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European Journal of Pharmacology 513 (2005) 1–8

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Protocatechuic aldehyde suppresses TNF-α-induced ICAM-1 and VCAM-1 expression in human umbilical vein endothelial cells

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Received 29 November 2004; received in revised form 26 January 2005; accepted 31 January 2005

Abstract

Adhesion molecules, which play a crucial role in the development of atherogenesis, are produced by endothelial cells following stimulation with various inflammatory cytokines. The current studies examined the effect of a potent water-soluble antioxidant, protocatechuic aldehyde (derived from the Chinese herb, *Salvia miltiorrhiza*), on the expression of adhesion molecules in human umbilical vein endothelial cells (HUVECs) stimulated with tumor necrosis factor-α (TNF-α).

Protocatechuic aldehyde appeared to specifically downregulate the TNF-α-induced cell surface expression of vascular adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) on HUVECs as well as the release of soluble VCAM-1and ICAM-1 from HUVECs in a dose–response manner at pharmacologically relevant concentrations (0.15–1.35 mM). We also observed a dose-dependent lowering of mRNA expression of VCAM-1 and ICAM-1 in the presence of protocatechuic aldehyde. Furthermore, protocatechuic aldehyde (0.15, 0.45, and 1.35 mM) notably inhibited TNF-α-induced upregulation of U937 cell adhesion to HUVECs to 83.7%, 60.9%, and 40.8%, respectively.

A gel shift assay further showed that protocatechuic aldehyde inhibited the TNF-α-activated NF-κB and AP-1 DNA binding activities in a dose-dependent manner. Collectively, these results indicate that protocatechuic aldehyde inhibits TNF-α-stimulated VCAM-1 and ICAM-1expression in HUVECs through a mechanism that involves NF-κB and AP-1.

Keywords: Protocatechuic aldehyde; Endothelial cell; Adhesion; NF-κB (nuclear factor-κB); AP-1 (activator protein-1)

1. Introduction

Activation of the vascular endothelium, increased adhesion of circulating leukocytes to the injured endothelial layer, and their subsequent extravasation into the vessel wall is a critical early event in the development of atherosclerosis (Price and Loscalzo, 1999; Joris et al., 1983). Endothelial cells recruit leukocytes by selectively expressing adhesion molecules on the surface: e.g., vascular cell adhesion molecules (VCAM-1), intercellular adhesion molecules (ICAM-1), and endothelial cell selectin (E-selectin) (Faggiotto et al., 1984; Iiyama et al., 1999). Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), commonly found in atherosclerotic lesions, can induce chemotactic factors, other cytokines, and cell adhesion molecules, all of which contribute to the inflammatory process (Zibara et al., 2000; Ross, 1999; Springer, 1994).

Actions of antioxidants on cell adhesion molecules expression may play an important role in the prevention of atherogenesis (Marui et al., 1993; Wu et al., 1999; Noguchi et al., 2003). *Salvia miltiorrhiza*, a herb often used in popular folk medicine in China, has been found to show beneficial effects on the circulatory system (Lei and Chiou, 1986; Zhu et al., 2001). Aqueous extracts of *S. miltiorrhiza* that are rich in antioxidants have been described as effective in reducing atherosclerosis in experimental studies in vitro and in vivo (Wu et al., 1998; Chen et al., 2001a,b).

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doi:10.1016/j.ejphar.2005.01.059

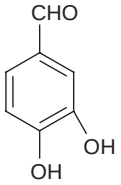

Fig. 1. Chemical structure of protocatechuic aldehyde.

Although the precise mechanisms by which *S. miltiorrhiza* may reduce atherogenesis have not been completely defined, it has been hypothesized that the antiatherosclerotic activity of *S. miltiorrhiza* is associated with its antioxidative activity. Studies have shown that the reduction of atherosclerosis by *S. miltiorrhiza* relies heavily on its antioxidant potential to prevent endothelial damage and inhibit low-density lipoprotein (LDL) oxidative modification in hypercholesterolemic animals. Salvianolic acid B, a water-soluble polyphenolic antioxidant isolated from the roots of *S. miltiorrhiza*, has been reported to exert a protective action against TNF-α injury in the human aortic vascular endothelial cells by reducing cell adhesion molecules (Chen et al., 2001a,b).

Protocatechuic aldehyde (Fig. 1) is another pure water-soluble antioxidant compound found in the root of *S. miltiorrhiza*. No studies exist that examine the influence of protocatechuic aldehyde on the regulation of adhesion molecules expression and monocyte adhesion to endothelial cells. We therefore examined whether protocatechuic aldehyde modulates TNF-α-induced expression of adhesion molecules and the consequent propensity of monocyte adhesion to human umbilical vein endothelial cells (HUVECs). Furthermore, we examined the activities of transcriptional factor nuclear factor-κB (NF-κB) and transcriptional factor activator protein-1 (AP-1), two key molecules in the regulation of adhesion molecules expression.

2. Materials and methods

2.1. Materials

Protocatechuic aldehyde (purity>99.5%) was purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Special precautions were taken to avoid direct exposure of protocatechuic aldehyde to light and air during experiments. Protocatechuic aldehyde was dissolved in warm culture medium just before incubation with HUVECs. Recombinant human TNF-α was from Sigma (Steinheim, Germany). The ImProm-II™ Reverse Transcription System, the CellTiter 96®Aqueous One Solution Cell Proliferation Assay (MTS), the PCR system, and the Gel shift core system were purchased from Promega (Madison, WI, USA), the ICAM-1, E-selectin, and VCAM-1 enzyme-linked immunosorbent assay (ELISA) kits were products of BioSource International (Camarillo, CA).

2.2. Cell culture

Human umbilical vascular endothelial cells (HUVECs) were obtained from Cascade Biologics (Portland, Oregon) as cryopreserved primary cultures, and grown in culture flasks (Costar) in endothelial cell growth medium M200 (Cascade Biologics) supplemented with 2% LSGS (low serum growth supplement; Cascade Biologics) according to Cascade Biologics's recommended protocol. The growth medium was changed every other day until cells reached confluence. Cells of passages 3 and 4 were grown in monolayers at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air, and used for experiments at >80% confluency.

Twenty-four hours before experiments, the control medium was removed and replaced with LSGS-free medium containing 0.4% fetal bovine serum. For experiments, HUVECs were cultured in medium containing 0.4% fetal bovine serum with or without the protocatechuic aldehyde for 18 h, then, cultures were further carried out for additional 6 h with TNF-α (2 ng/ml).

2.3. Assessment of cell viability

For evaluation of cytotoxicity, cells were seeded at 5000 cells/well into 96-well culture plates (Costar) and grown for 48 h. The cells were incubated with various concentrations of the agents in serum-free M200 medium containing 0.4% fetal bovine serum for 24 h, and then 20 μl of MTS was added to each well and further incubated at 37 °C for 2 h. The absorbance of the solubilized formazan was read at 490 nm using a Victor™ 1420 Multilabel Counter (Wallac, Turku, Finland). Cells incubated in control media were considered 100% viable.

2.4. Cell adhesion assay

The monocytoid cell line U937 was used in studies as previously described (Clerck et al., 1994) with minor modifications. Briefly, regularly passaged U937 cells were labeled with fluorescent dye, 2,7-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethylester (BCECF-AM, Acros, Geel, Belgium), at 10 μM final concentration in RPMI-1640 medium (Invitrogen) containing 10% fetal bovine serum at 37 °C for 1 h. The labeled cells were harvested by centrifugation and washed three times with phosphate buffered saline (PBS) before suspension in the medium, and added to HUVEC in six-well culture plates at 4×10^5 cells/ml. The co-incubation was done at 37 °C for 1 h, and unbound U937 cells were removed by stringent washing four times with medium (RPMI-1640, 1% fetal bovine serum), and finally twice with PBS. U937 cells bound to HUVECs were lysed with 50 mM Tris–HCl, pH

8.0, containing 0.1% SDS, and fluorescence measured on a spectrofluorometer (Wallac, Turku, Finland) at 485 nm excitation and 535 nm emission wavelength.

2.5. Measurement of adhesion molecules secretion by HUVECs

The levels of soluble VCAM-1, ICAM-1, and E-selectin antigens in HUVECs culture supernatants were determined by enzyme-linked immunosorbent assay methods according to the manufacturer's recommended protocol.

2.6. Cell ELISA

Cell ELISA was performed as previously reported with minor modifications (Manduteanu et al., 2004). Briefly, HUVECs in 96-well plates were pretreated with or without protocatechuic aldehyde then treated with TNF-α. Following the treatments, the cells were fixed by 1% glutaraldehyde and then exposed to monoclonal mouse anti-human VCAM-1, ICAM-1 (BioSource International), or E-selectin (Dako, Glostrup, Denmark) antibody at 1:1000 dilution in the PBS containing 1% skim milk for 2 h at room temperature. Cells were washed and then incubated with a horseradish peroxidase-conjugated goat anti-mouse IgG (BioSource International). Finally, the expression of VCAM-1, ICAM-1, or E-selectin was quantitated by the addition of peroxidase substrate solution containing 40 mg O-phenylenediamine and 10 μl 30% H_2O_2 in 100 ml 0.05 M citrate–phosphate buffer. After incubation for 30 min at 37 °C, the reaction was stopped by addition of 5N H_2SO_4, and the absorbance of each well was measured at 490 nm.

2.7. RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR)

For multiplex semi-quantitative RT-PCR analysis, total RNA was isolated from HUVECs using the Trizol reagent (Invitrogen). Total RNA (2 μg) was reverse-transcribed into cDNA using an oligo(dT) primer, and then amplified with specific primers. The following primers with the predicted size were used for amplification: VCAM-1(336 bp) 5′-GCAAGGTTCCTAGCGTGTAC-3′ (forward) and 5′-GGCTCAAGCATGTCATATTCAC-3′(reverse), ICAM-1(289 bp) 5′-CGACTGGACGAGAGGGATTG-3′ (forward) and 5′-TTATGACTGCGGCTGCTACC-3′(reverse), E-selectin(537 bp) 5′-AACTTCCATGAGGCCAAACG-3′ (forward) and 5′-TTGTCGTTGCCAGTGTTCAG-3′(reverse), and GAPDH (452 bp) 5′-ACCACAGTCCATGCCATCAC-3′ (forward) and 5′-TCCACCACCCTGTTGCTGTA-3′(reverse). The cycle number was determined from a linear amplification curve as being within the linear amplification range. The PCR products were subjected to 2% agarose gel electrophoresis. Quantitative data normalized to GAPDH were obtained from a densitometer and analyzed with the included Quantity One 4.4.0 software (BIO-RAD).

2.8. Nuclear protein extract and western blot

Nuclear proteins were extracted and quantified as described (Dschietzig et al., 2001). Protein samples (20 μg) were resolved by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Schleicher and Schuell Biosciences, Keene, NH) at room temperature. Membranes were pre-incubated for 2 h at room temperature with PBS-Tween 20 (0.05%)/2% skim milk, and then incubated for 1 h at room temperature with rabbit anti-human-NF-κB polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). After washing, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Antigen detection was performed with an Enhanced Chemiluminescence (ECL) kit (Santa Cruz Biotechnology), and blots were exposed to Biomax MRfilm (Kodak, Shantou, China).

2.9. Fluorescenic non-radioactive electrophoretic mobility shift assay (fEMSA)

Fluorescent EMSA was performed according to the method of Ruscher et al. (2000) with minor modifications. The double-stranded gel-shift oligonucleotides for NF-κB, and AP-1 were end-labeled with Cy5-end-labeling (Amersham Pharmacia Biotech). The binding reaction was performed for 20 min at 25 °C in a total volume of 10 μl of binding buffer that contained 10 mM Tris–HCl, pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 4% glycerol, 0.5 μg of double stranded poly (dI-dC). DNA–protein complexes were resolved on 6% non-denaturing polyacrylamide gels at 250 V for 30 min in 0.5 × Tris borate-EDTA (1 × Tris borate-EDTA which contains 90 mM Tris–borate, pH 8.3, 2 mM EDTA) at 4 °C. After electrophoresis, gels were scanned with a Typhoon 9410 scanner (Amersham Pharmacia) at an excitation wavelength of 630 nm and an emission wavelength of 670 nm.

2.10. Statistical analysis

All values are expressed as the mean ± S.E.M. of independent determinations. Statistical analysis was performed with analysis of variance (ANOVA) and Tukey test using software originlab originPro 7.5.

3. Results

3.1. Modulation of endothelial adhesiveness by increased concentrations of protocatechuic aldehyde

We evaluated the effects of protocatechuic aldehyde on the binding of U937 cells to TNF-α stimulated HUVECs. TNF-α is a potent inducer of endothelial adhesiveness.

Adhesion was markedly increased when HUVECs were treated with TNF-α. Pretreatment with protocatechuic aldehyde (1.35, 0.45, 0.15 mM) significantly reduced the adhesion of U937 cells to TNF-α-stimulated HUVECs in a dose-dependent fashion (Fig. 2).

3.2. Effects of protocatechuic aldehyde on ICAM-1, VCAM-1, and E-selectin secretion and cell surface expression

Exposure of cells to TNF-α (2 ng/ml) for 6 h induced strong upregulation of the cell surface expression of VCAM-1, ICAM-1, and E-selectin (Fig. 3). The effects of different concentrations of protocatechuic aldehyde on TNF-α-induced cell surface adhesion molecules expression are depicted in Fig. 3. Protocatechuic aldehyde failed to significantly modulate the TNF-α-induced cell surface expression of E-selectin at any of the concentrations used (Fig. 3). In contrast, when treated with protocatechuic aldehyde (0.15,0.45, and 1.35 mM), the TNF-α-induced cell surface expression of VCAM-1was notably reduced to 27.2%, 22.1%, and 18.6%, respectively, and ICAM-1 was significantly reduced to 84.5%, 75.1%, and 43.3%, respectively (Fig. 3).

Protocatechuic aldehyde at doses of 0.15, 0.45, and 1.35mM induced significant dose-dependent inhibition of VCAM-1 and ICAM-1 protein secretion (Fig. 4). Protocatechuic aldehyde did not attenuate the level of the TNF-α-induced release of E-selectin (Fig. 4).

3.3. Effects of protocatechuic aldehyde on ICAM-1, VCAM-1, and E-selectin mRNA expression

To investigate whether protocatechuic aldehyde inhibits adhesion molecules production at the mRNA expression level in HUVECs, we performed an RT-PCR analysis on RNA isolated from HUVECs cultured in the presence of

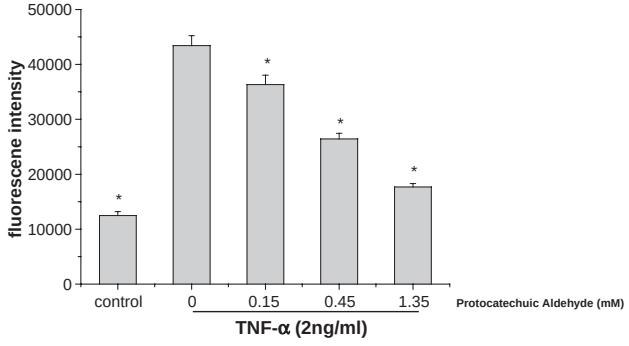

Fig. 2. Protocatechuic aldehyde reduced adhesion of U937 cells to HUVECs monolayers. HUVECs were pre-incubated with the indicated doses of protocatechuic aldehyde for 18 h and then stimulated with TNF-α (2 ng/ml) for 6 h. Fluorescence-labelled monocytic U937 cells were added to the HUVEC monolayer and allowed to adhere for 60 min. The adhered cells were measured as described in Materials and methods. The data represent the mean ± S.E.M. (n=4). Asterisks indicate a statistically significant difference when compared to results from TNF–stimulated cells in the absence of protocatechuic aldehyde (*P<0.05).

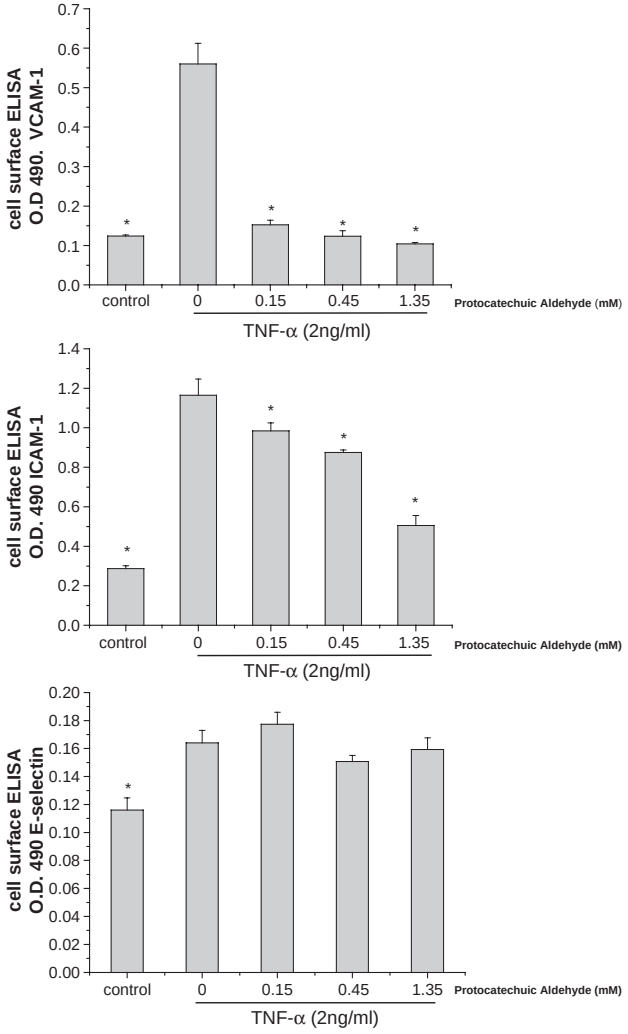

Fig. 3. Effects of protocatechuic aldehyde on the cell surface expression of VCAM-1, ICAM-1, and E-selectin on HUVECs surface. The cells were pretreated with protocatechuic aldehyde (0.15, 0.45, and 1.35 mM) for 18 h and then activated with TNF-α (2 ng/ml) for 6 h. After the incubation, the expression of VCAM-1, ICAM-1, and E-selectin on HUVECs was assessed by cell ELISA. The data represent the mean ± S.E.M. (n=4). Asterisks indicate a statistically significant difference when compared to results from TNF-α-stimulated cells in the absence of protocatechuic aldehyde (*P<0.05).

TNF-α with or without Protocatechuic Aldehyde. As shown in Fig. 5, TNF-α induced marked increase in VCAM-1 and ICAM-1 mRNA expression, whereas treatment with protocatechuic aldehyde dose-dependently inhibited TNF-α-induced VCAM-1 and ICAM-1 mRNA expression. Protocatechuic aldehyde failed to significantly modulate the TNF-α-induced expression of E-selectin mRNA at any of the concentrations used.

3.4. Effect of protocatechuic aldehyde on TNF-α-induced NF-κB p65 and AP-1 activation

To determine whether activation of NF-κB and/or AP-1 was involved in the effect of protocatechuic aldehyde on TNF-α-stimulated VCAM-1 and ICAM-1 expression, we

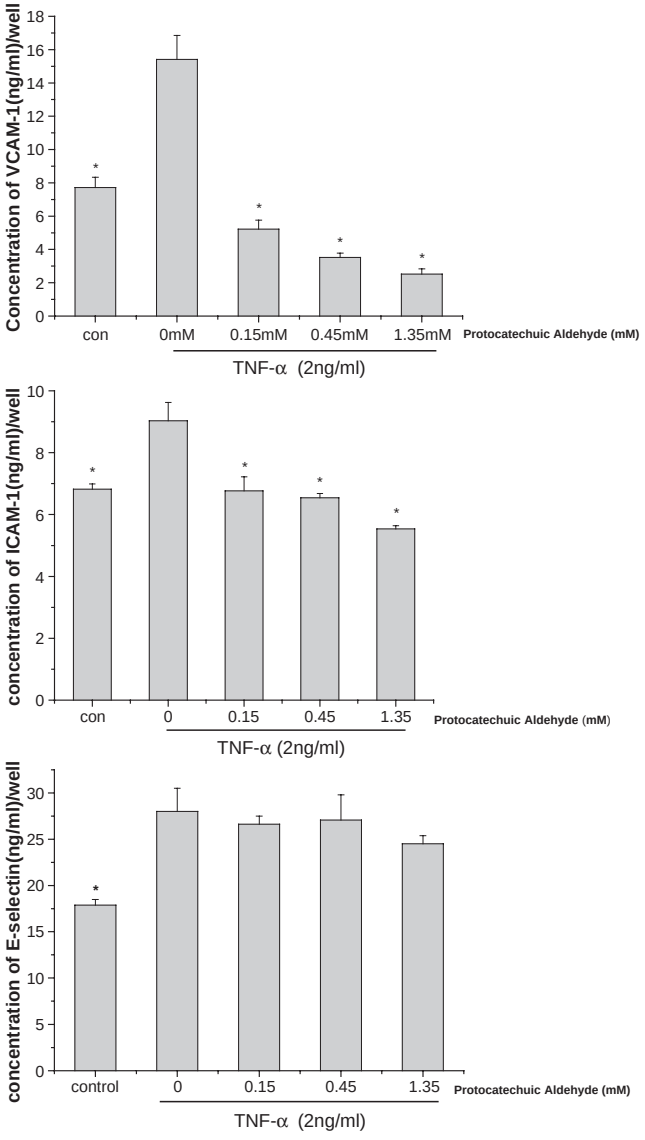

Fig. 4. Effects of various concentrations of protocatechuic aldehyde on VCAM-1, ICAM-1, and E-selectin secretion in HUVECs stimulated with TNF-α. The cells were pretreated with protocatechuic aldehyde (0.15, 0.45, and 1.35 mM) for 18 h and then activated with TNF-α (2 ng/ml) for 6 h. The data represent the mean ± S.E.M. (n=4). Concentrations of VCAM-1, ICAM-1, and E-selectin in the culture medium were examined. Asterisks indicate a statistically significant difference when compared to results from TNF-α-stimulated cells in the absence of protocatechuic aldehyde (*P<0.05).

measured NF-κB and AP-1 DNA-binding activities in HUVEC nuclear extracts, and also measured the amount of NF-κB p65 nuclear translocation. TNF-α-stimulated HUVECs showed a marked increase in nuclear translocation of NF-κB p65, as measured by Western blot (Fig. 6). As shown in Fig. 7, TNF-α-stimulated HUVECs also showed increased NF-κB and AP-1 DNA-binding activities, whereas pretreatment of HUVECs with protocatechuic aldehyde for 18 h markedly attenuated TNF-α-stimulated NF-κB and AP-1 activation in a dose-dependent manner.

4. Discussion

S. miltiorrhiza is commonly used in traditional Oriental herbal medicine and has been demonstrated to have

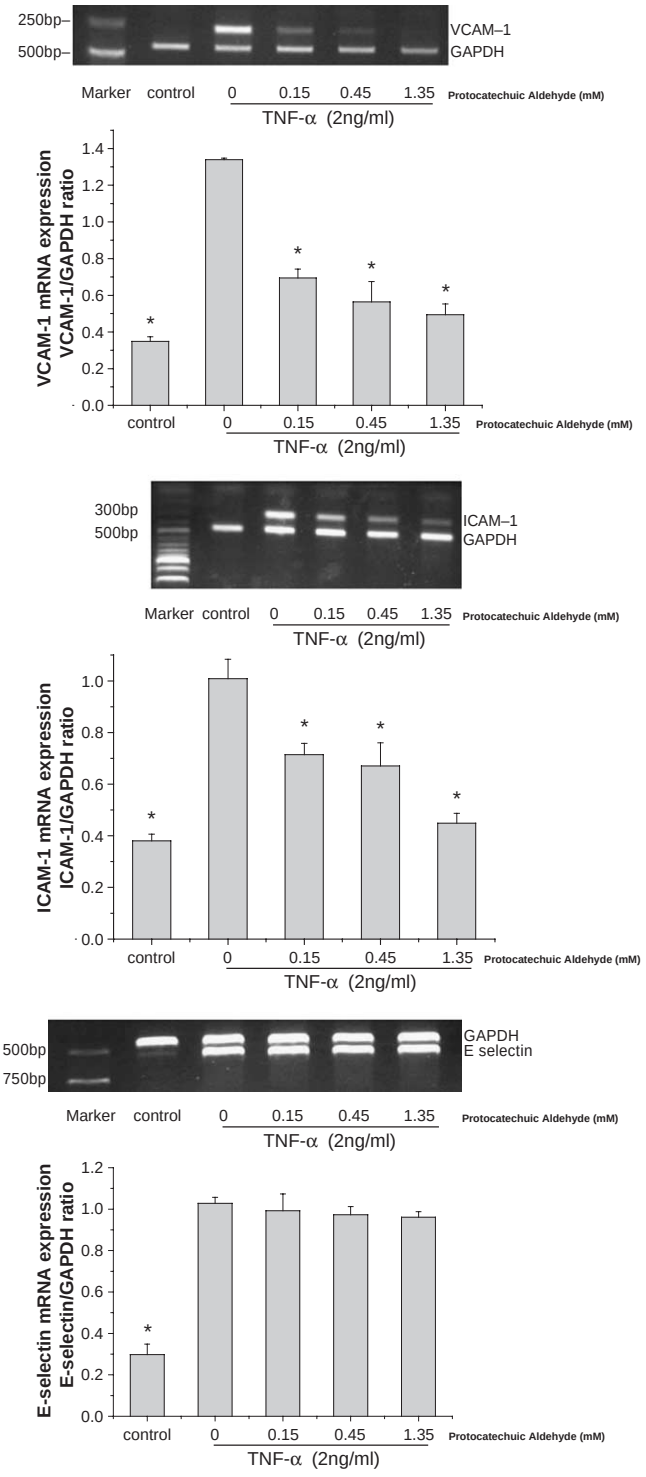

Fig. 5. RT-PCR shows the dose-dependent effect of protocatechuic aldehyde on adhesion molecules mRNA expression in TNF-α-induced HUVECs. Data are expressed as mean ± SEM (n=3). Asterisks indicate a statistically significant difference when compared to results from TNF-α-stimulated cells in the absence of protocatechuic aldehyde (*P<0.05).

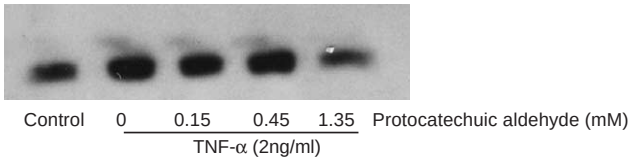

Fig. 6. Western blotting analysis of NF-κB p65 protein levels in cultured HUVECs nuclear extracts. Data shown are representative of three independent experiments.

beneficial effects on cardiovascular diseases (Ji et al., 2000; Zhu et al., 2001). Previous studies have demonstrated that *S. miltiorrhiza* is an antioxidative, antithrombogenic, and anti-inflammatory plant. Its non-polar extracts contain tanshinones, which can inhibit platelet aggregation and protect the myocardium against ischemia-induced derangements (Yagi et al., 1989; Lin et al., 2001). In contrast, aqueous extracts consist primarily of antioxidants such as salvianolic acid B, danshensu, and protocatechuic aldehyde, which are thought to mediate the antiatherogenic effects of *S. miltiorrhiza*.

One of the earliest events in atherogenesis is the adhesion of monocytes to the endothelium, followed by their infiltration and differentiation into macrophages. This key step is mediated by the interaction of monocytes with molecules expressed on the surface of endothelial cells (Price and Loscalzo, 1999; Faggiotto et al., 1984). These adhesion molecules primarily mediate the adhesion of monocytes and lymphocytes, cells specifically found in atherosclerosis lesions, to the vascular endothelium (O'Brien et al., 1993; Faggiotto et al., 1984; Iiyama et al., 1999). The first steps in endothelial-monocyte adhesion appear to depend on the interaction of P- and E-selectin with carbohydrate ligands on the surfaces of the leukocytes. Firm adhesion follows if the leukocytes encounter activating signals while rolling along the endothelium; this is facilitated by interaction of very late antigen-4 (VLA-4) with VCAM-1 or interaction of lymphocyte function antigen-1 (LFA-1) with ICAM-1 (Marlin and Springer, 1987; Elices et al., 1990). However, it was not previously known whether protocatechuic aldehyde selectively inhibits cytokine-induced adhesion molecules expression and reduces monocyte adhesion to endothelial cells.

In the present study, we investigated the effects of protocatechuic aldehyde on expression of endothelial adhesion molecules and monocyte adhesion to HUVECs. We found that TNF-α-induced VCAM-1 and ICAM-1 expression was selectively blocked by protocatechuic aldehyde treatment in a concentration-dependent manner, but did not affect secretion of E-selectin. This is consistent with the previously reported effect of salvianolic acid B and aqueous ethanolic extracts of *S. miltiorrhiza* on human aortic endothelial cells (Chen et al., 2001a). It should be pointed out that that co-culture of HUVECs with protocatechuic aldehyde and TNF-α was not cytotoxic, as determined by MTS assay (data not shown).

It has been demonstrated that oxidative stress upregulates the expression of cell adhesion molecules via redox-sensitive transcriptional activation, and that this is inhibited by the classical antioxidant, vitamin E (Martin et al., 1997). In addition, several kinds of natural antioxidants, including catechins, flavonoids, and related polyphenolic compounds, have been shown to inhibit adhesion molecule expression and the adhesion of monocytes to endothelial cells (Choi et al., 2004; Ludwig et al., 2004). Since atherosclerosis is a chronic inflammatory disease associated with increased oxidative stress in the vascular endothelium, it is possible that the antiatherogenic effects of antioxidants are mainly due to their antioxidative properties. Aqueous ethanolic extracts of *S. miltiorrhiza* are rich in the polyphenolic compounds that are effective in protecting liver microsomes, hepatocytes, and erythrocytes against oxidative damage (Liu et al., 1992). Such extracts have been reported to reduce endothelial damage and decrease the severity of atherosclerosis in New Zealand white rabbits fed a high cholesterol diet. Since protocatechuic aldehyde is a potent antioxidant, it would be reasonable to presume that the effects of this compound on reduced VCAM-1and ICAM-1 expression in activated HUVECs may be mainly due to its antioxidative properties. This finding, in conjunction

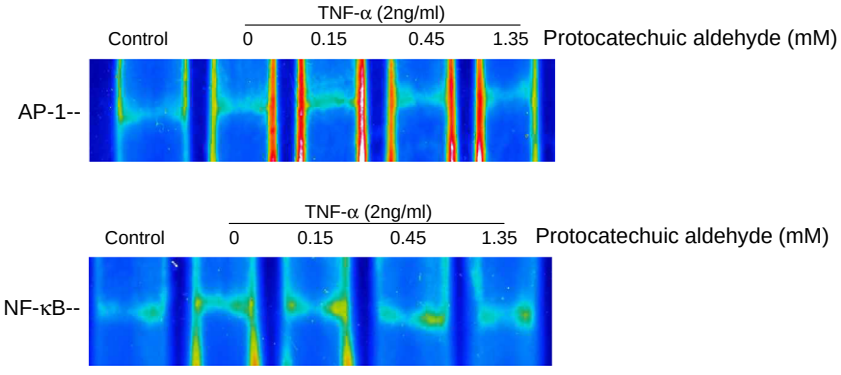

Fig. 7. False color image of NF-κB and AP-1 DNA binding activity measured by fEMSA. HUVECs were in the presence of 0, 0.15, 0.45, and 1.35 mM protocatechuic aldehyde for 18 h and then treated with or without TNF-α (2 ng/ml) for 6 h. Nuclear extracts were prepared and NF-κB and AP-1 DNA binding activities were measured by fEMSA.

with the previous reports of Chen et al. (2001a) and Wu et al. (1998), seems to indicate that protocatechuic aldehyde may play an important role in the anti-artherosclerotic effects of *S. miltiorrhiza*.

This effect may involve a number of molecules, including transcription factors. AP-1, a transcription factor involved in the progression of atherosclerosis, regulates the genes encoding matrix metalloproteinases, cytokines, chemokines, adhesion molecules, inducible nitric oxide synthase, cell cycle proteins, and the Fas ligand (Angel and Karin, 1991). Another transcription factor, NF-κB, is recognized as one of the major transcription factors influencing key steps in the development of atherosclerotic lesions. The activation of NF-κB is involved in endothelial dysfunction and seems to be linked to the redox sensitivity of itself. NF-κB mobilization (and its suppression) is involved in the signal transduction pathways for TNF-α-induced adhesion molecule expression (Beg et al., 1993; Neish et al., 1995a,b). However, maximum induction of transcription by cytokine stimulation requires the combined action of different transcription factors in addition to NF-κB: AP-1, SP-1, GATA, and IRF are required for VCAM-1 induction (Iademarco et al., 1992; Neish et al., 1992), SP-1 and AP-1 are needed for ICAM expression, and CRE/ATF and AP-1 facilitate expression of E-selectin (Collins et al., 1995; Neish et al., 1995b; Voraberger et al., 1991). We observed that the activations of TNF-α-stimulated AP-1 and NF-κB were both attenuated by protocatechuic aldehyde, suggesting that protocatechuic aldehyde at least partially impairs the AP-1 and NF-κB pathways specific to cytokine-stimulated induction of VCAM-1 and ICAM-1 expression.

In conclusion, the present results demonstrate that protocatechuic aldehyde selectively inhibits cytokine-induced VCAM-1 and ICAM-1 expression and reduces monocyte adhesion to endothelial cells through a mechanism involving NF-κB and AP-1. Since monocyte adhesion to endothelial cells and subsequent recruitment into the vascular wall is a crucial step in the pathogenesis of atherosclerosis, our data seem to support the idea that protocatechuic aldehyde has inhibiting effects on pro-atherosclerotic mechanisms in vitro.Technology Research and Development Project.

Acknowledgements

This work was supported by grants from State 863 High Technology Research and Development Project of China (No. 2003AA2Z2042) and National Natural Science Foundation of China (No. 30271617).

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