British Journal of Nutrition (2007), **97**, 692–698
DOI: 10.1017/S0007114507666409
© The Authors 2007
https://doi.org/10.1017/S0007114507666409 Published online by Cambridge University Press

Ellagic acid inhibits IL-1β-induced cell adhesion molecule expression in human umbilical vein endothelial cells

Ya-Mei Yu[1]*, Zhi-Hong Wang[1], Chung-Hsien Liu[2] and Chin-Seng Chen[3]

[1]*Department of Nutrition, China Medical University, 91, Hsueh-Shih Road, Taichung, Taiwan*
[2]*School of Medicine, Chung Shan Medical University, 110, Sec 1, Chien-kuo N Road, Taichung, Taiwan*
[3]*Department of Biotechnology, Chang Jung Christian University, 396 Chang Jung Road, Sec 1, Kway Jen, Tainan, Taiwan*

(Received 8 June 2006 – Revised 6 October 2006 – Accepted 6 November 2006)

Expression of cell adhesion molecules by endothelium and the attachment of monocytes to endothelium may play a major role in atherosclerosis. Ellagic acid (EA) is a phenolic compound found in fruits and nuts including raspberries, strawberries, grapes and walnuts. Previous studies have indicated that EA possesses antioxidant activity *in vitro*. In the present study, we investigated the effects of EA on the formation of intracellular reactive oxygen species, the translocation of NFκB and expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 and endothelial leucocyte adhesion molecule (E-selectin) induced by IL-1β in human umbilical vein endothelial cells (HUVEC). We found that EA significantly reduced the binding of human monocytic cell line, U937, to IL-1β-treated HUVEC. The production of reactive oxygen species by IL-1β was dose-dependently suppressed by EA. Supplementation with increasing doses of EA up to 50 μmol/l was most effective in inhibiting the expression of VCAM-1 and E-selectin. Furthermore, the inhibition of IL-1β-induced adhesion molecule expression by EA was manifested by the suppression of nuclear translocation of p65 and p50. In conclusion, EA inhibits IL-1β-induced nuclear translocation of p65 and p50, thereby suppressing the expression of VCAM-1 and E-selectin, resulting in decreased monocyte adhesion. Thus, EA has anti-inflammatory properties and may play an important role in the prevention of atherosclerosis.

Ellagic acid: Cell adhesion molecule: NFκB: Reactive oxygen species: Atherosclerosis

Activation of the vascular endothelium, increased adhesion of mononuclear cells to the injured endothelial layer, and their subsequent extravasations into the vessel wall are initial events in atherogenesis. Endothelial cells recruit leucocytes by expressing adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and endothelial leucocyte adhesion molecules (E-selectin; Cybulsky & Gimbrone, 1991). Several inflammatory cytokines including IL-1, TNF and interferon produced by activated monocytes and macrophages may stimulate the endothelium to up-regulate genes encoding chemokines, other cytokines and adhesion molecules (Ross, 1999).

Previous studies have indicated that NF-κB/Rel transcription factors may play an important role in the development of atherosclerosis (Collins, 1993; Qwarnstrom *et al.* 1994). Activation of NF-κB by inflammatory stimuli has been demonstrated in cultures of endothelial cells using electrophoretic mobility shift assays (Collins, 1993). A variety of genes induced in the atherosclerotic lesion have been shown to be regulated by NF-κB proteins, including the genes encoding TNF-α (Baeuerle & Henkel, 1994), IL-1β (Hiscott *et al.* 1993), VCAM-1 (Neish *et al.* 1992) and ICAM-1 (Poston *et al.* 1992).

It is well established that dietary polyphenolic compounds play significant roles in the prevention of atherosclerosis and CVD (Gaziano *et al.* 1992; Gey *et al.* 1993). Polyphenolic compounds affect the development of atherosclerosis not only through modulation of serum lipids but also by influencing the immune and inflammatory processes associated with the development of this disease. Previous studies have indicated that polyphenolic compounds such as vitamin E or tea flavonoid may exert their effects through modulation of cytokines, adhesion molecules and interaction of immune cells with endothelial cells (Martin *et al.* 1997; Islam *et al.* 1998; Ludwig *et al.* 2004). Ellagic acid (EA) is a phenolic compound found in fruits including grape juice (10·2 mg/100 g), grape wine (5·6 mg/100 g), blueberries (0·9 mg/100 g), blackberries (42·4 mg/100 g), raspberries (17·9 mg/100 g) and strawberries (19·8 mg/100 g) (de Ancos *et al.* 2000; Sellappan *et al.* 2002; Mertens-Talcott *et al.* 2003). Previous studies have indicated that EA scavenges both oxygen and hydroxyl radicals, and inhibits lipid peroxidation (Cozzi *et al.* 1995; Laranjinha *et al.* 1996; Iino *et al.* 2001). In our laboratory, we found that EA reduced oxidative stress and atherosclerosis in a hyperlipidaemic rabbit model (Yu *et al.* 2005). Therefore, the present study was designed to examine the effect of EA on monocyte adhesion to cultured human endothelial cells and the

---

Abbreviations: EA, ellagic acid; E-selectin, endothelial leucocyte adhesion molecule; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular adhesion molecule-1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; VCAM-1, vascular cell adhesion molecule-1.

***Corresponding author:** Dr Ya-Mei Yu, fax +886 4 22083762, email ymyu@mail.cmu.edu.tw

https://doi.org/10.1017/S0007114507766409 Published online by Cambridge University Press

expression of adhesion molecules (VCAM-1, ICAM-1 and E-selectin) and to elucidate its possible mechanism.

Methods

Cell culture

Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase type II (Biochrom KG, Berlin, Germany) digestion of human umbilical veins by standard techniques and cultured in EC medium (MCDB 131; Gibco-BRL, Life Technologies GmbH, Karlsruhe, Germany) at 37°C in a humidified atmosphere of 5 % CO_2 and 95 % air as described previously (Stangl *et al.* 2001). All experiments were performed with HUVEC from passages one to three. HUVEC were seeded at 1×10^4 cells/well in ninety-six-well plates. After 3 d, the medium was replaced by fresh EC medium before treatment.

Cell viability assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Chen *et al.* 2002). The principle of this assay is that mitochondria dehydrogenase in viable cells reduces MTT to a blue formazan. Briefly, cells were grown in ninety-six-well plates and incubated with various concentrations of EA (which was dissolved in dimethyl sulphoxide) for 24 h; 100 μl MTT (0·5 mg/ml) were then added to each well and incubation continued at 37°C for an additional 4 h. The medium was then carefully removed, so as not to disturb the formazan crystals which had formed. Dimethyl sulphoxide (100 μl), which solubilizes formazan crystals, was added to each well and the absorbance of the solubilized blue formazan was read at 530 nml/l (reaction) and 690 nml/l (background) using a DIAS Microplate Reader (Dynex Technologies, Chantilly, VA, USA). The reduction in optical density caused by EA was used as a measurement of cell viability, normalized to cells incubated in control medium, which were considered 100 % viable.

Estimation of the production of reactive oxygen species

The production of intracellular reactive oxygen species (ROS) induced by IL-1β was estimated by a fluorometric assay using 2′,7′-dichlorofluorescein-diacetate (DCFH-DA) as a probe according to the method reported by Bass *et al.* (1983). HUVEC (1×10^6) were incubated with IL-1β and EA, suspended in PBS containing 2 % fetal calf serum, and then incubated again with 5 mmol/l 2′,7′-dichlorofluorescein-diacetate for 30 min at 37°C. The formation of 2′,7′-dichlorofluorescein was determined by flow cytometry. The excitation wavelength was 488 nm, and green fluorescence collected through a 530 nm band-pass filter was measured on a logarithmic scale. The formation of ROS was expressed as relative fluorescence intensity.

Real-time PCR for vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and endothelial leucocyte adhesion molecule

The real-time PCR assay for adhesion molecules was conducted according to the method reported by Li & Wang (2002). Total cellular RNA was isolated from samples (HUVEC) using the Trizol reagent according to the manufacturer's instructions (Gibco BRL). RT reactions were carried out for each RNA sample in thin-welled PCR tubes using the First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Each reaction tube contained 4·2 μg total RNA in a volume of 21 μg containing 1× RT buffer, 5·5 mmol/l $MgCl_2$, 500 μmol/l of each dNTP, 2·5 μmol/l of oligo-$d(T)_{12-18}$ primers, 40 U/μl RNase inhibitor, 2 U/μl *Escherichia coli* RNaseH and 50 U/μl of SuperScript II RT. RT reaction was carried out at 65°C for 5 min, 42°C for 50 min and 70°C for 15 min. The RT reaction mixture was then placed at 4°C for immediate PCR amplification or stored at −20°C for later use. Real-time PCR was performed in optical real-time PCR tubes. The following primers were used: VCAM-1: forward 5′-AAGCGGAGACAGGAGACAC-3′, reverse 5′-TGGCAGGTATTATTAAGGAGGATG-3′; ICAM-1: forward 5′-TGGTTCACAGGTTCAGATTAC-3′, reverse 5′-GACAAGAGGACAAGGCATAGC-3′; E-selectin: forward 5′-TGTGAGATGCGATGCTGTC-3′, reverse 5′-AACCTCTTCTGTCCATTGTCC-3′; glyceraldehyde-3-phosphate dehydrogenase: forward 5′-CCCACTCCTCCACCTTTG-3′, reverse 5′-CTTCCTCTTGTGCTCTTGC-3′.

Each tube contained 1 μl of each RT product (200 ng total RNA), 5·5 mmol/l $MgCl_2$, 400 μM-dNTP, 500 nmol/l primer (forward and reverse), 0·005 U/μl iTaq DNA polymerase and 20 nmol/l SYBR Green I in a total volume of 25 μl. Amplification conditions were 3 min at 95°C for activation, then run for forty cycles at 95°C for 15 s and 60°C for 1 min. All reactions were performed in the Bio-Rad iCycle Sequence Detection System using the iCycle V3.1 program. The threshold cycle (*Ct*) and melting point (*Mt*) were obtained during each reaction. The relative quantification was calculated based on its $2^{-\Delta Ct}$ value, $\Delta Ct = Ct$ (sample) − Ct (control).

Measurement of NF-κB activity

Nuclear extracts were prepared as described previously (Dschietzig *et al.* 2001). Bradford reagent determined protein concentrations. For analysis of NF-κB activity, a TransAM NF-κB Family kit was used (Active Motif, Rixensart, Belgium). In this assay, ninety-six-well plates were coated with an oligonucleotide containing the consensus binding sequence for NF-κB 5′-GGGACTTTCC-3′. Specific primary antibodies included in the kit detected the binding of NF-κB family transcription factors to their consensus sequence. Experiments were analysed by an ELISA-based assay. A total of 10 μg nuclear extract was used in each experiment and processed according to the manufacturer's protocol. Briefly, nuclear extracts were incubated with the oligonucleotide-coated wells for 60 min. Where indicated a competitor for NF-κB binding (NF-κB wild-type consensus oligonucleotide) was added in molar excess prior to the probe. The wells were then washed and incubated with the primary antibodies for p65, p50, c-Rel, p52 and RelB for 60 min. After incubation with a horseradish peroxidase-conjugated secondary antibody, a substrate was added to produce blue colour and then for quantitation by a standard ELISA reader. The absorbance was read at 450 nm and the blanks were subtracted from all measurements. The data presented are the result of three independent experiments.

Monocyte-endothelial cell adhesion

HUVEC (2×10^5) were distributed into six-well plates and allowed to reach confluence. They were then incubated for 18 h with medium supplemented with EA at concentrations of 25 and 50 μmol/l according to the MTT test, followed by incubation for 6 h with 10 ng/ml IL-1β in the continued presence of EA. U937 cells, originally derived from a human histiocytic lymphoma and used for the monocyte-endothelial cell adhesion assay, were grown in RPMI-1640 medium (Gibco, New York, USA) containing 10 % fetal bovine serum and subcultured at a 1 : 5 ratio three times per week, labelled for 30 min at 37°C with calcein AM (10 nmol/l; Molecular Probe; Invitrogen) in RPMI-1640 medium and washed with PBS to remove free dye, and then resuspended in 10 % M-199 medium. Labelled U937 cells (1×10^6) were added to each HUVEC-containing well and incubated for 1 h. Non-adherent cells were removed by two gentle washes with PBS. Then, adherent U937 cells were determined by a fluorescence plate reader at an excitation wavelength of 485 nm and emission at 530 nm; HUVEC cell monolayers served as the blank.

Statistics

Results are presented as means and standard deviations. Statistical significance was determined by one-way ANOVA. Differences were considered significant at $P < 0.05$.

Results

Concentrations of ellagic acid for human umbilical vein endothelial cells

Cell viability was assayed by the MTT test. After 24 h incubation with 10, 25, 50, 75, 100 and 150 μmol/l EA, cell viability was 125·2 (SD 4·9), 122·3 (SD 4·4), 106·7 (SD 2·7), 91·3 (SD 1·8), 75·8 (SD 1·4) and 78·4 (SD 1·4) %, respectively, of control levels, the three highest concentrations causing a significant reduction in cell viability. Therefore, according to the MTT test we chose 25 and 50 μmol/l to do all the experiments.

Ellagic acid inhibits IL-1β-induced reactive oxygen species production in human umbilical vein endothelial cells

Fig. 1(A) shows the results of ROS production induced by IL-1β. The production of ROS decreased after addition of 25 and 50 μmol/l EA (Fig. 1(B, C)).

Ellagic acid inhibits IL-1β-induced cell surface expression of vascular cell adhesion molecule-1 and endothelial leucocyte adhesion molecule but not expression of intercellular adhesion molecule-1 in human umbilical vein endothelial cells

The effects of EA on IL-1β-induced VCAM-1, ICAM-1 and E-selectin expression by HUVEC were studied by pretreating HUVEC for 18 h with 25 or 50 μmol/l EA before addition of 10 ng/ml IL-1β. This resulted in reduced cell surface expression of VCAM-1 and E-selectin, but had no effect on cell surface expression of ICAM-1 (Fig. 2(A–C)).

Ellagic acid attenuates activation of NF-κB expression and nuclear translocation of NF-κB p65 and p50 in IL-1β-stimulated human umbilical vein endothelial cells

To examine whether the inhibitory effect of EA on the cytokine-induced expression of adhesion molecules is medicated via NF-κB, we measured the nuclear translocation of p65 and p50 protein of the NF-κB family of transcription factors. Incubation of IL-1β (10 ng/ml) for 6 h induced the nuclear translocation of p65 and p50 (Fig. 3(A, B)). Preincubation of HUVEC with 50 μmol/l EA prior to IL-1β stimulation did significantly prevent the nuclear translocation of p65 and p50 (Fig. 3(A, B)).

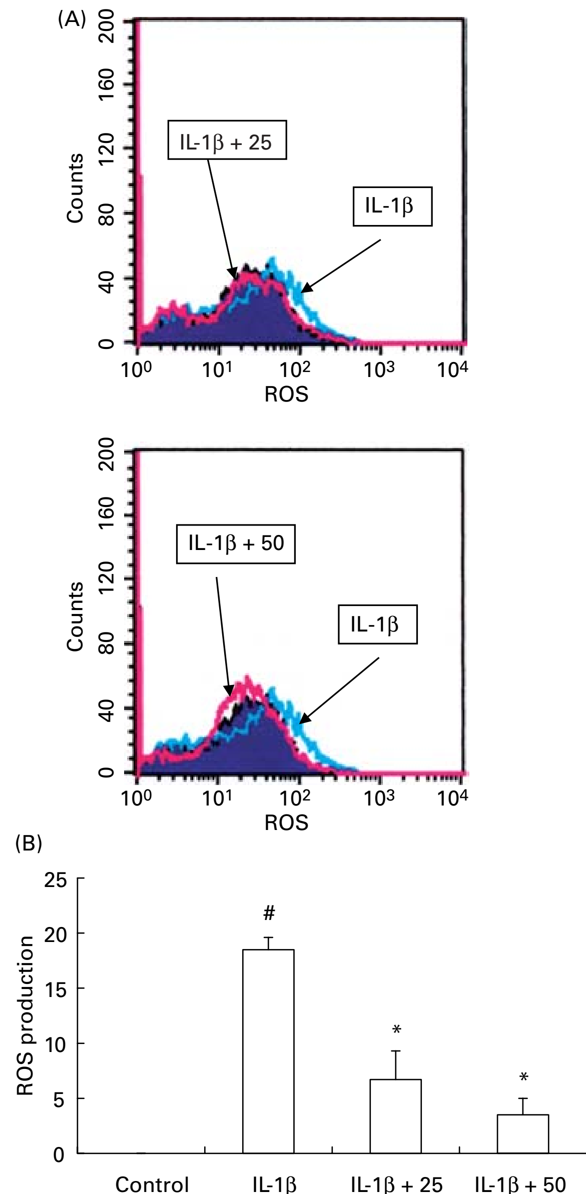

Fig. 1. Effect of ellagic acid on IL-1β-induced reactive oxygen species (ROS) production in human umbilical vein endothelial cells (HUVEC). HUVEC were stimulated with IL-1β after preincubation with 25 (IL-1β + 25) and 50 (IL-1β + 50) μmol/l ellagic acid. HUVEC were labelled with H_2O_2-sensitive fluorescent probe and were detected by flow cytometry (A). Mean ROS production was expressed as % of control (*n* 3) (B). Mean values were significantly different from those of the control group: #$P < 0.05$. Mean values were significantly different from those of the IL-1β group: *$P < 0.05$.

https://doi.org/10.1017/S0007114507766409 Published online by Cambridge University Press

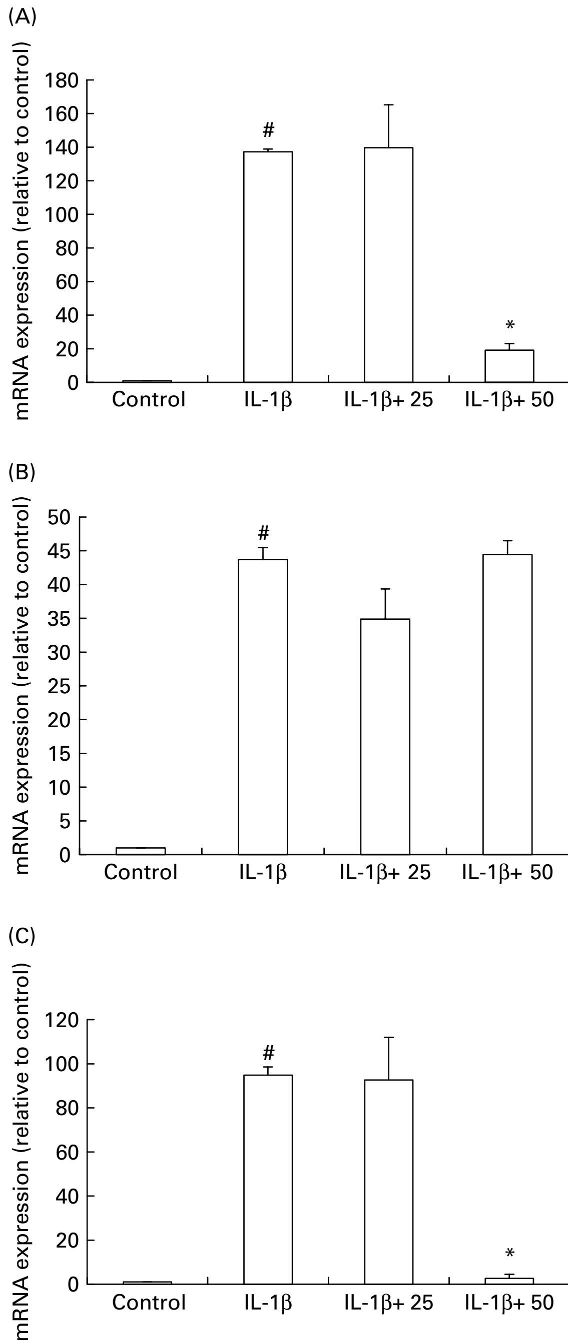

Fig. 2. Effect of ellagic acid on the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin in human umbilical vein endothelial cells (HUVEC). HUVEC were pretreated 25 (IL-1β + 25) and 50 (IL-1β + 50) μmol/l ellagic acid for 18 h and then induced by IL-1β for 6 h. The expression of VCAM-1 (A), ICAN-1 (B) and endothelial leucocyte adhesion molecule (E-selectin) (C) were measured by real-time PCR. Values are means with their standard deviations depicted by vertical bars (*n* 3). Mean values were significantly different from those of the control group: #$P<0.05$. Mean values were significantly different from those of the IL-1β group: *$P<0.05$.

Ellagic acid inhibits adhesion of U937 cells to IL-1β-stimulated human umbilical vein endothelial cells

To explore the effects of EA on endothelial cell leucocyte interactions, we examined the adhesion of U937 cells to

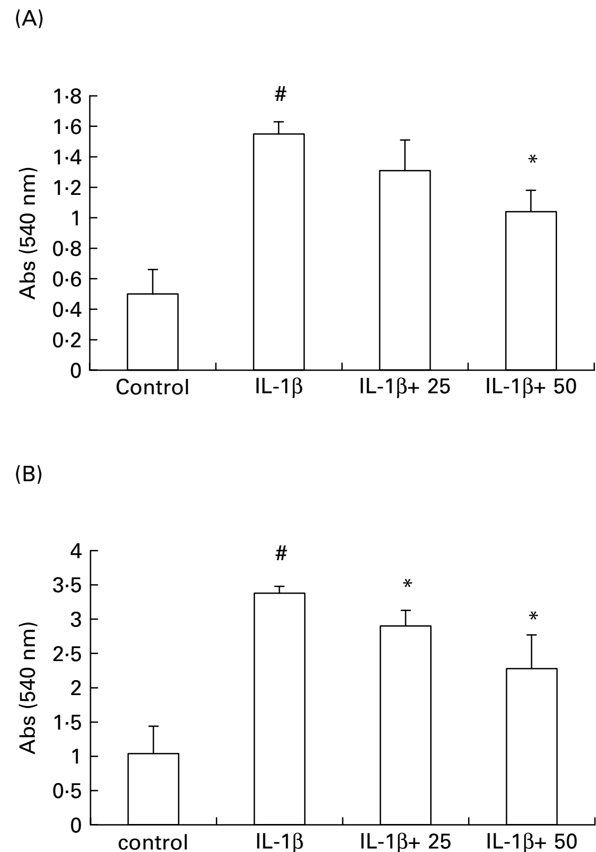

Fig. 3. Effect of ellagic acid on IL-1β-induced activation of NF-κB p-65 (A) and p-50 (B). Human umbilical vein endothelial cells (HUVEC) were pretreated with 25 (IL-1β + 25) and 50 (IL-1β + 50) μmol/l ellagic acid for 18 h and induced by IL-1β (10 ng/ml) for 6 h. Nuclear extracts were prepared and analysed for activation of NF-κB family. Five micrograms of nuclear protein was used in each experiment. Values are means with their standard deviations depicted by vertical bars (*n* 3). Mean values were significantly different from those of the control group: #$P<0.05$. Mean values were significantly different from those of the IL-1β group: *$P<0.05$.

cytokine-activated HUVEC. Control confluent HUVEC showed minimal binding to U937 cells, but adhesion increased when the HUVEC were treated with IL-1β (Fig. 4(A, B)). Pretreatment of HUVEC with 50 μmol/l EA reduced the number of U937 cells adhering to IL-1β-stimulated HUVEC (Fig. 4(A, B)).

Discussion

An early stage in atherosclerosis is the adhesion of monocytes to the arterial wall, followed by their infiltration and differentiation into macrophages. This key stage is mediated by the interaction of monocytes with adhesion molecules expressed by endothelial cells. In the present study, we found that 50 μmol/l EA treatment (50 μmol/l EA is equivalent to the dietary intake of approximately 200 g blackberries or 350 g strawberries; Walgren *et al.* 1998; Mertens-Talcott *et al.* 2003; Whitley *et al.* 2003) effectively blocked VCAM-1 and E-selectin expression in IL-1β-induced HUVEC. It significantly reduced the binding of human monocytic cell line U937 to IL-1β-induced HUVEC. Previous studies also

https://doi.org/10.1017/S0007114507766409 Published online by Cambridge University Press

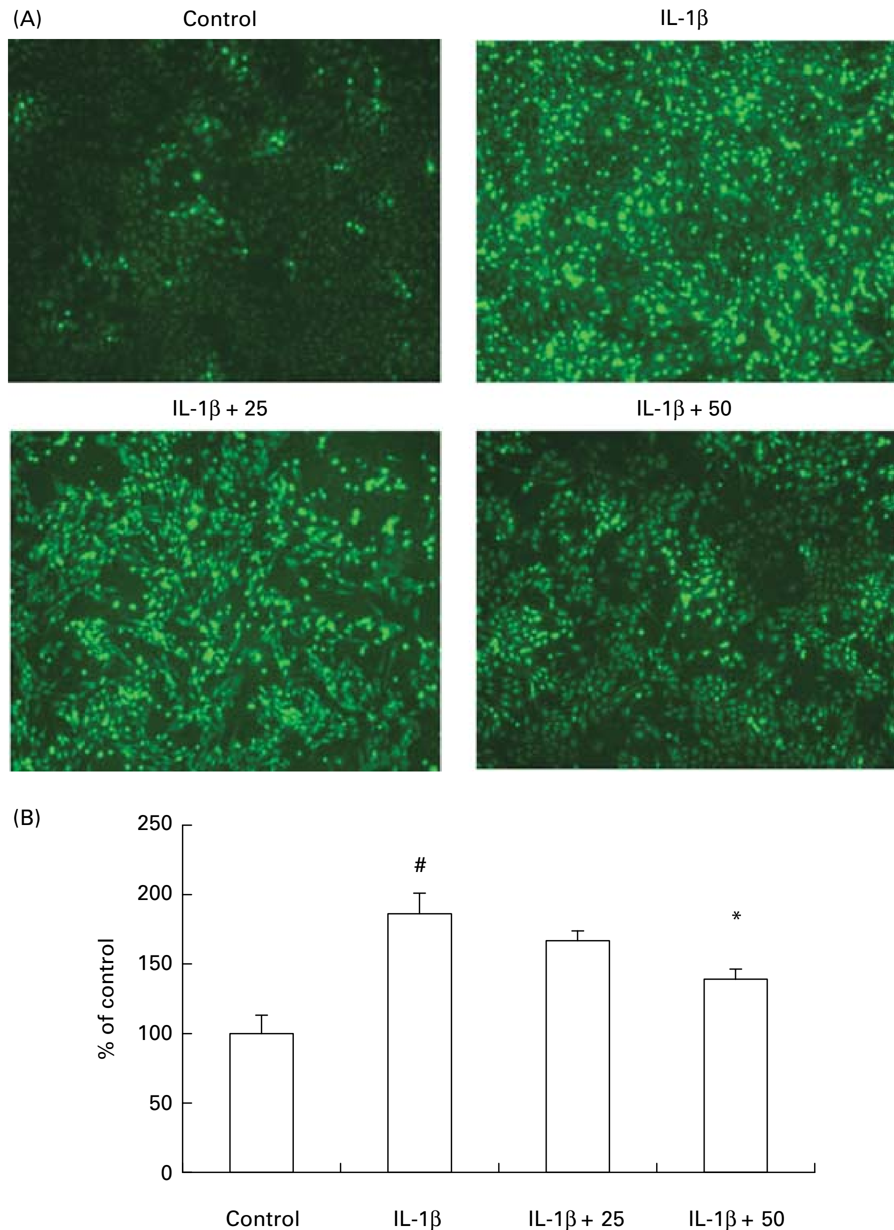

Fig. 4. Reduction effect of ellagic acid on IL-1β-induced adhesion of U937 cells to human umbilical vein endothelial cells (HUVEC). (A), Representative images of the reduction of IL-1β-induced adhesion of U937 cells to HUVEC monolayers after pretreatment of 25 (IL-1β + 25) and 50 (IL-1β + 50) μmol/l ellagic acid for 18 h. (B), HUVEC were pretreated with 25 (IL-1β + 25) and 50 (IL-1β + 50) μmol/l ellagic acid for 18 h and induced by IL-1β (10 ng/ml) for 6 h. Fluorescence-labelled U937 cells were added to the HUVEC monolayer and allowed to adhere for 30 min. Values are means with their standard deviations depicted by vertical bars (*n* 3). Mean values were significantly different from those of the control group: #$P < 0{\cdot}05$. Mean values were significantly different from those of the IL-1β group: *$P < 0{\cdot}05$.

showed that other polyphenolic compounds, such as vitamin E (40 μmol/l), probucol (50 μmol/l) or tea flavonoid (60 μmol/l epigallocatechin-3-gallate), reduce cytokine-induced adhesion molecule expression and monocyte adhesion to endothelial cells (Islam *et al.* 1998; Zapolska-Downar *et al.* 2001; Ludwig *et al.* 2004). In the present study, EA reduced cytokine-induced expression of VCAM-1 and E-selectin but not ICAM-1. A similar result was seen when HUVEC were pretreated with probucol; probucol reduced IL-1β-induced VCAM-1 surface protein and mRNA expression, but not ICAM-1 expression (Zapolska-Downar *et al.* 2001). Previous studies indicated that VCAM-1, but not ICAM-1, plays a critical role in the initiation of atherosclerosis (Cybulsky *et al.* 2001). VCAM-1 is expressed in vascular lesions in early atherosclerosis and has been found to be elevated in serum from patients with early atherosclerosis, suggesting that this adhesion protein is one of the key molecules involved in the atherogenic process (Cybulsky & Gimbrone, 1991; Rohde *et al.* 1998).

The NF-κB family controls the expression of genes involved in the inflammation and immune response (Baeuerle, 1991). In the cytoplasm, inactive NF-κB exists as a heterodi-

https://doi.org/10.1017/S0007114507666409 Published online by Cambridge University Press

meric complex of subunits p50 and p65 that binds to a cytoplasmic protein, IκB (Baeuerle & Henkel, 1994). Upon activation, IκB is rapidly degraded, and the p50/p65 heterodimer is translocated from the cytoplasm into the nucleus where the dimer interacts with regulatory κB elements in promoters and enhancers, thereby controlling gene transcription (Baeuerle & Baltimore, 1988; Grilli *et al.* 1993; Chenbg *et al.* 1994). NF-κB is activated by a multitude of stimuli, including inflammatory cytokines and reactive oxygen intermediates (Baeuerle & Baltimore, 1988; Grilli *et al.* 1993; Chenbg *et al.* 1994; Muller *et al.* 1997), which are activated in atherosclerotic lesions (Brand *et al.* 1996; Barnes & Karin, 1997; D'Acquisto *et al.* 2002). In the present study, we demonstrated that EA reduced cytokine-induced expression of VCAM-1 and E-selectin and prevented the nuclear translocation of p65 and p50 in endothelial cells. The present results suggest that the inhibitory mechanisms of EA might interrupt a signalling cascade involving VCAM transcription-mediated activation of NF-κB.

Several studies have indicated that ROS are implicated in the activation of NF-κB (Muller *et al.* 1997). The current study shows that the ROS production stimulated by IL-1β was decreased by EA pretreatment (Fig. 2(A–C)). Based on the present result, we propose that the inhibitory effect of EA on VCAM-1 expression and NF-κB activation may be due to its antioxidant properties and that it may act by directly scavenging free radicals. In one of our previous studies, we found that EA is approximately 2–3-fold more potent than Trolox in antioxidative ability. Our previous results showed that it scavenged α-α-diphenol-β-picrylhydrazyl (DPPH), alkoxyl radical (RO°) and peroxyl radical (ROO°) and inhibited LDL oxidation (Yu *et al.* 2005). Since atherosclerosis is a chronic inflammatory disease associated with increased oxidative stress in the vascular endothelium, it would be conceivable that the anti-atherogenic effects of EA might due to its antioxidative properties. The inhibition of cytokine-induced VCAM-1 expression has been described for other substances with antioxidant properties such as tea flavonoid epigallocatechin-3-gallate, probucol, magnolol, protocatechuic aldehyde and other flavonoids (Zapolska-Downar *et al.* 2001; Chen *et al.* 2002; Ludwig *et al.* 2004; Zhou *et al.* 2005).

In conclusion, EA inhibits IL-1β-induced VCAM-1 and E-selectin expression in HUVEC through a mechanism that involves NF-κB. It reduces the binding of human monocytic cell line U937 to IL-1β-induced HUVEC, which might be due to its antioxidant properties.

Acknowledgements

The research was supported by grants from the National Science Counsel of Taiwan (NSC 93-2320-B-039-017) and China Medical University (CMU 93-NT-01).

References

Baeuerle PA (1991) The inducible transcription activator NFκB: regulation by distinct protein subunits. *Biochim Biophys Acta* **1072**, 63–80.

Baeuerle PA & Baltimore D (1988) IκB: a specific inhibitor of the NFκB transcription factor. *Science* **242**, 540–546.

Baeuerle PA & Henkel T (1994) Function and activation of NF-κB in the immune system. *Annu Rev Immunol* **12**, 141–179.

Barnes PJ & Karin M (1997) Nuclear factor-kappa B: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* **336**, 1066–1071.

Bass DA, Parce JW, Decharelet LR, Szejda P, Seeds MC & Thomas M (1983) Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. *J Immunol* **130**, 1910–1917.

Brand K, Page S, Rogler G, Bartsch A, Brandl R, Knuechel R, Page M, Kalschmidt C, Baeuerle PA & Neumeier D (1996) Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. *J Clin Invest* **97**, 1715–1722.

Chen YH, Lin SJ, Chen JW, Ku HH & Chen YL (2002) Magnolol attenuates VCAM-1 expression *in vitro* in TNF-α-treated human aortic endothelial cells and *in vivo* in the aorta of cholesterol-fed rabbits. *Br J Pharmacol* **135**, 37–47.

Chenbg Q, Cant CA, Moll T, Hofer-Warbinek R, Wagner E, Birnstiel ML, Bach FH & de Martin R (1994) NF-κB subunit-specific regulation of the I kappa B-alpha promoter. *J Biol Chem* **296**, 13551–13557.

Collins T (1993) Biology of disease: endothelial nuclear factor-κB and the initiation of the atherosclerotic lesion. *Lab Invest* **68**, 499–508.

Cozzi R, Ricordy R, Bartolini F, Ramadori L, Perticone P & De Salvia R (1995) Taurine and ellagic acid: two differently-acting natural antioxidants. *Environ Mol Mutagen* **26**, 248–254.

Cybulsky MA & Gimbrone J (1991) Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* **251**, 788–791.

Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Liyama M, Davis V, Gutierrez-Ramos JC, Connelly PW & Milstone DS (2001) A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest* **107**, 1255–1262.

D'Acquisto F, May MJ & Ghosh S (2002) Inhibition of nuclear factor kappa B (NF-κB): an emerging theme in anti-inflammatory therapies. *Mol Intervent* **2**, 22–35.

de Ancos B, Gonzalez EM & Cano MP (2000) Ellagic acid, vitamin C and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *J Agric Food Chem* **48**, 4565–4570.

Dschietzig T, Richter C, Pfannenschmidt G, Bartsch C, Laule M, Baumann G & Stangl K (2001) Dexamethasone inhibits stimulation of pulmonary endothelins by proinflammatory cytokines: possible involvement of a nuclear factor κB dependent mechanism. *Intensive Care Med* **27**, 751–756.

Gaziano JM, Manson J, Buring JE & Hennekens CH (1992) Dietary antioxidants and cardiovascular disease. *Ann N Y Acad Sci* **669**, 249–259.

Gey KF, Stahelin HB & Eichholser M (1993) Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: Panel Prospective Study. *J Clin Invest* **71**, 3–6.

Grilli M, Chiu JJS & Lenardo MJ (1993) NFκB and Rel: participants in a multiform transcriptional regulatory system. *Int Rev Cytol* **143**, 1–62.

Hiscott J, Marois J, Garoufalis J, *et al.* (1993) Characterization of a functional NF-kappa B site in the human interleukin-1β promoter: evidence for a positive autoregulatory loop. *Mol Cell Biol* **13**, 6231–6240.

Iino T, Nakahara K, Miki W, Kiso Y, Ogawa Y, Kato S & Takeuchi K (2001) Less damaging effect of whisky in rat stomachs in comparison with pure ethanol. Role of ellagic acid, the nonalcoholic component. *Digestion* **64**, 214–221.

Islam KN, Devaraj S & Jialal I (1998) alpha-Tocopherol enrichment of monocytes decrease agonist-induced adhesion to human endothelial cells. *Circulation* **98**, 2255–2261.

https://doi.org/10.1017/S0007114507766409 Published online by Cambridge University Press

Laranjinha J, Vierira O, Almeida L & Madeira V (1996) Inhibition of metmyoglobin/H_2O_2-dependent low density lipoprotein lipid peroxidation by naturally occurring phenolic acid. *Biochem Pharmacol* **51**, 395–402.

Li X & Wang X (2002) Application of real-time polymerase chain reaction for the quantitation of interleukin-1β mRNA upregulation in brain ischemic tolerance. *Brain Res Protocols* **5**, 211–217.

Ludwig A, Lorenz M, Grimbo N, Steinle F, Meiners S, Bartsch C, Stangl K, Baumann G & Stangl V (2004) The tea flavonoid epigallocatechin-3-gallate reduces cytokine-induced VCAM-1 expression and monocyte adhesion to endothelial cells. *Biochem Biophys Res Commun* **316**, 659–665.

Martin A, Foxall T, Blumberg JB & Meydani M (1997) Vitamin E inhibits low density lipoprotein-induced adhesion of monocytes to human aortic endothelial cells *in vitro*. *Arterioscler Thromb Vasc Biol* **17**, 429–436.

Mertens-Talcott SU, Talcott ST & Percival SS (2003) Low concentrations of Quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells. *J Nutr* **133**, 2669–2674.

Muller JM, Rupec RA & Baeuerle PA (1997) Study of gene regulation by NF-kappa B and AP-1 in response to reactive oxygen intermediates. *Methods* **11**, 301–312.

Neish AS, Williams AJ, Palmer HJ, Whitley MZ & Clollins T (1992) Functional analysis of the human vascular cell adhesion molecule-1 promoter. *J Exp Med* **176**, 1583–1593.

Poston RN, Haskard DO, Coucher JR, Gall NP & Johnson-Tidey RR (1992) Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am J Pathol* **140**, 665–673.

Qwarnstrom EE, Ostberg CO, Turk GL, Richardson CA & Bomsztyk K (1994) Fibronectin attachment activates the NF-κB p50/p65 heterodimer in fibroblasts and smooth muscle cells. *J Biol Chem* **269**, 30765–30768.

Rohde LE, Lee RT, Rivero J, Jamacochian M, Arroyo LH, Briggs W, Rifai N, Libby P, Creager MA & Ridker PM (1998) Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* **18**, 1765–1770.

Ross R (1999) Atherosclerosis is an inflammatory disease. *Am Heart J* **138**, S419–S420.

Sellappan S, Akoh CC & Krewer G (2002) Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *J Agric Food Chem* **50**, 2432–2438.

Stangl V, Gunther A, Jarrin A, Bramlage P, Moobed M, Staudt A, Baumann G & Stangl K (2001) Homocysteine inhibits TNF-alpha-induced endothelial adhesion molecule expression and monocyte adhesion via nuclear factor-κB dependent pathway. *Biochem Biophys Res Commun* **280**, 1093–1100.

Walgren RA, Walle UK & Walle T (1998) Transport of quercetin and its glucosides across human intestinal epithelial Caco-2 cells. *Biochem Pharmacol* **55**, 1721–1727.

Whitley AC, Stoner GD, Darby MV & Walle T (2003) Intestinal epithelial cell accumulation of the cancer preventive polyphenol ellagic acid – extensive binding to protein and DNA. *Biochem Pharmacol* **66**, 907–915.

Yu YM, Chang WC, Wu CH & Chiang SY (2005) Reduction of oxidative stress and apoptosis in hyperlipidemic rabbits by ellagic acid. *J Nutr Biochem* **16**, 675–681.

Zapolska-Downar D, Zapolski-Downar A, Markiewski M, Diechanowicz A, Kaczmarczyk M & Naruszewicz M (2001) Selective inhibition by probucol of vascular cell adhesion molecule-1 (VCAM-1) expression in human vascular endothelial cells. *Atherosclerosis* **155**, 123–130.

Zhou Z, Liu Y, Miao AD & I Wang SQ (2005) Protocatechuic aldehyde suppresses TNF-α-induced ICAM-1 and VCAM-1 expression in human umbilical vein endothelial cells. *Eur J Pharmacol* **513**, 1–8.

https://doi.org/10.1017/S0007114507766409 Published online by Cambridge University Press