Anti inflammatory effect of asiaticoside on human umbilical vein endothelial cells induced by ox-LDL

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Abstract Early diagnosis and changes associated with atherosclerosis are crucial in clinical medicine. However, atherosclerosis is a multifactorial disease. Asiaticoside (AA), a triterpenoid derived from *Centella asiatica*, has anti-inflammatory activity. Endothelium-derived nitric oxide is important in modulating vascular tone in a distinct vessel size-dependent manner; it plays a dominant role in conduit arteries and endothelium-dependent hyperpolarisation in resistance vessels. This study evaluated the effects of AA administration on human umbilical endothelial cells with oxidised low-density lipoprotein-induced inflammation. We measured the levels of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Our results indicated that 10–30 μ M AA modulated endothelial hyper

permeability, adenosine triphosphate levels, ICAM-1 expression, VCAM-1 expression, E-selectin levels, and PECAM-1 expression to 90% ($p < 0.005$), 80% ($p < 0.05$), 105% ($p < 0.01$), 65% ($p < 0.005$), 70% ($p < 0.05$), and 105% ($p < 0.01$), respectively. Taken together, our data suggest that AA inhibits the augmentation of endothelial permeability, thus preventing the early events of atherosclerosis.

Keywords Asiaticoside · Adhesion molecule · Inflammation · Atherosclerosis · Endothelium

Introduction

Excessive cholesterol accumulation in macrophages can lead to their transformation into foam cells, leading to the initiation and progression of atherosclerotic lesions. Atherosclerosis, primarily in coronary artery disease (CAD) or carotid stenosis, is the main cause of myocardial infarction and stroke, which together are responsible for more than 50% deaths worldwide (Moran et al. 2014). Vascular endothelial cell is a barrier and a common target of oxidized low-density lipoprotein ox-LDL (ox-LDL), high glucose and other risk factors (Muller et al. 2011). Ox-LDL levels in the bloodstream have been identified as a risk factor for development of coronary artery disease and plaque formation (Berliner et al. 2001; Diaz et al. 1997; Holvoet et al. 2001). Exposure of the vascular

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endothelium to ox-LDL results in EC damage and dysfunction, including the disruption of cell–cell adhesion (Ma et al. 2015) as well as impairment of nitric oxide (NO) release (Xu et al. 2015). In vivo, ox-LDL affects both the large vessels and the microvasculature; it also promotes leukocyte adhesion and transmigration in the microvasculature (Wang et al. 2016).

ECs play a major role in determining and maintaining the inflammatory reactions in response to various inflammatory stimuli (Shah et al. 2015). However, in animal models, the overexpression of VCAM-1 and ICAM-1 has been observed in areas prone to the development of atherosclerotic lesion (Shu et al. 2015). Several studies have investigated the role of NO in the development and progression of vascular inflammation and atherogenesis. Of these, some studies have demonstrated that endothelial NO synthase (eNOS) has an athero-protective effect. For instance, preclinical studies in apolipoprotein E-deficient mice have indicated that the genetic deletion of eNOS accelerates atherosclerosis progression (Kuhlencordt et al. 2001). Similarly, pharmacological eNOS inhibition in rabbits fed with cholesterol-supplemented diets promoted fatty streak formation (Naruse et al. 1994); by contrast, increase in the NO levels, by using L-arginine (NO precursor) or synthetic NO donors, improved endothelial-dependent vasorelaxation and inhibited fatty streak formation in the same rabbit model (Sukhovshin et al. 2015). Thus, NO is an essential mediator of vascular tone and permeability, and the inhibition of its synthesis contributes to the initiation of atherosclerotic events.

Despite recent advancements, the use of herbal medicine as an alternative treatment of atherosclerosis remains limited. *Centella asiatica* (L.) Urban (*Syn. Centella coriacea* Nannfd., *Hydrocotyle asiatica* L., *Hydrocotyle lunata* Lam., and *Trisanthus cochinchinensis* Lour.) is a tropical medicinal plant from the Apiaceae family, native to Southeast Asian countries, such as India, Sri Lanka, China, Indonesia, and Malaysia, as well as to South Africa and Madagascar (Xu et al. 2012); it is commonly known as Gotu kola, Asiatic pennywort, Indian pennywort, Indian water navelwort, wild violet, and tiger herb. Because of its medicinal advantages, this plant has been cultivated widely in some countries, including Turkey. Over centuries, it has also been used in Ayurveda and traditional Chinese medicine. In particular, in Sri Lankan and Indian Ayurvedic medicine, it has been

used for treating skin diseases, syphilis, rheumatism, mental illness, epilepsy, hysteria, dehydration, and leprosy. The triterpenoid glycoside asiaticoside (AA) in *C. asiatica* extracts can be used for numerous curative purposes, including wound repair (Kimura et al. 2008); in addition, topical treatment with *C. asiatica* extracts promotes fibroblast proliferation and collagen synthesis, thus facilitating wound and ulcer healing (Bian et al. 2013).

Several studies have suggested the strong neuro-protective and anticancer efficacy of AA (Tang et al. 2009; Schechter et al. 2005; Gurfinkel et al. 2006; Park et al. 2007). For instance, AA treatment induced apoptotic death in human hepatoma and malignant glioma cells by enhancing intracellular calcium release (Lee et al. 2002; Cho et al. 2006); it also induced apoptosis in melanoma cells by increasing reactive oxygen species levels (Park et al. 2005).

Few studies have also reported the anti-atherosclerotic and anticancer activities of AA through tyrosinase-related protein 1 (TRP1) and TRP2 inhibition (Fong et al. 2015; Kwon et al. 2014). However, the effects of AA on early atherosclerosis events has not been elucidated thus far. Therefore, this study determined the effects of AA on the ox-LDL-induced increase in endothelial permeability and monocyte–EC adhesion as well as verified whether the restoration of the NO bioavailability by AA normalises the EC barrier function and whether EC adhesion is prevented by reducing adhesion molecule expression.

Materials and methods

Asiaticoside (AA) with a purity of 95.8% by HPLC was purchased from Chromadex (Irvine, CA, USA) and dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 10 mM. Chemicals were obtained from the following companies: M200 and low-serum growth supplement (LSGS) kit from Cascade Biologicals (Portland, OR, USA); antibiotics (5000 U/mL penicillin and 5000 µg/mL streptomycin) (Sigma, St. Louis, MO, USA), amphotericin B (0.25 µg/mL), fetal bovine serum (FBS), Roswell Park Memorial Institute medium (RPMI)-1640, were from Hyclone (Logan, UT, USA); phosphate buffered saline (PBS), bovine serum albumin (BSA), trypsin/EDTA solution, were from Sigma (St. Louis, MO, USA); oxidized low-density lipoprotein (ox-LDL) was from Kalen

Biomedical (Germantown, MD, USA); simvastatin with a purity $\geq 98\%$ by HPLC, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DMSO, and human sICAM-1 high-sensitivity ELISA kit and human sVCAM-1 ELISA kit, human E-selectin, PECAM-1 and H_2O_2 were from BenderMed Systems (Vienna, Austria); nitrate/nitrite fluorimetric assay kit was from Cayman Chemical; and ATP colorimetric/fluorimetric assay kit was from BioVision (Milpitas, CA, USA).

Cell culture

Human umbilical vein endothelial cells (HUVECs/7th passage) from Cascade Biologics were grown in fibronectin coated plates (Thermo Fisher Scientific, Waltham, MA, USA) coated flasks in M200 medium supplemented with a low-serum growth supplement (LSGS) kit (Sigma) consisting of 2% fetal bovine serum, hydrocortisone, human epidermal growth factor, basic fibroblast growth factor, and heparin (Sigma). Cells were harvested at a confluency of 90% or more. Cells at passages two to six were used in all experiments.

Cell viability

The MTT assay and the ATP colorimetric/fluorimetric assay kit were used for the quantitative measurement of cell viability and AA cytotoxicity.

MTT assay

HUVECs were treated with various concentrations of AA (0, 10, 20, 30, 40, 50, 100 μM) for 24 h. Cell viability in response to AA treatment was assessed using the MTT method after removal of spent medium. In the assay, 100 μL of M200 medium containing LSGS was added to each well followed by 20 μL of MTT solution (5 mg/mL). After 4 h of incubation, the purple formazan salts formed were dissolved with 100 μL of DMSO (100%). Optical density was measured at 570 nm with a reference wavelength of 650 nm using a microplate reader (Tecan M200 Infinite; Männedorf, Switzerland).

ATP assay

The ATP colorimetric/fluorimetric assay kit was used to determine mitochondria activity upon HUVECs

exposure to AA. HUVECs were grown to confluence in 6-well plates for 72 h and were then washed with $1 \times \text{PBS}$, followed by the addition of AA in a range of concentrations (0, 10, 20, 30, 40, 50 and 100 μM) in separate wells. After 24 h incubation, the cells were washed with ice-cold $1 \times \text{PBS}$. The ATP assay was then performed according to the manufacturer's instructions. Optical density was measured at 570 nm using a microplate reader.

Membrane permeability assay

The in vitro endothelial permeability assay kit was used to determine the change in HUVECs monolayer permeability upon exposure to ox-LDL and AA. Permeability assays were carried out according to the manufacturer's protocols with modifications (EMD Millipore, Billerica, MA, USA). Fluorescein isothiocyanate (FITC)-conjugated dextran (Sigma) was at 1:50 dilution, and the volume reduced to 100 μL . Ox-LDL was used to induce HUVECs hyperpermeability at 100 $\mu\text{g/mL}$ for 24 h. Various concentrations of AA (0, 10, 20, 30 μM) were added concurrently with ox-LDL for 24 h of treatment. Simvastatin at 1×10^{-7} M concentration was used as a drug control. Data were expressed as a permeability index (%) using the formula stated by Ang et al. (2011).

Adhesion molecule expression

To determine the effect of AA on the level of ox-LDL-induced adhesion molecule expression, HUVECs were grown to confluence in a 24-well plate. Ox-LDL (100 $\mu\text{g/mL}$) was used to stimulate HUVECs concurrently with AA at various concentrations (0, 10, 20, 30) for 24 h. Following stimulation, the HUVECs supernatant was collected for the sICAM-1, E-Selectin, PECAM-1 and sVCAM-1 ELISA assays using commercial kits. Optical density was measured using a microplate reader (Tecan M200 Infinite).

Measurement of nitric oxide production

To evaluate the effect of AA on NO bioavailability, HUVECs were grown to confluence in a 24-well plate. Ox-LDL and AA at various concentrations (0, 10, 20, 30 μM) were added concurrently. After 24 h incubation, the media were collected for a nitrate/nitrite fluorimetric assay using a commercial kit.

Fluorescence intensity was assessed by using a fluorescence microplate reader (Tecan M200 Infinite).

Western blot analysis

Each plate was washed with 3–4 mL $1 \times$ PBS twice and the remaining fluid in each plate was discarded. Then 100 μ L cell lysis buffer (50 mM Tris-base (pH 7.5), 0.5 M NaCl, 1 mM EDTA (pH 8.0), 1% NP-40, 1% glycerol and protease inhibitor cocktail tablets) was added to lyse cells in each plate. The cells were scraped down and collected in appropriate 1.5-mL microcentrifuge tubes on ice, which were then vortexed once every 10 min three times and centrifuged for 20 min at 12,000 rpm, 4 °C. The supernatants were transferred to another set of 1.5-mL microcentrifuge tubes. These were the total protein samples. Protein concentration in each sample was determined by Lowry assay and Western blot analysis was conducted in order to determine the results.

Immunofluorescent microscopy

HUVECs cells were seeded in 12-well plates (1×10^5 cells per well). 24 h after cell seeding, 100 μ M Ox-LDL was added first followed by 30 μ M AA. Twenty-four hours after administration cells were fixed with 4% paraformaldehyde in $1 \times$ PBS at RT for 15 min. Cells were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 20 min. Non-specific binding of the fixed cells was blocked with 10% Copper chaperone (Sigma) for superoxide dismutase at room temperature (RT) for 1 h and the cells were incubated with diluted primary antibody solutions (Invitrogen, CA, USA) in $1 \times$ PBS for 48 h at 4 °C. Then, the cells were incubated with Alexa Fluor 488 donkey anti-goat IgG secondary fluorescence antibody (Invitrogen, Carlsbad, CA, USA) avoiding light exposure for 1 h at 37 °C. Finally, the cells were stained with 1 μ g/mL DAPI for 5 min to detect cell nuclei (blue staining). The fluorescence was visualized using a fluorescence microscope coupled with an image analysis system.

RNA isolation and northern analysis

Northern analysis was performed as previously described (Scott et al. 2007). After extraction, 20 μ g of total RNA was fractionated in formaldehyde-denaturing agarose gel and transferred to Hybond-N

filters (Amersham Laboratories, Pittsburgh, PA, USA) by capillary blotting. Hybridization was with 32 P-labeled DNA probes in 50% formamide, $5 \times$ SSC ($1 \times$ SSC = 0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate), 0.02 mol/L sodium phosphate pH 7.2, $5 \times$ Denhardt's solution, 0.2% sodium lauryl phosphate, 10% dextran sulfate, and 0.1 mg/mL denatured sheared salmon sperm DNA (Sigma) at 42 °C for 18 h. Filters were washed in $2 \times$ SSC, 0.25% sodium lauryl sulfate for 30 min with 2 changes and then for 60 min in $0.2 \times$ SSC, 0.25% sodium lauryl sulfate with 3 changes at 45 °C. Autoradiograms were developed after 2 days of exposure. Hybridization to study ICAM-1 gene expression was performed with a 1.9-kb cDNA clone (Simmons et al. 1998) (donated by Dr Brian Seed, Department of Molecular Biology, Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA).

Statistical analysis

The experimental data are expressed as mean $\pm$ standard error of the mean (SEM). Groups of data were compared by one-way ANOVA with posthoc analyses (Dunnett test) for pairwise comparisons. Statistical significance was defined as $p < 0.05$.

Results

Effect of AA on cell viability

Human umbilical ECs (HUVECs) were incubated with 0, 10, 20, 30, 40, 50 and 100 μ M AA for 24 h. In the MTT assay (Fig. 1a), AA had no detectable effect on the viability of the HUVECs at 10–30 μ M concentration, as indicated by the unaffected mitochondrial dehydrogenase. By contrast, in the adenosine triphosphate (ATP) assay (Fig. 1b), compared with controls, incubation with 40, 50, and 100 μ M AA significantly reduced the ATP levels in the HUVECs (85 ± 4 , 63 ± 2 , and $21 \pm 5\%$, respectively; all $p < 0.05$).

Effect of AA on endothelial permeability

Exposure to 100 μ M ox-LDL increased the permeability index of the HUVECs (Fig. 2). Treatment of these HUVECs with 10, 20, and 30 μ M AA

Fig. 1 **a** MTT assay for HUVEC viability. Data are expressed in percentage of cell viability control, compared with the normal control group. **b** ATP levels in AA-treated HUVECs. Data are

expressed as mean $\pm$ SEM, from at least three independent experiments. control vs treated * $p < 0.05$; *** $p < 0.005$ by ANOVA

Fig. 2 Effect of AA on ox-LDL-induced endothelial permeability in HUVECs. AA was incubated concurrently with ox-LDL (100 μmol/mL) for 24 h. NC no cell, PC induced, Sim 2 μM simvastatin. Data are expressed as mean $\pm$ SEM, from at least three independent experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$ by ANOVA

significantly reduced this hyperpermeability to $139 \pm 0.7\%$ ($p < 0.01$), $90 \pm 0.92\%$ ($p < 0.005$), and $65 \pm 0.3\%$ ($p < 0.005$), respectively. The strongest inhibitory effect on endothelial hyperpermeability reduction from the ox-LDL-induced baseline was induced by AA. The drug control simvastatin (2 μM) reduced the permeability index from $112 \pm 7.34\%$ to 79% (baseline) ($p < 0.005$).

Effect of AA on adhesion molecule levels

Ox-LDL (100 μmol/mL) significantly increased the expression of sICAM-1 and sVCAM-1 from $100 \pm 5\%$ (baseline) to $172 \pm 3\%$ (Fig. 3a) and $100 \pm 3\%$ (baseline) to $178 \pm 1.2\%$ (Fig. 3b), respectively. Treatment with 10, 20, and 30 μM AA of non-induced HUVECs significantly reduced the expression of sICAM-1 [$140 \pm 1.2\%$

($p < 0.01$), $87 \pm 2\%$ ($p < 0.01$), and $65 \pm 3.4\%$ ($p < 0.05$), respectively] and VCAM-1 [$153 \pm 2.7\%$ ($p < 0.01$), $102 \pm 2.4\%$ ($p < 0.01$), and $102 \pm 1.78\%$ ($p < 0.05$), respectively]. However, 10 μM AA had no significant effect on the sICAM-1 and sVCAM expression in ox-LDL-induced HUVEC. Compared with controls, 2 μM simvastatin significantly increased sICAM-1 and sVCAM expression to 70 ± 0.5 and $140 \pm 1.8\%$, respectively. Ox-LDL (100 μmol/mL) reduced the expression of PECAM-1 and E-selectin from the baseline to $225 \pm 2.3\%$ (Fig. 3c) and $174 \pm 6\%$ (Fig. 3d), respectively. Treatment with 10, 20, and 30 μM AA significantly reduced the expression of ox-LDL-induced PCAM-1 expression to $139 \pm 2\%$ ($p < 0.005$), $105 \pm 0.4\%$ ($p < 0.005$), and $104 \pm 1\%$ ($p < 0.01$), respectively, and that of E-selectin expression to $145 \pm 6\%$ ($p < 0.005$), $70 \pm 1.0\%$ ($p < 0.005$), and $71 \pm 0.03\%$ ($p < 0.01$), respectively. Furthermore, simvastatin (2 μM) significantly increased PECAM-1 and E-selectin expression to 145 ± 3 and $127 \pm 4\%$, respectively.

Effect of AA on NO production

The concurrent 24-h supplementation of ox-LDL with 10, 20, and 30 μM AA significantly decreased NO production in the HUVECs [$79 \pm 3\%$ ($p < 0.05$), $89 \pm 3\%$ ($p < 0.05$), and $104 \pm 5\%$ ($p < 0.01$), respectively; Fig. 4]. Simvastatin (2 μM) significantly increased NO levels to $74 \pm 6\%$ ($p < 0.05$).

Effect of AA on H₂O₂, interleukin-18, and ICAM mRNA levels

Ox-LDL significantly increased H₂O₂ (Fig. 5a), interleukin (IL)-18 (Fig. 5b), and ICAM mRNA levels

Fig. 3 Effect of AA on the ox-LDL-induced sICAM-1, sVCAM-1, PECAM, and E-Selectin expression in HUVECs. AA was concurrently incubated with ox-LDL (100 $\mu\text{g}/\text{mL}$) for 24 h. *NC* non-induced, *PC* induced, and *Sim* 2 μM simvastatin. sICAM-1 (a), sVCAM-1 (b), PECAM (c), and E-selectin (d) expression. Data are expressed as mean $\pm$ SEM, from at least three independent experiments. * $p < 0.05$; ** $p < 0.01$ by ANOVA

Fig. 4 Effect of AA on ox-LDL-attenuated NO production in HUVECs. *NC* non-induced, *PC* induced, and *Sim* 2 μM simvastatin. Data are expressed as mean $\pm$ SEM, from at least three independent experiments. * $p < 0.05$; ** $p < 0.01$, and *** $p < 0.005$ by ANOVA

(Fig. 5c) in the HUVECs; however, AA treatment reduced these levels, compared with 2 μM simvastatin. ICAM-1, VCAM-1, and PECAM-1 protein levels were significantly increased in ox-LDL-treated cells, compared with AA-treated cells (Fig. 6a). Moreover, treatment with 100 $\mu\text{g}/\text{mL}$ ox-LDL also induced nuclear localisation of ICAM-1 (Fig. 6b), which was then down regulated by addition of 30 μM AA. Therefore, AA could attenuate ox-LDL-induced activation of inflammation in our HUVECs.

Discussion

AA is an active ingredient in *C. asiatica*, responsible for its pharmacological activity. AA has various therapeutic effects, such as heat removal, diuresis promotion, detumescence, and detoxification; it has been used in the treatment of urinary tract infections, skin diseases, jaundice, diarrhoea, measles, as well as larynx and gall bladder injuries. A recent study demonstrated that AA has nerve protection effects; however, its role in anti-inflammatory cytokine production and subcellular structure maintenance remains unclear (Mook-Jung et al. 1999). In this study, we demonstrated, for the first time, that AA can be used for controlling cell adhesion molecule expression and NO production and thus its potential use in the regulation of cellular trafficking. We analysed the ability of AA to inhibit ox-LDL-induced ICAM-1, VCAM-1, PECAM, and E-selectin expression in HUVECs. We noted that AA did not induce any permanent changes in the endothelial structure.

Leukocyte–EC adhesion is the first step in the cellular migration process; here, the expression of cell adhesion molecules on ECs is a requirement as ICAM-1, VCAM-1, and E-selectin receptors are present on circulating leukocytes. Leukocytes become arrested on the endothelium by binding to these receptors and

Fig. 5 Effect of AA on ox-LDL-induced H_2O_2 , IL-8 and VCAM-1 production in HUVECs. (A–C) AA significantly reduced the production of H_2O_2 , IL-8 and VCAM-1 in dose dependent manner. Data are presented as percentages of H_2O_2 and IL-8, compared with ox-LDL-treated cells, and expressed as mean $\pm$ SEM, from at least three independent experiments. * $p < 0.05$; ** $p < 0.01$, and *** $p < 0.005$ by ANOVA

migrate across into the underlying tissues. As a functional consequence of the inhibition of cell adhesion molecules, AA inhibited ox-LDL-induced EC adhesion. To analyse the mechanism of the inhibition of cell adhesion by AA, we studied the time kinetics of ICAM-1 inhibition and found that AA should be added prior to or simultaneously with ox-LDL to be effective. Thus, AA potentially interferes at an early stage of ox-LDL-induced signalling.

Several studies, including the current study, have shown that the presence of the hydroxyl group on a polyphenol is useful for imparting the free radicals' anti-inflammatory or antioxidant activities. The endothelial dysfunction in early atherosclerosis and its reversibility with L-arginine in cardiovascular diseases suggest NO deficiency in a cell type central to early atherosclerosis pathophysiology. Furthermore, because endothelial dysfunction is associated with both soluble ICAM-1 and total parasite biomass, impaired NO production in atherosclerosis potentially enhances endothelial adhesion molecule expression as well as exacerbates cytoadherence and sequestration. In myocarditis, ICAM-1 expression in the coronary

vessel endothelium is also inversely associated with NO-dependent endothelial function (Ang et al. 2011).

Despite its protective effect, NO can become harmful if overproduced, as noted in this study. At concentrations of 10–30 μ M, AA effectively suppressed endothelial hyperpermeability, monocyte adhesion, and adhesion molecule expression in our HUVEC monolayer and restored NO levels to 104% of the control. Zhang et al. (2011) and Yeo et al. (2007) reported that AA significantly increased eNOS levels in HUVECs, providing a possible explanation of the mechanism of NO restoration by AA; furthermore, AA stimulated NO production in a dose-dependent manner in the absence and presence of the inducer TNF- α . However, these findings are contradicted by those of Luo et al. (2015), who indicated that AA does not upregulate eNOS expression in HUVECs. Nevertheless, in this study, we found that the high IL-18 and H_2O_2 levels induced by ox-LDL were reduced by AA treatment.

We further examined the detrimental effect of ox-LDL on the endothelium in this study. Karman et al. (1997) reported that in porcine aortic ECs, 100 μ g/mL

Fig. 6 Western blot results for ICAM-1, VCAM-1, and PECAM-1 levels. The protein band intensities were detected through densitometry ($*p < 0.05$) (A). After incubation, cells were fixed and stained for ICAM-1 (green); nuclei were stained

blue by using DAPI. Cells were observed using a confocal microscope with a constant photomultiplier (B). (Color figure online)

ox-LDL significantly increased albumin clearance, affecting its endothelial barrier function, but native LDL had no such effect. In this study, we used simvastatin, a well-known drug from the statin group and an inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A reductase, as the positive control. Simvastatin is considered the first-line therapy for

hypercholesterolemia. A study indicated that simvastatin can reduce vascular inflammation by suppressing ICAM-1 and VCAM-1 expression and thus the monocyte–EC adhesion (Zapolska-Downar et al. 2004).

Several studies have indicated that AA is an anti-inflammatory agent (Lee et al. 2016; Di Tomo et al.

2015; Giribabu et al. 2014; Sharma et al. 2014; Pakdeechote et al. 2014). It is also an established cardioprotective compound (Belcaro et al. 2015, 2017; Leng et al. 2013). However, its effect on the endothelial barrier function remains unclear. In the present study, the increased endothelial permeability to FITC-dextran appears to have been significantly suppressed by AA. Low-dose AA concentrations (10–30 μM) were effective in attenuating the hyperpermeability in HUVEC; 30 μM AA showed the highest inhibition of HUVEC hyperpermeability. These data suggest that AA exerts its anti-inflammatory activity by improving the endothelial barrier function to both fluid and macromolecules. Ox-LDL triggers a series of inflammatory responses which induce the expression of adhesion molecules involved in circulating monocyte recruitment to the vascular endothelium.

In summary, the current study demonstrated that AA, a natural complex isolated from *C. asiatica* Bunge, can potentially interrupt the progression of early atherosclerotic events. NO bioavailability restoration is the key factor regulating the AA-induced inhibition of ox-LDL-induced endothelial hyperpermeability, adhesion molecule expression, and monocyte–EC adhesion. Therefore, beneficial goals in treating atherosclerosis should prohibit the stimulation of NO bioavailability and exploit it against impaired ECs. These findings demonstrate the potential therapeutic application of AA in preventing the early events of atherosclerosis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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