate buffered saline (PBS), 2′,7′-dichlorofluorescin diacetate (DCFH-DA), Vitamin C were purchased from Sigma–Aldrich (St. Louis, MO, USA). Annexin V-FITC Vibrant Apoptosis Assay Kit was purchased from Invitrogen (Eugene, OR, USA). Delphinidin was obtained from Polyphenols Laboratories AS (Sandnes, Norway), which was dissolved in DMSO. To measure the levels of LDH, MDA, SOD and NO, the corresponding reagent kits were purchased from Nanjing Institute of Jiancheng Bioengineering (Nanjing, China). Mouse anti-human Bcl-2 and Bax antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals in our study were of commercial grade.

2.2. Cell culture and treatment

HUVECs were made in our lab according to Jaffe et al. [19]. The cells were cultured on gelatin-coated plastic dishes with M199 medium that was supplemented with 20% FBS at 37°C in 5% CO2/95% air (the normal group). All experiments were performed on the cells from 3 to 10 passages. When the cells reached sub-confluence, they were pre-treated for 2 h with culture medium containing different concentrations of delphinidin (25, 50, 100 and 200 μmol/L) or Vitamin C (250 μg/mL). Next, the culture supernatant was collected, and the cells were washed twice with PBS (pH 7.4). Then, the cells were exposed to oxLDL (100 μg/mL) diluted in culture medium for 24 h at 37°C until further assays.

2.3. Cell viability measurement

HUVECs were seeded at a density of 5 × 10⁴ cells/mL in 96-well plates, and the cell viability was measured with the MTT assay [20]. Briefly, at the indicated time points after the above-mentioned treatments, the culture supernatant was removed. The cells were washed with PBS and incubated with MTT (5 mg/mL) in culture medium at 37°C for another 3 h. After the MTT removal, the colored formazan was dissolved in 100 μL DMSO. The absorption values were measured at 490 nm with a Sunrise Remote Microplate Reader (Grodg, Austria). The viability of HUVECs in each well was shown as the percentage of control cells.

2.4. Intracellular ROS measurement

In our study, the relative fluorescence of growing concentrations of DCF was measured first, in order to test the sensitivity of the fluorimeter and to confirm the linearity measurement. The measurement of intracellular ROS was based on ROS-mediated conversion of non-fluorescent 2′,7′-DCFH–DA into DCFH [21,22]. The intensity of fluorescence reflected enhanced oxidative stress. After the incubation studies as described before, HUVECs, which were previously seeded in black 96-well plates, were washed with PBS (pH 7.4) and then incubated with DCFH–DA (20 μM) in PBS at 37°C for 2 h. At the end of incubation, the DCFH fluorescence of the cells from each well was measured at an emission wavelength of 530 nm and an excitation wavelength of 485 nm with a FLX 800 microplate fluorescence reader (Biotech Instruments Inc., USA). The background was obtained from cell-free conditions. The results were expressed as the percentage of control (non-stimulated HUVECs) fluorescence intensity.

2.5. Cell lysate preparation

The cells were seeded at a density of 1 × 10⁵ cells/mL in 24-well plates and were allowed to attach for 24 h before the treatments. Upon completion of the incubation studies, the culture supernatant was collected to measure the MDA, LDH and NO release. The cells were scraped from the plates into ice-cold RIPA lysis buffer (50 mM Tris with pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS and 0.05 mM EDTA) and protein concentration was determined with respect to a standard curve of NaNO2 diluted as the percentage of control cells.

2.6. Measurement of LDH and NO release

As an indicator of cell injury, LDH was detected after the exposure to oxLDL with an assay kit according to the manufacturer’s protocol. The enzyme activity was expressed as units per liter and the absorbance was measured at 440 nm. Nitric oxide generation was indirectly monitored by measuring nitrite/nitrate (NOₓ) levels, and the stable end product of NO was determined by the Griess reaction [23] and expressed as mM/L. The nitrite concentration was determined with respect to a standard curve of NaNO2 diluted in saline solution. The absorbance was measured by a microplate reader at 490 nm. Nitrite concentrations were calculated by comparing nitrite levels in the culture medium with those from the standard curve, and the reported NOₓ concentrations were normalized in term of 10⁶ cells to correct for the growth rate difference between the samples.

2.7. Assay for intracellular contents of SOD and MDA

The activities of SOD and the concentration of MDA were all determined by commercially available kits. All the procedures were performed according to the manufacturer’s instructions. The activities of enzymes were expressed as nanounits per milligram protein. The assay of SOD activity was based on its ability to inhibit the oxidation of hydroxylamine by O²⁻ produced from the xanthine–xanthineoxyase system. One unit of SOD activity was defined as the amount reducing the absorbance at 550 nm by 50%. The activity of MDA was measured at a wavelength of 532 nm by reacting with thiobarbituric acid (TBA) to form a stable chromophoric production. The MDA level was expressed as nanomole per milliliter.
2.8. Flow cytometric evaluation of apoptosis

HUVECs growing in 25-cm² culture flasks were harvested, washed and double-stained by an Annexin V-FITC apoptosis detection kit. Annexin V has a strong and Ca²⁺-dependent affinity for phosphatidylserine (PS), which translocates from the internal to the external surface of the plasma membrane as a probe for detecting apoptosis [24]. Cells without membrane integrity showed red staining (propidium iodide, PI) throughout the nucleus and therefore were easily distinguished between the early apoptotic cells and the late apoptotic cells or necrotic cells. Samples were incubated at room temperature for 15 min in the dark with Annexin V and PI and quantitatively analyzed by a FACS flow cytometer [25,26].

2.9. Western blot analysis

Cells were harvested and spun down at 1000 × g for 10 min, and were lysed for 1 h in ice-cold total lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 0.5% nadeoxycolate, protease inhibitors). After quantification by the Bradford method, 50 µg protein was re-suspended in Laemmli’s buffer, separated on 12% SDS-PAGE and Western blotted. The sample loading was verified by staining membranes with Ponceau red and amidoblack. Blots were probed with anti-Bcl-2 antibodies and anti-Bax antibodies, followed by the HRP-conjugated antibody. Then, they were treated with ECL reagents for 1 min and exposed to CL-Xposure films. Finally, the blots were scanned, and densitometric analysis was performed on the scanning images with Scion Image-Release Beta 4.02 software (http://www.scioncorp.com).

2.10. Statistic analysis

Statistical analyses were performed with the SPSS package (version 10.0, SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± S.D. of 3–5 independent experiments. Statistical comparison between the control and the treatment group was carried by ANOVA and a group-by-group comparison was performed using a t-test with Tukey’s correction for multiple comparison. Differences between the groups were considered statistically significant at P < 0.05.

3. Results

3.1. The viability loss in HUVECs induced by oxLDL

We first carried out the concentration-dependent studies of viability loss in HUVECs induced by oxLDL. After treatments with increasing concentrations of oxLDL for 24 h, cell viability was examined by MTT method. As shown in Fig. 2A, cell viability gradually lost with the increase of oxLDL concentration. The degree of cell injury reached the maximum at 200 µg/mL among all the concentrations surveyed.

3.2. Effects of delphinidin on the viability of oxLDL-induced HUVECs

We evaluated the effect of delphinidin on the viability of oxLDL-treated HUVECs using the MITT analysis. As shown in Fig. 2B, the survival rate of HUVECs was about 46.16 ± 6.41% after exposure to oxLDL (100 µg/mL) for 24 h. However, pre-incubation of HUVECs with different concentrations of delphinidin (25, 50, 100, 200 µmol/L) or Vitamin C (250 µg/mL) for 2 h markedly increased the viability of oxLDL-treated HUVECs in a dose-dependent manner. The treatments with delphinidin (50, 100, and 200 µmol/L) and with Vitamin C (250 µg/mL) significantly increased the viability of HUVECs to 71.19 ± 4.09%, 75.64 ± 5.03%, 80.80 ± 5.34% and 76.73 ± 10.29%, respectively. In addition, no difference was detected in cell viability between cells treated with delphinidin (25–200 µmol/L) alone and controls (data not shown). Thus, delphinidin appeared to be effective in protecting HUVECs against oxLDL-induced injury.

To further investigate the protective effects of delphinidin, we employed LDH assay, another indicator of cell toxicity. As shown in Fig. 2C, LDH release in HUVECs was minimal in the vehicle-treated control group (173.36 ± 24.53 U/L), and there was a dramatic increase (689.69 ± 71.16 U/L) after 24 h exposure to oxLDL (100 µg/mL). HUVECs were pre-incubated with 25–200 µmol/L of delphinidin or 250 µg/mL of Vitamin C for 2 h and then exposed to 100 µg/mL of oxLDL for 24 h. Values are shown as mean ± S.D. (n = 5); and **P < 0.01 compared with the vehicle-treated control group; ***P < 0.01 compared with 24 h oxLDL-treated cells.

Fig. 2. Viability loss in HUVECs induced by oxLDL (A). HUVECs were treated for 24 h with various concentrations of oxLDL. Effects of delphinidin and Vitamin C on viability loss (B) and LDH release (C) in HUVECs after exposure to oxLDL (100 µg/mL). HUVECs were pre-incubated with 25–200 µmol/L of delphinidin or 250 µg/mL of Vitamin C for 2 h and then exposed to 100 µg/mL of oxLDL for 24 h. Values are shown as mean ± S.D. (n = 5); and **P < 0.01 compared with the vehicle-treated control group; ***P < 0.01 compared with 24 h oxLDL-treated cells.
3.3. Intracellular radical scavenging ability of delphinidin

The intracellular ROS concentration was determined by measuring the intensity of DCFH fluorescence. When DCFH-DA-labeled cells were incubated in the medium for 2h, a sudden increment in fluorescence intensity indicated the oxidation of DCFH-DA by intracellular radicals (Fig. 3A and B). The production of DCFH fluorescence in HUVECs with oxLDL significantly increased to 220.10 ± 19.10% of the vehicle-treated control group; whereas pre-incubation with delphinidin (25–200 μmol/L) significantly reduced the increased fluorescence induced by oxLDL in a concentration-dependent manner. The effect of Vitamin C (250 μg/mL) was similar to that of delphinidin.

3.4. Measurement of SOD activities and MDA content

Treatment of HUVECs with oxLDL (100 μg/mL) for 24h decreased the activities of SOD. However, pre-incubation with delphinidin (25–200 μmol/L) significantly attenuated the change of SOD activities (Fig. 4A). At 200 μmol/L of delphinidin, the oxLDL-induced decrease in SOD activities was largely restored. This concentration was the most effective among all the delphinidin concentrations tested and its effect was higher than that of Vitamin C (250 μg/mL).

In addition, HUVECs treated with 100 μg/mL oxLDL for 24h increased the intracellular MDA level; while pre-incubation of cells with 100 and 200 μmol/L of delphinidin or 250 μg/mL of Vitamin C markedly attenuated the increase, respectively (Fig. 4B).

3.5. Detection of NO release in cell culture medium in HUVECs

To determine whether delphinidin can promote the synthesis and release of NO in HUVECs, we measured the content of NO under different treatments. As shown in Fig. 4C, compared with the vehicle-treated control group, we found a significant decrease at the NO level from cell culture medium after exposure to oxLDL (100 μg/mL) for 24h; whereas pre-incubation with delphinidin
compared with 24 h oxLDL-treated cells. (

were pre-incubated with 25–200 mol/L of delphinidin or 250 μg/mL of Vitamin C for 2 h and then exposed to 100 μg/mL of oxLDL for 24 h. Values are means ± S.D. (n = 5); and **P < 0.01 compared with the vehicle-treated control group; ††P < 0.01 compared with 24 h oxLDL-treated cells.

(25–200 μmol/L) for 2 h triggered a similar increase in a dose-dependent manner.

3.6 Evaluation of oxLDL-induced apoptosis by flow cytometric analysis

To quantitatively gain insight into anti-apoptotic effects of delphinidin in oxLDL-induced HUVECs, we measured a display of PI vs. Annexin V-FITC fluorescence by flow cytometric analysis (Fig. 5A and B). In the vehicle-treated control group, 4.97 ± 1.11% cells were positive for Annexin V-FITC binding, which represented apoptotic cells. After exposure to oxLDL (100 μg/mL) for 24 h, the percentage of apoptosis increased to 58.23 ± 3.36%. However, pre-incubation with delphinidin (25–200 μmol/L) for 1 h prior to oxLDL exposure arrested the apoptosis in a dose-dependent manner, and the apoptosis value was decreased to 53.94 ± 6.69%, 42.97 ± 4.79%, 20.09 ± 3.43% and 9.69 ± 1.95%, respectively. Moreover, no difference was detected in cell viability between cells treated with delphinidin (25–200 μmol/L) alone and the controls (data not shown).

3.7 Anti-apoptotic effect of delphinidin related to Bcl-2 and Bax expression

To investigate the anti-apoptotic effects of delphinidin on oxLDL-treated HUVECs, we measured the expression of Bcl-2 and Bax proteins by Western blot. As shown in Fig. 6, compared with the vehicle-treated control group, there was a significant decrease in Bcl-2 protein expression (Fig. 6A and B) and a significant increase in Bax protein expression (Fig. 6A and C) after exposure to oxLDL (100 μg/mL) for 24 h. Again, pre-incubation with delphinidin (100 μmol/L) or Vitamin C (250 μg/mL) markedly attenuated these changes, respectively.

4. Discussion

Oxidative stress changes many functional responses of endothelial cells and therefore it is regarded as a critical pathogenic factor in the development of cardiovascular diseases [27]. Endothelial dysfunction can affect the development and stability of plaque, which is one of the most important factors in atherosclerosis [28]. Low-density lipoproteins (LDL) play a major role in the pathogenesis of atherosclerosis in which oxidative modification of LDL seems to be a key event [29]. One of the most important toxic effects of high-concentration oxLDL is cell death. Cell death induced by oxLDL can be in the form of necrosis and/or apoptosis [30]. Recent studies have indicated that oxLDL can induce apoptosis in a variety of cells, including endothelial cells [31–34]. For this reason, a lot of people have used antioxidant agents against ROS stimulus in cardiovascular diseases, which has been demonstrated in studies of sphingosine 1-phosphate, propofol, and Vitamin C [35–37]. As a kind of polyphenols, delphinidin has been shown to play positive roles for the antioxidant effects in a wide range of chemical oxidation systems [38]. Here, for the first time, we showed that it has a potential role in improving endothelial injury associated with a reduction of oxidative stress. Thus, delphinidin not only prevents oxidative injury in HUVECs, but also potently interferes with apoptosis due to attenuated exogenous oxidative stress.

In our study, exposing HUVECs to oxLDL (100 μg/mL) for 24 h showed a significant decrease in cell viability. On the other hand, the cytotoxic effect could be markedly abrogated by delphinidin pre-treatment at 50 μmol/L or higher concentrations. This result indicates that delphinidin has an ability to protect HUVECs against oxLDL-induced injury. Accumulating evidence has revealed that oxLDL can cause endothelial cell injury by inducing mitochondrial dysfunction [39]. The mitochondrial-membrane permeability transition increases the ROS formation by inhibiting respiratory chain [40]. To confirm the ability of delphinidin to scavenge ROS in a cellular environment, we pre-treated HUVECs with delphinidin of different concentrations before exposure to oxLDL. We also used non-toxic fluorescence probe DCFH-DA to assess oxidative responses since it is suitable for reliable measurement [41]. DCFH-DA is a useful indicator of ROS and oxidative stress. The non-polar and non-ionic DCFH-DA goes through cell membranes and then is hydrolyzed by intracellular esterases to non-fluorescent 2′,7′-dichlorofluorescin (DCFH). Given the presence of ROS such as hydrogen peroxide (H₂O₂), lipid hydroperoxides and peroxinitrite,
Fig. 5. Effects of delphinidin against oxLDL-induced apoptosis in HUVECs by flow cytometric analysis. HUVECs were harvested and labeled with a combination of PI and Annexin V-FITC, and analyzed by flow cytometer. The figures show representative flow cytometric histograms of the cells. HUVECs were pre-incubated with 25–200 μmol/L of delphinidin or 250 μg/mL of Vitamin C for 2 h and then exposed to 100 μg/mL of oxLDL for 24 h. Values are means ± S.D. (n = 5); and ## P < 0.01 compared with the vehicle-treated control group; ** P < 0.01 compared with 24 h oxLDL-treated cells.

DCFH are oxidized to fluorescent 2′,7′-dichlorofluorescein (DCF). The oxidation is amplified by intracellular Fe^{2+}. The reaction of H_2O_2 with Fe^{2+} can generate hydroxyl radicals (HO^−), which can oxidize DCFH. Superoxide anions do not directly oxidize DCFH, but in some biological systems, SOD transforms superoxide anions into H_2O_2 that can then oxidize DCFH. In addition, DCFH can be oxidized by intracellular oxidases and oxidants generated during the reduction of H_2O_2. The oxidation of DCFH may be derived from several ROS intermediates. Therefore, DCFH indirectly reflects the effect of intracellular antioxidant activities in scavenging ROS and protecting DCFH from oxidation. As we expected, incubation of HUVECs with oxLDL strikingly increased intracellular ROS, and it can be significantly suppressed by pre-treatment with delphinidin (50–200 μmol/L). The effects of delphinidin were similar to those of Vitamin C (250 μg/mL) in scavenging ROS in HUVECs induced by oxLDL. These observations strongly suggest that a significant inhibition of oxLDL-induced ROS production contributes to restoring the viability of HUVECs. However, it remains unclear whether delphinidin initiates a quenching effect on oxLDL-induced ROS directly or in some indirect way. Hence, further experiments are required to confirm these results. Lipid peroxidation is one of the primary events in free-radical-mediated cell injury [42]. MDA is a by-product of lipid peroxidation induced by excessive ROS, which has been widely used as a biomarker of oxidative stress [34]. On the other hand, cells are often equipped with several antioxidants to prevent free-radical damages. SOD and other enzymatic/non-enzymatic antioxidants play a pivotal role in preventing cellular damage caused by ROS [13]. Therefore, the intracellular ROS can be effectively eliminated by the combined action of SOD and other endogenous antioxidants, providing a repairing mechanism for oxidized membrane components. In this study, we observed significant decreases in SOD in HUVECs after exposure to oxLDL, indicating the impairment in antioxidant defenses. In addition, an obvious elevation of MDA production was accompanied by an increase of LDH release. Nonetheless, when HUVECs were pre-incubated with delphinidin, these oxLDL-induced cellular events...
were largely blocked. Our study also showed that in terms of protecting HUVECs against oxLDL-induced oxidative damage, the effects of delphinidin (50–200 μmol/L) and Vitamin C (250 μg/mL) were similar. Taken together, our results suggest that enhancement of endogenous antioxidant preservation and attenuation of lipid peroxidation can represent a major mechanism of cellular protection by delphinidin.

Derived from the action of NOS, NO is one of the most important mediators in regulating endothelial functions [43,44]. It is synthesized by the enzyme NOS in which NO and l-arginine are products, and l-citrulline is the substrate. There are three NOS isoforms: neuronal NOS (nNOS, NOS-1), inducible NOS (iNOS, NOS-2), and endothelial NOS (eNOS, NOS-3) [45]. Decreased NO content is associated with increased oxidative flux and therefore impaired endothelium-dependent vasodilation. Indeed, several studies have reported that down-regulation of NO production is implicated in the pathogenesis and clinical course of all known cardiovascular diseases and it is also related to future risk of adverse cardiovascular events [46,47]. Our study showed that pre-incubation with different concentrations of delphinidin further increased in the production of extracellular NO release compared with oxLDL-induction alone. Thus, delphinidin can obviously increase the NO content.

A role of ROS mediating apoptosis of endothelial cells has been documented by that the radical scavengers Vitamin C and N-acetylcysteine inhibit apoptosis of endothelial cells in response to exposure to oxidized LDL, which increases oxidative flux within endothelial cells [48] and highlights the importance of oxidative stress in induction of apoptosis [49]. Recently, oxLDL has been suggested as an inducer of apoptosis in several types of cells as a consequence of disrupting mitochondrial electron transport [50]. Our results showed that oxLDL, a major source of ROS, at 100 μg/mL, could markedly increase the permeability in HUVECs. This was demonstrated by LDH leakage, indicating the presence of apoptotic components in oxLDL-induced cell injury. Agents that inhibit production of ROS or enhance cellular antioxidant defense can prevent apoptosis and protect cells from damaging effects of oxygen radicals [51]. Consistent with these reports, we showed that pre-incubation of delphinidin with HUVECs could distinctly protect against oxLDL-induced apoptosis. Thus, we propose that the ability of delphinidin to inhibit ROS production may be responsible for their anti-apoptotic activities.

Bcl-2 is an anti-apoptotic regulator and Bax is a pro-apoptotic regulator [52]. Bcl-2 and Bax can be expressed at the same time, which plays an important role in sustaining morph and function of HUVEC. The cells are viable when Bcl-2 is overexpressed; whereas they are killed given the overexpression of Bax [53]. The ratio of anti- and pro-apoptotic proteins partly determines how cells respond to apoptotic or survival signals [54]. Our study showed that oxLDL caused a down-regulation of Bcl-2 protein and an up-regulation of Bax protein. In contrast, pre-incubation with delphinidin resulted in an up-regulation of Bcl-2 protein and a down-regulation of Bax protein, indicating that delphinidin is able to inhibit the change of Bax/Bcl-2 in HUVECs induced by oxLDL.

In summary, our study clearly shows that delphinidin can attenuate oxLDL-induced stress injury in HUVECs. The underlying mechanisms of this protective effect may involve a combination of inhibiting intracellular ROS, restoring the activities of endogenous antioxidants, and suppressing endothelial cell apoptosis subsequent to the amelioration of ROS. Thus, the antioxidant properties of delphinidin are highly valuable for treating oxidative diseases. Since oxidative stress-induced endothelial cell injury plays a key role in atherosclerosis, our findings thus highlights a novel and crucial application of delphinidin in the treatment of cardiovascular diseases.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by National Natural Science Foundation of China (grant number 30730079), “the 11th Five-year Plan” for National Key Technology Research and Development Program (2008BAI58B06), and the Innovation Project of Chongqing Key Laboratory of Nutrition and Food Safety (2006CA1003).

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