Epigallocatechin-3-O-Gallate Inhibits the Angiotensin II-Induced Adhesion Molecule Expression in Human Umbilical Vein Endothelial Cell Via Inhibition of MAPK Pathways

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Key Words

VCAM • ICAM • Catechins • Mitogen-activated protein kinase • EGCG • Angiotensin II • Atherosclerosis • Green tea

Abstract

Epigallocatechin-3-O-gallate (EGCG) is the main catechin, which is derived from *Camellia sinensis* plant. Vascular cell adhesion molecules (VCAMs) and intercellular adhesion molecules (ICAMs) mediate the binding of inflammatory cells onto the vascular wall-promoting the early phase of atherosclerosis. In the present study, we investigated the mechanism(s) by which EGCG inhibits angiotensin II (Ang II)-induced elevation of the membrane associated VCAM-1 and ICAM-1 in human umbilical vein endothelial cells (HUVEC). Ang II induced a 40% increase of VCAM-1 and ICAM-1 in the plasma membrane. EGCG (10 to 50 μ M) inhibited the effect of Ang II in a concentra-

tion-dependent manner. In parallel, the Ang II-induced elevation of the mRNA expressions of VCAM-1 and ICAM-1 in HUVEC were completely inhibited by 50 μ M EGCG. Since mitogen-activated protein kinase (MAPK) families are involved in vascular inflammation in response to stressful stimuli, we investigated the effects of EGCG on the MAPK signal transduction pathway stimulated by Ang II. EGCG (30 to 50 μ M) completely inhibited the Ang II-induced phosphorylation of ERK (extracellular signal-regulated kinase) 1/2 and p38 MAPK. PD98059, an inhibitor of ERK1/2 inhibited the Ang II-induced increase of VCAM-1 but not of ICAM-1 in the plasma membranes. In contrast, SB203580, an inhibitor of p38 MAPK inhibited both the Ang II-induced enrichment of ICAM-1 and VCAM-1. From these results, it may be concluded that EGCG inhibits the Ang II-induced elevation of VCAM-1 and ICAM-1 in the HUVEC plasma membranes via inhibition of the p38 MAPK and the ERK1/2 signalling pathways resulting in an inhibition of the VCAM-1 and ICAM-1 transcription.

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Introduction

Several sources of evidence support the finding that adhesion molecules play a crucial role in the development of atherosclerosis and plaque instability. Expression of intercellular adhesion molecule-1 (ICAM-1) has been observed in human atherosclerotic plaques [1]. During the early phases of atherosclerosis, selectins and their ligands are involved in the rolling and tethering of leukocytes onto the vascular wall. ICAMs and vascular cell adhesion molecules (VCAMs), as well as some of the integrins, induce firm adhesion of inflammatory cells onto the vascular wall. Platelet endothelial cellular adhesion molecules (PECAMs) are involved in extravasations of cells from the blood compartment into the vessel and underlying tissue [2].

Accumulating evidence implicates angiotensin II (Ang II) in the development of cardiovascular diseases such as hypertension, atherosclerosis and restenosis after vascular injury [3]. Ang II accelerated the rate of atherosclerosis by increasing monocyte binding to the endothelium [4]. Angiotensin-converting enzyme inhibition prevented arterial nuclear factor-kappa B (NF- κ B) activation, monocyte chemoattractant protein-1 expression and macrophage infiltration in a rabbit model [5]. Moreover, it has been shown that the Ang II-induced leukocyte adhesion on human coronary endothelial cells was induced by E-selectin [6]. Ang II induced an intracellular oxidative stress in endothelial cells, which stimulates IkappaB (IkB) degradation and NF- κ B activation. This activation enhanced the expression of VCAM-1 involved in the early stages of atherosclerosis [7].

Flavonoids are biologically active polyphenolic compounds widely distributed in plants. Flavonoid consumption may be associated with decreased risk of some chronic degenerative diseases in humans [8]. In this context, quercetin, a naturally occurring dietary flavonol, inhibited lipopolysaccharide-induced expression of endothelial cell intracellular adhesion molecule-1 [9]. Resveratrol, a polyphenolic phytoalexin present in red wine, suppressed the IL-6-induced ICAM-1 gene expression in endothelial cells [10]. Epigallocatechin-3-gallate (EGCG), a flavanol that is enriched in green tea, reduced TNF α -induced VCAM-1 expression and monocyte adhesion to endothelial cells [11]. Naringin, a bioflavonoid found in citrus fruit peel, significantly reduced fatty streak formation and neointimal macrophage infiltration and also inhibited the expression of ICAM-1 in endothelial cells [12].

The aim of this study was to investigate possible intracellular mechanisms by which Ang II induces the

expression of VCAM-1 and ICAM-1, and mechanisms by which EGCG is enabled to prevent the stimulatory effects of Ang II on VCAM-1 and ICAM-1 expression in human umbilical vein endothelial cells (HUVEC).

Materials and Methods

Materials

All reagents were purchased from Sigma Chemical, unless otherwise specified.

Cell isolation and culture

HUVECs were obtained from Clonetics (USA). HUVECs were grown in medium 199 (Gibco BRL, USA) containing 0.1 mg/ml heparin, 25 μ g/ml endothelial cell growth factor (Biomedical technology, USA), 2 mM L-glutamine, 100 U/ml penicillin G, 100 μ g/ml streptomycin and 20% FBS. At confluence, the cells were subcultured at a 1:3 ratio and then cultured in an atmosphere of 95% air and 5% CO₂ at 37°C.

Detection of plasma membrane associated ICAM-1 and VCAM-1 molecules

The assay for the determination of cell plasma membrane molecules was carried out by cell surface enzyme immunoassay (EIAs) using rabbit polyclonal antibodies against VCAM-1 (Ab E1/6), ICAM-1 (Ab HU5/3). HUVECs were seeded 1×10^4 cells/well onto 96-well plates and then cultured for 3 days in an atmosphere of 95% air and 5% CO₂ at 37°C. After treatment with Ang II or EGCG, HUVECs were fixed by 1% glutaraldehyde for 30 min at 4°C. EIAs were carried out by incubating monolayers first with saturating concentrations of ICAM-1 Ab or VCAM-1 Ab for 1 hour and then with horseradish peroxidase-conjugated secondary Ab (Santa Cruz Biotechnology, USA) for 1 hour at 37°C. Layers were washed 3 times with PBS with 0.05% Tween 20. The surface expression of each protein was quantified at a wavelength of 450 nm 30 minutes after addition of the chromogenic substrate (TMB, 3,3',5,5'-tetramethylbenzidine) and stopping the reaction with 2 M H₂SO₄.

Semi-quantitative RT-PCR analysis

Total RNA from 10^6 cells was isolated using RNeasy B (TEL-TEST, USA). RNA concentrations were determined by spectrophotometry at 260 nm. RNA (2 μ g) was dissolved in 20 μ l of the reaction mixture containing dATP, dTTP, dCTP, and dGTP at 2.5 mM each, 20 U RNasin, 100 pM random hexamers and 200 U superscript II reverse transcriptase (Invitrogen, USA). Incubation was carried out at 42°C for 20 min. The temperature of the reaction was then raised to 94°C for 5 min to inactivate the enzyme and finally reduced to 4°C. An aliquot of cDNA (5 μ l of RT mixture) was dissolved in 50 μ l of the reaction solution containing 10 μ l PCR buffer (final Mg²⁺ concentration was 1.5 mM), dATP, dTTP, dCTP, and dGTP at 2.5 mM each, 10 pM up- and downstream primers (VCAM-1, ICAM-1, GAPDH; Bionics, Korea) and 1 U Taq polymerase (SolGent, Korea). For semi-quantitative analyses, linearity of amplification in rela-

tion to the number of PCR cycles was established in preliminary experiments for VCAM-1, ICAM-1, and GAPDH. Optimal amplification to detect differences among the samples corresponded to 30 cycles for VCAM-1, 27 cycles for ICAM-1, 25 cycles for GAPDH. The following primer pairs were used: VCA M-1: sense 5'-ATG ACA TGC TTG AGC CAG G-3'; Antisense 5'-GTG TCT CCT TCT TTG ACA CT-3', ICAM-1: sense 5'-CAG TGA CCA TCT ACA GCT TTC CGG-3'; Antisense 5'-GCT GCT ACC ACA GTG ATG ATG ACA A-3', GAPDH: sense 5'-ATG ACA ACA GCC TCA AGA TCA TCA G-3'; Antisense 5'-CTG GTG GTC CAG GGG TCT TAC TCC T-3'. Amplification products obtained with PCR (VCAM-1 260 bp, ICAM-1 529 bp, GAPDH 515 bp) were electrophoretically separated on 1.5% agarose gel. Ethidium bromide stained bands of VCAM-1, ICAM-1 and GAPDH were photographed with DS-34 Polaroid camera. Band intensity was densitometrically measured with the gel analysis macro attached supplied with the NIH Image (available free from: <http://rsb.info.nih.gov/ni-image/>). VCAM-1 and ICAM-1 signals were normalized to cDNA levels of the housekeeping gene GAPDH and expressed as a ratio.

Western blot analysis

HUVECs were starved for 12 hours and treated with desired drugs for desired times. Cells were lysed in ice-cold buffer (20 mM Tris-HCl pH 7.4, 1 % Triton X-100, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM β -glycerolphosphate, 1 mM Na_3VO_4 , 1 mM PMSF, 1 $\mu\text{g}/\text{ml}$ leupeptin). The lysates were sonicated and centrifuged (12,000 rpm, 20 min). Protein concentration was measured by the method of Bradford. Equal amount of protein (10 μg) was run on 12% SDS-PAGE, blotted onto polyvinylidene difluoride membrane. Then the membrane was incubated with anti-phospho ERK 1/2, anti-phospho p38 MAPK (Cell Signaling Technology, USA), respectively. The antigen-antibody complexes were visualized with the use of appropriate secondary antibodies and the enhanced chemiluminescence (ECL) plus reagent kits (Amersham, UK), as recommended by the manufacturer.

Statistical Analysis

Results are shown as means $\pm$ SEM or SD from at least 3 independent experiments. Statistical significance between the means was assessed by one-way ANOVA followed by Turkey's multiple comparison test and P value of <0.05 was taken as statistically significant.

Results

Effects of EGCG on the Ang II-induced enrichment of endothelial adhesion molecules in the HUVEC plasma membranes

In order to investigate whether Ang II induces expression of VCAM-1 and ICAM-1 in endothelial cells and to demonstrate that EGCG is capable of inhibiting the expression, HUVEC were pre-treated with EGCG at the indicated concentrations for 30 minutes and then

Fig. 1. Effect of EGCG on the Ang II-induced increase of the VCAM-1 (A) and ICAM-1 (B). HUVEC were treated with EGCG for 30 min before stimulation with 1 μM Ang II for 8 hours. VCAM-1 and ICAM-1 protein level was determined by ELISA as described in Materials and Methods. Results are expressed as % of control. ICAM-1: Mean $\pm$ SEM, $n=13$, * $P < 0.01$ for Ang II effect vs control, ** $P < 0.05$ for 30 μM EGCG+Ang II effect vs Ang II effect, *** $P < 0.001$ for 50 μM EGCG+Ang II vs Ang II effect. VCAM-1: Mean $\pm$ SEM, $n=13$, * $P < 0.001$ for Ang II effect vs control, *** $P < 0.001$ for 10, 30, and 50 μM EGCG+Ang II effect vs Ang II effect.

stimulated with 1 μM Ang II for 8 hours. As indicated in Fig. 1, stimulation with Ang II caused a $41.7 \pm 5.1\%$ ($P < 0.001$, $n=13$) (A) and a $35 \pm 4\%$ ($P < 0.01$, $n=13$) (B) significant increase of VCAM-1 and ICAM-1 in the plasma membrane amount, respectively, compared to the control level (=100%). EGCG (30 and 50 μM) completely inhibited the Ang II-induced effect on ICAM-1 (Fig. 1A).

Fig. 2. Effect of EGCG on the Ang II-induced *ICAM-1* and *VCAM-1* mRNA expression in HUVEC. HUVEC were treated with EGCG for 30 minutes before Ang II (1 μM) application for 4 hours. *ICAM-1* (A) and *VCAM-1* mRNA (B) expression was analysed by RT-PCR as described in the Materials and Methods section. PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining, and then the bands of the products (*ICAM-1*: 529 bp, *VCAM-1*: 260 bp, *GAPDH*: 515 bp) were photographed under UV illumination. Diagrams below summarized results from densitometric analysis derived from three independent experiments (Mean±SD; n=3, *P<0.05 for Ang II effect vs control; **P<0.05 for EGCG+Ang II effect vs Ang II effect).

Interestingly, a complete inhibition of the Ang II-effect on *VCAM-1* was observed also at a concentration of 10 μM EGCG (Fig. 1B). EGCG did not affect the basal *VCAM-1* and *ICAM-1* expression (data not shown).

Effects of EGCG on the Ang II-induced VCAM-1 and ICAM-1 mRNA expression in HUVEC

To investigate the effect of EGCG on the Ang II-stimulated *ICAM-1* and *VCAM-1* expression, HUVEC were pre-treated with EGCG at the indicated concentrations for 30 minutes and stimulated with 1 μM Ang II for 8 hours. As shown in Fig. 2A, stimulation with Ang II caused a 80±16% significant increase of the expression level of *ICAM-1* (Mean±SD, n=3). Treatment of the cells

with 10 and 30 μM EGCG had no effect on the *ICAM-1* expression. Notably, treatment of the cells with 50 μM EGCG completely abolished the effect of Ang II. Ang II induced a 63±19% increase of the *VCAM-1* expression level (Fig. 2B). Treatment of the cells with 30 and 50 μM EGCG resulted in a complete inhibition of the *VCAM-1* expression (Mean±SD, n=3).

Effects of EGCG on the extracellular signal-regulated kinase 1/2 and p38 MAPK activation by Ang II in HUVEC

To demonstrate whether EGCG affects activation of MAPK pathways, we analyzed the phosphorylation levels of extracellular signal-regulated kinase 1/2 (ERK1,

Fig. 3. Effect of EGCG on Ang II-induced p38 MAPK (A) and ERK 1/2 (B) activation in HUVEC. HUVECs were pre-treated with EGCG in the indicated concentrations (10, 30, and 50 μM) for 30 minutes, then stimulated with 1 μM Ang II for 5 minutes. Activities of p38 MAPK and ERK 1/2 (p42/44 MAPK) were determined by Western blot analysis as described in the Methods and Materials section. Equally loading of the gel has been detected by detection of similar amounts of total p38 MAPK and ERK 1/2 (total p42/44).

p44 MAPK, ERK2 also known as p42 MAPK) and p38 MAPK. For this purpose, cells were pre-treated with 10 to 50 μM EGCG for 30 minutes and then stimulated with 1 μM Ang II for 5 minutes. As shown in Fig. 3, the Ang II-stimulated increases of ERK1/2 and p38 MAPK phosphorylation was inhibited by EGCG in a concentration-dependent manner, respectively. EGCG at a concentration of 50 μM completely abolished the effect of Ang II on the p38 MAPK and on the ERK1/2 phosphorylation. These results show that the effect of EGCG on Ang II-induced VCAM-1 and ICAM-1 expression in HUVEC may be mediated by an inhibition of ERK 1/2 and p38 MAPK signalling pathway.

Effects of PD98059 and SB203580 on Ang II-induced enrichment of VCAM-1 and ICAM-1 in the HUVEC plasma membrane

To demonstrate whether the Ang II-induced expression of VCAM-1 and ICAM-1 is induced by the ERK1/2 and/or by the p38 MAPK pathway, HUVEC were pre-treated with PD98059, an inhibitor of ERK1/2 kinase, and SB203580, an inhibitor of p38 MAPK at the indi-

Fig. 4. Effect of PD98059, an inhibitor of ERK 1/2 (p42/44 MAPK) and SB203580, an inhibitor of p38 MAPK, on the Ang II-induced increase of the VCAM-1 (A) or ICAM-1 (B) in plasma membrane of HUVEC. HUVEC were treated with Ang II (1 μM) for 8 hours. Inhibitor of MAPK was treated 30 minutes before Ang II application. Adhesion molecule formation was measured by ELISA as described in Materials and Methods. Results are expressed as % of control. ICAM-1: Mean±SEM, n=12, *P<0.05 for Ang II vs control, **P<0.05 for SB203580+Ang II effect vs Ang II effect. VCAM-1: Mean±SEM, n=12, *P<0.01 for Ang II vs control, **P<0.001 for PD98059+Ang II effect vs Ang II effect. **P<0.001 for SB203580+Ang II effect vs Ang II effect.

cated concentrations for 30 minutes and stimulated with 1 μM Ang II for 8 hours, respectively. As shown in Fig. 4, stimulation with Ang II caused a 49±11.5% (P<0.01, n=12) and a 72±19% (Mean±SEM, P<0.05, n=12) significant increase of VCAM-1 and ICAM-1 in the plasma membranes of HUVEC, respectively, compared to control levels (=100%). SB203580 (10 μM) completely inhibited the Ang II effect on both VCAM-1 (Fig. 4A) and

ICAM-1 (Fig. 4B). PD98059 also abolished the effect of Ang II on the amount of VCAM-1 in the HUVEC plasma membranes (Fig. 4A). However, 30 μ M PD98059 did not influence the effect of Ang II on the ICAM-1 plasma membrane amount (Fig. 4B). These results indicate that p38 MAPK as well as ERK 1/2 activation may be essential for Ang II-stimulated VCAM-1 expression in HUVEC. However, p38 MAPK rather than ERK 1/2 activation may play a role for Ang II-stimulated ICAM-1 expression in HUVEC.

Discussion

The present study demonstrates that activation of the HUVEC with Ang II results in an expression of *VCAM-1* and *ICAM-1* mRNA and in an enrichment of VCAM-1 and ICAM-1 in the plasma membranes. Remarkably, EGCG is an inhibitor of the Ang II-stimulated VCAM-1 and ICAM-1 expression in HUVEC. Moreover, since EGCG inhibited the Ang II-induced stimulation of the p38 MAPK and ERK1/2 and inhibition of these kinases by pharmacological agents resulted in an inhibition of the plasma membrane associated VCAM-1 and ICAM-1, we may conclude that EGCG prevents expression of the adhesion molecules via inhibition of the MAPK pathways. In this context, it is well known that the MAPK families are involved in vascular inflammation in response to stressful stimuli [13]. Our conclusion is based on the experiments demonstrating that Ang II rapidly increased the phosphorylation of ERK 1/2 and p38 MAPK and EGCG inhibited Ang II-stimulated ERK 1/2 and p38 MAPK phosphorylation in HUVEC. Therefore, it seems that ERK 1/2 and p38 MAPK activation may be required for the Ang II-induced expression of the vascular adhesion molecule expression in HUVEC. This hypothesis may be further strengthened by the observation that PD98059, an inhibitor of ERK 1/2, decreased VCAM-1 but not ICAM-1 expression stimulated by Ang II in HUVEC. On the other hand, SB203580, an inhibitor of p38 MAPK, decreased both VCAM-1 and ICAM-1 expression stimulated by Ang II in HUVEC. Therefore, the activation of p38 MAPK as well as ERK 1/2 may be required for Ang II-induced vascular adhesion molecule expression in HUVEC. However, we do not have an explanation why PD98059 did not affect ICAM-1 expression stimulated by Ang II in HUVEC. EGCG inhibits the activation of p38 MAPK as well as ERK 1/2 stimulated by Ang II, which results in decrease of VCAM-1 and ICAM-1 expression in HUVEC.

It has been well established that activation of nuclear factor (NF)- κ B by Ang II plays an essential role in stimulating expression of vascular adhesion molecules, which are essential for vascular inflammation [14]. In this perspective, Ang II activated NF- κ B in vascular smooth muscle cells (VSMCs) and signalling distal to mitogen-activated protein kinase kinase-1 (MEK1) involved extracellular signal-regulated kinase (ERK) [15]. Moreover, Ang II enhances VCAM-1 expression induced by the interleukin-1 β and the effect on VCAM-1 was independent of ERK activation in VSMCs [16]. On the other hand, Ang II induced NF κ B-dependent transcription in primary endothelial cell lines, leading to the upregulation of ICAM-1 and VCAM-1 expression [17]. Ang II also activates the inflammatory p38 MAPK in endothelial cells and pharmacological interference of the p38 MAPK pathway using the inhibitor SB 202190 prevented Ang II-mediated activation of the NF κ B target, VCAM [17]. It has been also reported that the ONOO⁻ peroxide activated ERK1/2 in neutrophils via the Ras/Raf-1/MEK signal transduction pathway, leading to an up-regulation of surface expression of CD11b/CD18 and consequently to an increased neutrophil adhesion to endothelial cells [13]. These findings emphasize the important role of the MAPK pathways as key regulators of the expression of the adhesion molecules and the process of the vascular inflammation.

On the other hand, EGCG prevented the induction of VCAM-1 expression in a concentration-dependent manner after stimulation with TNF- α . However, EGCG did not influence TNF- α -stimulated NF- κ B activation [11]. Recently, several phytochemicals have been shown to inhibit the expression of adhesions molecules and the process of cellular adhesions on the endothelial cells. In this context, resveratrol inhibited IL-6-induced ICAM-1 gene expression, in part, by interfering with Rac-mediated pathways via the attenuation of STAT3 phosphorylation [10]. Anthocyanins inhibited TNF- α -induced VCAM-1, ICAM-1, and cyclooxygenase-2 levels, which is through NF- κ B-dependent pathway [18]. Genistein, a principal component of most isoflavone preparations, inhibited monocyte adhesion to TNF- α -stimulated human vascular endothelial cells. This inhibition was dependent on the activation of endothelial peroxisomal proliferator-activated receptor-gamma [19]. Phloretin, a plant-derived phytochemical, prevented TNF- α -stimulated upregulation of VCAM-1, ICAM-1 and E-selectin expression. Phloretin did not affect TNF- α -stimulated activation of nuclear factor kappaB (NF- κ B) but inhibited activation of interferon regulatory factor 1, a transcription factor in-

volved in the regulation of endothelial cell adhesion molecule expression [20]. Olive oil and red wine antioxidant polyphenols transcriptionally inhibit endothelial adhesion molecule expression with relevance of NF- κ B, activator protein-1, and possibly GATA binding sites [21]. Inhibition of inducible VCAM-1 gene expression by grape seed proanthocyanidin extract was not through a NF- κ B-dependent pathway in HUVEC [22]. Quercetin down-regulated both phorbol 12-myristate 13-acetate (PMA)- and TNF- α -induced ICAM-1 expression via inhibiting both activator protein-1 (AP-1) activation and the c-Jun NH2-terminal kinase (JNK) pathway in human endothelial cell line ECV304 [23]. Therefore, the action mechanisms by which flavonoids suppress endothelial adhesion molecule expression may be different depending on the cytokines or the cell line used.

In conclusion, EGCG inhibits Ang II-induced VCAM-1 and ICAM-1 expression in HUVEC, which results from an inhibition of p38 MAPK and ERK 1/2 phosphorylation.

These results might contribute to the elucidation of

the mechanism of the inhibitory actions of EGCG and may help to better understand the beneficial effects of the green tea catechins in human epidemiological studies and in animal atherosclerosis models (for review see [24, 25]). However, the *in vivo* relevance of these results obtained from *in vitro* cell culture studies regarding the concentration of EGCG needs to be verified since EGCG concentrations exceed the plasma concentration of the catechins after drinking four to eight cups of green tea per day. However, enrichment of EGCG on tissues in a concentration higher than 10 μ M cannot be excluded.

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References

- Poston RN, Haskard DO, Coucher JR, Gall NP, Johnson-Tidey RR: Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am J Pathol* 1992;140:665-673.
- Blankenberg S, Barbaux S, Tiret L: Adhesion molecules and atherosclerosis. *Atherosclerosis* 2003;170:191-203.
- Nakashima H, Suzuki H, Ohtsu H, Chao JY, Utsunomiya H, Frank GD, Eguchi S: Angiotensin ii regulates vascular and endothelial dysfunction: Recent topics of angiotensin ii type-1 receptor signaling in the vasculature. *Curr Vasc Pharmacol* 2006;4:67-78.
- Kim JA, Berliner JA, Nadler JL: Angiotensin ii increases monocyte binding to endothelial cells. *Biochem Biophys Res Commun* 1996;226:862-868.
- Hernandez-Presa M, Bustos C, Ortego M, Tunon J, Renedo G, Ruiz-Ortega M, Egido J: Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-kappa b activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. *Circulation* 1997;95:1532-1541.
- Grafe M, Auch-Schwelk W, Zakrzewicz A, Regitz-Zagrosek V, Bartsch P, Graf K, Loebe M, Gaehtgens P, Fleck E: Angiotensin ii-induced leukocyte adhesion on human coronary endothelial cells is mediated by e-selectin. *Circ Res* 1997;81:804-811.
- Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Arnal JF, Michel JB: Angiotensin ii stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappa b activation induced by intracellular oxidative stress. *Arterioscler Thromb Vasc Biol* 2000;20:645-651.
- Middleton E, Jr., Kandaswami C, Theoharides TC: The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacol Rev* 2000;52:673-751.
- Middleton E, Jr., Anne S: Quercetin inhibits lipopolysaccharide-induced expression of endothelial cell intracellular adhesion molecule-1. *Int Arch Allergy Immunol* 1995;107:435-436.
- Wung BS, Hsu MC, Wu CC, Hsieh CW: Resveratrol suppresses il-6-induced icam-1 gene expression in endothelial cells: Effects on the inhibition of stat3 phosphorylation. *Life Sci* 2005;78:389-397.
- Ludwig A, Lorenz M, Grimbo N, Steinle F, Meiners S, Bartsch C, Stangl K, Baumann G, Stangl V: The tea flavonoid epigallocatechin-3-gallate reduces cytokine-induced vcam-1 expression and monocyte adhesion to endothelial cells. *Biochem Biophys Res Commun* 2004;316:659-665.

- 12 Choe SC, Kim HS, Jeong TS, Bok SH, Park YB: Naringin has an antiatherogenic effect with the inhibition of intercellular adhesion molecule-1 in hypercholesterolemic rabbits. *J Cardiovasc Pharmacol* 2001;38:947-955.
- 13 Zouki C, Zhang SL, Chan JS, Filep JG: Peroxynitrite induces integrin-dependent adhesion of human neutrophils to endothelial cells via activation of the raf-1/mek/erk pathway. *Faseb J* 2001;15:25-27.
- 14 Zhang L, Cheng J, Ma Y, Thomas W, Zhang J, Du J: Dual pathways for nuclear factor kappaB activation by angiotensin II in vascular smooth muscle: Phosphorylation of p65 by I kappaB kinase and ribosomal kinase. *Circ Res* 2005;97:975-982.
- 15 Zhang L, Ma Y, Zhang J, Cheng J, Du J: A new cellular signaling mechanism for angiotensin II activation of NF-kappaB: An I kappaB-independent, RSK-mediated phosphorylation of p65. *Arterioscler Thromb Vasc Biol* 2005;25:1148-1153.
- 16 Jiang B, Xu S, Hou X, Pimentel DR, Cohen RA: Angiotensin II differentially regulates interleukin-1-beta-inducible NO synthase (iNOS) and vascular cell adhesion molecule-1 (VCAM-1) expression: Role of p38 MAPK. *J Biol Chem* 2004;279:20363-20368.
- 17 Costanzo A, Moretti F, Burgio VL, Bravi C, Guido F, Leviero M, Puri PL: Endothelial activation by angiotensin II through NF-kappaB and p38 pathways: Involvement of NF-kappaB-inducible kinase (NIK), free oxygen radicals, and selective inhibition by aspirin. *J Cell Physiol* 2003;195:402-410.
- 18 Kim HJ, Tsou I, Park JM, Chung JJ, Shin SC, Chang KC: Anthocyanins from soybean seed coat inhibit the expression of TNF-alpha-induced genes associated with ischemia/reperfusion in endothelial cell by NF-kappaB-dependent pathway and reduce rat myocardial damages incurred by ischemia and reperfusion in vivo. *FEBS Lett* 2006;580:1391-1397.
- 19 Chacko BK, Chandler RT, Mundhekar A, Khoo N, Pruitt HM, Kucik DF, Parks DA, Kevil CG, Barnes S, Patel RP: Revealing anti-inflammatory mechanisms of soy isoflavones by flow: Modulation of leukocyte-endothelial cell interactions. *Am J Physiol Heart Circ Physiol* 2005;289:H908-915.
- 20 Stangl V, Lorenz M, Ludwig A, Grimbo N, Guether C, Sanad W, Ziemer S, Martus P, Baumann G, Stangl K: The flavonoid phloretin suppresses stimulated expression of endothelial adhesion molecules and reduces activation of human platelets. *J Nutr* 2005;135:172-178.
- 21 Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, D'Amico A, De Caterina R: Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: Antiatherogenic properties of Mediterranean diet phytochemicals. *Arterioscler Thromb Vasc Biol* 2003;23:622-629.
- 22 Sen CK, Bagchi D: Regulation of inducible adhesion molecule expression in human endothelial cells by grape seed proanthocyanidin extract. *Mol Cell Biochem* 2001;216:1-7.
- 23 Kobuchi H, Roy S, Sen CK, Nguyen HG, Packer L: Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway. *Am J Physiol* 1999;277:C403-411.
- 24 Gouni-Berthold I, Sachinidis A: Molecular mechanisms explaining the preventive effects of catechins on the development of proliferative diseases. *Curr Pharm Des* 2004;10:1261-1271.
- 25 Mariappan D, Winkler J, Parthiban V, Doss MX, Hescheler J, Sachinidis A: Dietary small molecules and large-scale gene expression studies: An experimental approach for understanding their beneficial effects on the development of malignant and non-malignant proliferative diseases. *Curr Med Chem* 2006;13:1481-1489.