Korean J Physiol Pharmacol
Vol 9: 117–123, April, 2005

Inhibition of Angiotensin II-Induced Vascular Smooth Muscle Cell Hypertrophy by Different Catechins

Ying Zheng[1], Hye Jin Song[1], Seok Hee Yun[1], Yeon Jeong Chae[1], Hao Jia[1], Chan Hyung Kim[1], Tae Sun Ha[2], Agapios Sachinidis[3], Hee Yul Ahn[1], and Sandra T. Davidge[4]

Departments of [1]Pharmacology, [2]Pediatrics, College of Medicine, Chungbuk National University, Cheongju 361-763, [3]Center of Physiology and Pathophysiology, University of Cologne, Cologne 50931, Germany, [4]Department of Obstetrics and Gynecology and Physiology, Perinatal Research Centre, University of Alberta, Edmonton, Canada

A cumulative evidence indicates that consumption of tea catechin, flavan-3-ol derived from green tea leaves, lowers the risk of cardiovascular diseases. However, a precise mechanism for this cardiovascular action has not yet been fully understood. In the present study, we investigated the effects of different green tea catechins, such as epigallocatechin-3 gallate (EGCG), epigallocatechin (EGC), epicatechin-3 gallate (ECG), and epicatechin (EC), on angiotensin II (Ang II)-induced hypertrophy in primary cultured rat aortic vascular smooth muscle cell (VSMC). [^{3}H]-leucine incorporation was used to assess VSMC hypertrophy, protein kinase assay, and western blot analysis were used to assess mitogen-activated protein kinase (MAPK) activity, and RT-PCR was used to assess *c-jun* or *c-fos* transcription. Ang II increased [^{3}H]-leucine incorporation into VSMC. However, EGCG and ECG, but not EGC or EC, inhibited [^{3}H]-leucine incorporation increased by Ang II. Ang II increased phosphorylation of c-Jun, extracellular-signal regulated kinase (ERK) 1/2 and p38 MAPK in VSMC, however, EGCG and ECG , but not EGC or EC, attenuated c-Jun phosphorylation increased by Ang II. ERK 1/2 and p38 MAPK phosphorylation induced by Ang II were not affected by any catechins. Ang II increased *c-jun* and *c-fos* mRNA expression in VSMC, however, EGCG inhibited *c-jun* but not *c-fos* mRNA expression induced by Ang II. ECG, EGC and EC did not affect *c-jun* or *c-fos* mRNA expression induced by Ang II. Our findings indicate that the galloyl group in the position 3 of the catechin structure of EGCG or ECG is essential for inhibiting VSMC hypertrophy induced by Ang II via the specific inhibition of JNK signaling pathway, which may explain the beneficial effects of green tea catechin on the pathogenesis of cardiovascular diseases observed in several epidemiological studies.

Key Words: Angiotensin II, VSMC, Hypertrophy, Catechin, JNK

INTRODUCTION

Growth (hypertrophy and hyperplasia) of vascular smooth muscle cell (VSMC) is an important component in the initiation and progression of cardiovascular diseases such as atherosclerosis, restenosis and hypertension (Takahashi et al, 1997). VSMC hypertrophy represents an increase in cell size without a change in cell number (Berk et al, 1989). The increased mass of VSMC in chronic hypertension models is principally due to VSMC hypertrophy (Owens and Schwartz 1982; Owens & Schwartz 1983; Black et al, 1988).

Angiotensin II (Ang II), the main peptide of the renin-angiotensin system, stimulates cellular hypertrophy in cultured vascular smooth muscle (Berk et al, 1989; Geisterfer et al, 1988; Zheng et al, 2004). Signal transduction pathways underlying Ang II-induced growth response involve the activation of mitogen-activated protein kinases (MAPKs). Of these MAPKs, extracellular signal-regulated kinase (ERK 1/2), p38 MAPK and c-Jun N-terminal kinase (JNK) have been shown to be implicated in the hypertrophic responses of VSMC to Ang II (Servant et al, 1996; Ushio-Fukai et al, 1998; Yoshizumi et al, 2001; Zheng et al, 2004). Moreover, the survival kinase Akt/PKB and its downstream $p70^{S6K}$ play a role in Ang II-induced VSMC hypertrophy (Giasson & Meloche, 1995; Eguchi et al, 1999; Takahashi et al, 1999; Ushio-Fukai et al, 1999; Hixon et al, 2000). Activator protein-1 (AP-1) is the transcription factor participating in the regulation of hypertrophy related gene expression and composed of c-Jun and c-Fos heterodimer (Rivera & Greenberg, 1990). Ang II increases the expression of *c-jun* and *c-fos* in VSMC (McKay et al, 1998; Zheng et al, 2004). In addition, Ang II controls AP-1 transcriptional activity by phosphorylating ser-63 and ser-73 of c-Jun N-terminal residues (Pluverer et al, 1991; Kallunki et al, 1996). Thus, AP-1 as well as ERK1/2, p38

Corresponding to: Hee Yul Ahn, Department of Pharmacology, College of Medicine, Chungbuk National University, Cheongju 361-763, Korea. (Tel) 82-43-261-2850, (Fax) 82-43-272-1603, (E-mail) hyahn@chungbuk.ac.kr

ABBREVIATIONS: EGCG, epigallocatechin-3 gallate; EGC, epigallocatechin; ECG, epicatechin-3 gallate; EC, epicatechin; VSMC, vascular smooth muscle cell; Ang II, angiotensin II; MAPK, mitogen-activated protein kinase.

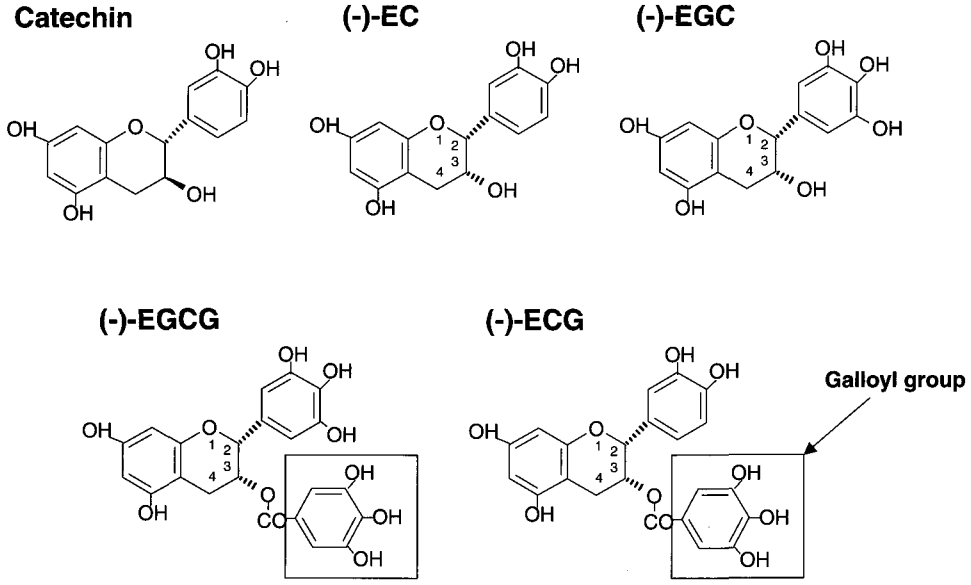

Fig. 1. Chemical structures of epigallocatechin-3 gallate (EGCG), epicatechin-3 gallate (ECG), epigallocatechin (EGC), and epicatechin (EC).

MAPK, JNK, Akt/PKB and the $p70^{S6K}$ play pivotal roles in Ang II-induced VSMC protein synthesis and cellular hypertrophy.

Epidemiological studies suggest that consumption of green tea is associated with reduced risk of cardiovascular diseases (Imai & Nakachi, 1995). Green tea consists mainly of catechins such as 2-(,4-dihydroxyphenyl)-3,4- dihydro-2*H*-1-benzopyran-3,5,7-triol (catechin), epicatechin (EC), epigallocatechin-3 gallate (EGCG), epicatechin-3 gallate (ECG), epigallocatechin (EGC), and catechin-3 gallate (CG) (Fig. 1). Of these catechins, EGCG, the most active catechin, has extensively been studied because of its diverse pharmacological effects. It has been reported that EGCG has anti-carcinogenic, anti-mutagenic, anti-oxidative, anti-inflammatory, anti-thrombogenic and anti-viral effects (Yamane et al, 1995; Santana-Rios et al, 2001; Kang et al, 2001; Benelli et al, 2002; Nagai et al, 2002; Yamaguchi et al, 2002). We have previously demonstrated that EGCG specifically suppressed Ang II-increased JNK activity followed by inhibition of VSMC hypertrophy (Zheng et al, 2004).

In the present study, we compared the effects of ECG, EGC, and EC on hypertrophy and on the intracellular signal transduction pathway of Ang II with those of EGCG in rat aortic VSMC.

METHODS

Reagents

The following drugs were used: anti-actin, -smooth muscle antibody (Sigma, USA); human Ang II, EGCG, EGC, ECG, EC (Sigma); JNK/SAPK assay kit, phospho-p38 MAPK (Thr 180/Thr 182) and p38 MAPK antibodies (Cell Signaling, USA); monoclonal anti-phospho MAPK and anti-MAPK 1/2 (ERK 1/2-CT) antibodies (Upstate, USA); and l-[4,5-^{3}H] leucine, horseradish peroxidase (HRP)-conjugated secondary antibody, rainbow protein molecular weight markers, enhanced chemiluminescence (ECL) reagent kits and ECL X-ray Hyperfilm (Amersham, UK). The protein assay kits were obtained from Bio-Rad (USA). All other chemicals obtained from commercial sources were of reagent grade.

Culture of rat aortic smooth muscle cells

The Chungbuk National University Animal Care and Use Committee (ACUC) approved all procedures. The thoracic aortae were retrieved from adult male rats (Sprague-Dawley, weighing 250 g, purchased from Dae Han Biolink, Korea) and were soaked in a collagenase mixture, and the adventitia and intima were removed. The remaining tissues, containing only smooth muscle and associated extracellular matrix, were minced in dissection medium (Ham's F12 medium containing penicillin 300 U/ml and streptomycin 300 μg/ml), then collected and placed in two 30-mm culture dishes with Dulbecco's modified Eagle medium (DMEM) containing 20% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. Vascular smooth muscle cells (VSMCs) were allowed to grow to confluence for 7~10 days. Cells from the second to the ninth passages were used in all the experiments. VSMCs were identified by the positive staining with anti-actin, -smooth muscle antibody using immunofluorescence microscopy.

Measurement of [^{3}H] leucine incorporation

Aliquots of 2×10^5 cells were subcultured in 30-mm dishes. After 48 h of starvation with DMEM containing 0.1% FBS, cells were treated with drugs to be tested for 30 min before stimulation with 100 nM Ang II for 24 h. Cells were incubated with 1 μCi/ml [^{3}H]leucine during the last 8 h prior to harvest. After washing once with ice-cold PBS, cells were treated with 5% trichloroacetic acid for 20

min and then washed twice with ice-cold PBS. Finally, cells were solubilized in 300 μl of 1 N NaOH plus 0.1% SDS and neutralized with 150 μl of 2 N HCl. Radioactivity was measured by a liquid scintillation counter (Beckman LS 3801, USA).

Assay of JNK/Stress-activated protein kinase (SAPK) activity

JNK/SAPK kinase assay was conducted as described in the JNK/SAPK assay kit (Cell Signaling, USA). After treating the growth-arrested VSMCs with drugs for the indicated times, cells were lysed and sonicated, followed by centrifugation at 14,000 rpm for 15 min at 4°C to retrieve the supernatant. Protein concentration was measured by the method of Bradford. Equal amount of protein (300 μg) was used to 'pull down' SAPK with 2 μg of c-Jun (1~89) fusion protein beads overnight. C-Jun (1~89) fusion protein beads contain high affinity binding sites for JNK/SAPK, just N-terminal to the two phosphorylation sites, ser 63 and ser 73. The beads were then washed with 1 lysis buffer (20 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 25 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na_3VO_4, 1 μg/ml leupeptin, with 1 mM phenylmethylsulphonylfluoride protease inhibitor added just before use), and then with 1× kinase buffer (25 mM Tris pH 7.5, 5 mM β-glycerolphosphate, 2 mM DTT, 0.1 mM Na_3VO_4, 0.1 mM $MgCl_2$) to remove non-specifically bound protein. The kinase reaction was carried out in the presence of cold ATP, followed by Western blot analysis using an antibody (1 : 1000) specific for JNK/SAPK-induced phosphorylation of c-Jun at ser 63, a site important for c-Jun-dependent transcriptional activity.

Western blot analysis of ERK 1/2 and p38 MAPK activities

VSMCs were starved for 48 h and treated with drugs to be tested for desired times. Cells were then lysed and sonicated, followed by centrifugation at 14,000 rpm for 15 min at 4°C to obtain the supernatant. Protein concentration was measured by the method of Bradford. Equal amount of protein (20 μg) was run on 10% SDS-PAGE, and the proteins were blotted onto polyvinylidene difluoride membrane. Then the membrane was incubated with anti-phospho ERK 1/2, anti-total ERK 1/2 antibody, anti-phospho p38 MAPK or anti-total p38 MAPK antibody. The antigen-antibody complexes were visualized with the use of appropriate secondary antibodies and the enhanced chemiluminescence (ECL) reagent kits, as recommended by the manufacturer.

Reverse transcription-polymerase chain reaction (RT-PCR)

The expression levels of *c-jun* and *c-fos* mRNA were analysed by RT-PCR. Quiescent cells were treated with drugs, and total RNA was isolated from each sample using RNAzol B (TEL-TEST, USA) according to the manufacturer's instruction. First-strand cDNA was synthesized from 2 μg of total RNA by using 200 units of reverse transcriptase, superscript II RNaseH$^-$ (Invitrogen, USA). PCR was performed in 50 μl of reaction mixture containing reverse transcription (RT) product, primers, dNTPs, $MgCl_2$ and *Taq* polymerase in 1×reaction buffer. The primer sequences for *c-jun, c-fos,* and β-actin (control marker) and the sizes of the amplified products were as follows:

c-jun (amplified PCR fragment: 1098 bp)
sense primer: 5'-GAAGTGACCGACTGTTCTATGACT-3';
anti-sense primer: 5'-CGCAACCAGTCAAGTTCTCAAGTT-3';
c-fos (amplified PCR fragment: 1164 bp)
sense primer: 5'-CCACGACCATGATGTTCTCGGGTT-3';
anti-sense primer: 5'-CTTCTCTGACTGCTCACAGGGCTA-3';
β-actin (amplified PCR fragment: 285 bp)
sense primer: 5'-TCATGAAGTGTGACGTTGACATCCGT-3';
anti-sense primer: 5'-CTTAGAAGCATTTGCGGTGCACG TTG-3'.

The PCR products (20 μl) were resolved on 0.8% agarose gels by electrophoresis, and the bands were visualized by ethidium bromide (ET-Br) staining under UV illumination.

Data analysis

Data are summarized as mean±SEM. Student's t-test was used to determine statistical differences. Differences were considered significant at $P<0.05$.

RESULTS

Effects of different catechins on Ang II-induced VSMC hypertrophy

To observe the phenotypic modulation of catechins on Ang II-stimulated hypertrophy *in vitro*, VSMC was pretreated with each catechin at the indicated concentrations for 30 min and then stimulated with 100 nM Ang II for 24 h. As

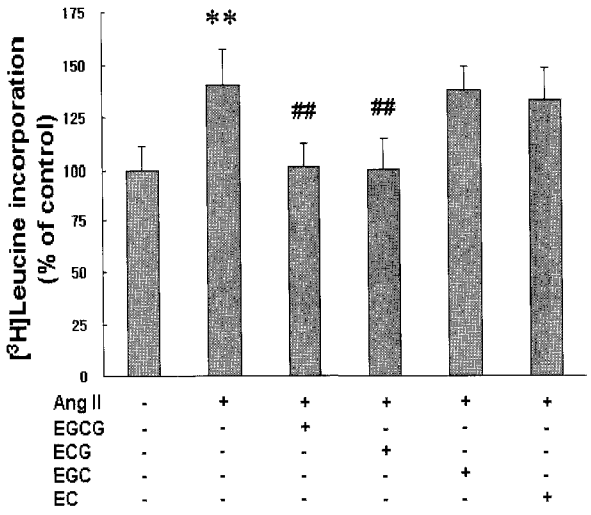

Fig. 2. Effect of EGCG, ECG, EGC, and EC on [^{3}H]-leucine incorporation into VSMC. After 48 hours of starvation with 0.1% FBS, cells were pretreated with 50 μM each of catechin for 30 min before stimulation with or without 100 nM Ang II for 24 hours. Data represent the percentage of [^{3}H]-leucine incorporation after treatment with or without each catechin, compared with control. Values are means±SEM. $^{**}P<0.01$ compared with control; $^{\#\#}P<0.01$ compared with the values obtained during Ang II stimulation alone (n=3).

shown in Fig. 2, stimulation with 100 nM Ang II caused a significant increase of [^{3}H]-leucine incorporation into VSMC, compared with control. Pretreatment with 50 μM EGCG, however, dose-dependently inhibited the increase of [^{3}H]-leucine incorporation. Pretreatment with 50 μM ECG also inhibited the increase of [^{3}H]-leucine incorporation. In contrast, pretreatment of the cell with 50 μM EGC or EC did not increase [^{3}H]-leucine incorporation (Fig. 2). These results indicate that EGCG and ECG with a galloyl group in the position 3 of the catechin structure decrease VSMC hypertrophy. In contrast, EGC and EC without galloyl group in the position 3 of the catechin structure do not decrease VSMC hypertrophy.

Effects of different catechins on JNK, ERK 1/2, and p38 MAPK activation induced by Ang II in VSMC

Next, we determined whether catechins modulate MAPKs (JNK, ERK 1/2, and p38 MAPK) activity in VSMC. Thus, cells were pretreated with 50 μM each of EGCG, ECG, EGC and EC for 30 min, and then stimulated with 100 nM Ang II for 10 min for JNK activation and for 5 min for ERK 1/2 and p38 MAPK activation. JNK activity was measured by phosphorylation of c-Jun, a downstream substrate of JNK, and ERK 1/2 and p38 MAPK activities were measured by phosphorylation of ERK 1/2 and p38 MAPK, respectively. As shown in Fig. 3, EGCG and ECG

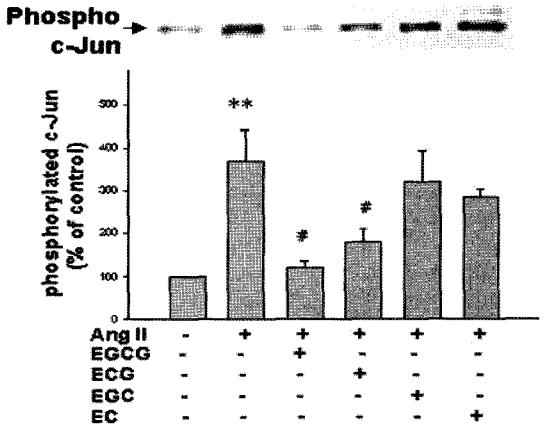

Fig. 3. Effect of EGCG, ECG, EGC, and EC on Ang II-induced JNK activation in VSMC. Cells were pretreated with 50 μM EGCG, ECG, EGC or EC for 30 min, and then stimulated with 100 nM Ang II for 10 min for JNK activation. Activities of JNK were measured by kinase assay as described in the *Methods* section, and representative blots are shown. Averaged data was quantified by densitometry and expressed as percentage increase in phosphorylation observed in unstimulated cells, which was defined as 100% (control). Values are means±SEM. $^{**}P<0.01$ compared with control; $^{\#}P<0.05$ compared with the values obtained during Ang II stimulation alone (n=3).

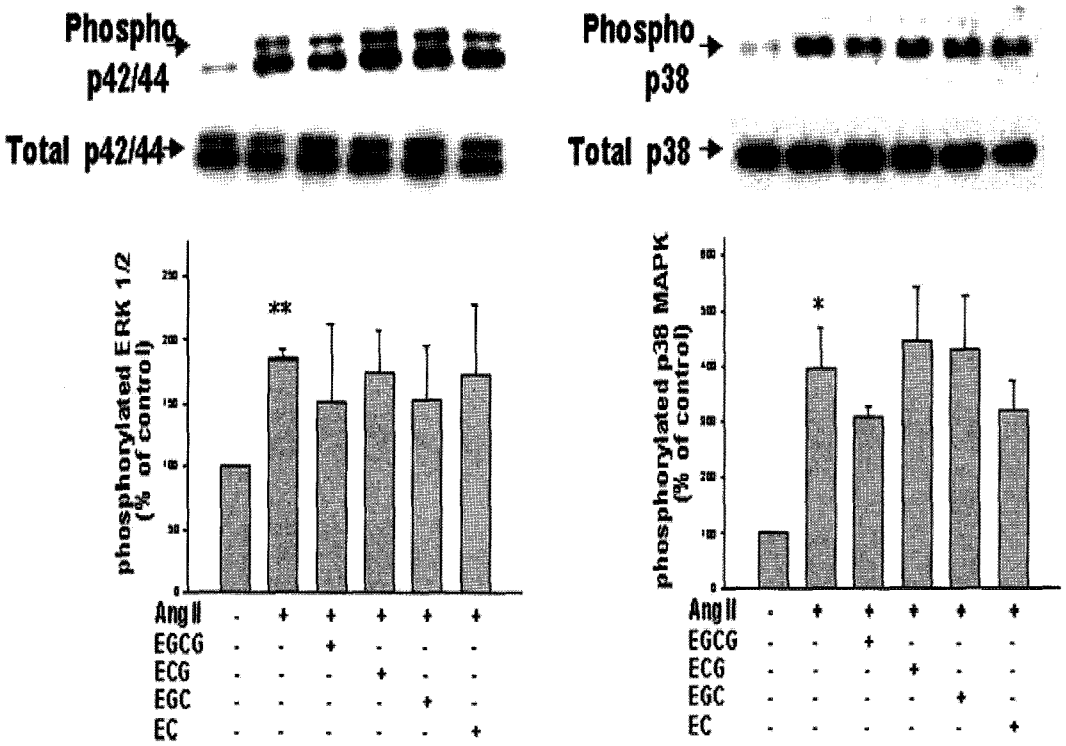

Fig. 4. Effect of EGCG, ECG, EGC, and EC on Ang II-induced MAPKs (ERK 1/2 and p38 MAPK) activation in VSMC. Cells were pretreated with 50 μM each of EGCG, ECG, EGC or EC for 30 min, and then stimulated with 100 nM Ang II for 5 min for ERK 1/2 and p38 MAPK activation. Activities of ERK 1/2 and p38 MAPK were measured by Western blot analysis as described in the *Methods* section, and representative blots are shown. No significant differences in the amounts of total ERK 1/2 and p38 MAPK were observed. Averaged data were quantified by densitometry and expressed as percentage increase in phosphorylation observed in unstimulated cells, which was defined as 100% (control). Values are means±SEM. $^{*}P<0.05$, $^{**}P<0.01$ compared with control (n=3).

inhibited Ang II-stimulated increase of c-Jun phosphorylation. However, EGC and EC did not affect c-Jun phosphorylation. EGCG, ECG, EGC and EC did not affect ERK 1/2 or p38 MAPK phosphorylation (Fig. 4). These results correspond with those of VSMC hypertrophy, suggesting that inhibitory effects of EGCG and ECG on Ang II-induced hypertrophy in VSMC may be partly mediated by inhibition of JNK activity, without influencing ERK 1/2 and p38 MAPK activities.

Effects of different catechins on c-fos and c-jun mRNA expression induced by Ang II in VSMC

The AP-1 transcription factor is composed of c-Jun and c-Fos homo/heterodimers and plays an important role in the JNK signaling pathway. Therefore, we hypothesized that EGCG and ECG might inhibit Ang II-induced VSMC hypertrophy through JNK signaling pathway, and examined whether EGCG and ECG affect the amount of Ang II-induced AP-1 transcription factor. Therefore, cells were pretreated with each catechin at indicated concentrations, then stimulated with 100 nM Ang II for 30 min, and induction of *c-fos* and *c-jun* mRNA expression were analyzed by RT-PCR. As seen in Fig. 5, an increase of *c-jun* mRNA expression induced by Ang II was attenuated by 50 μM EGCG, whereas 50 μM ECG, EGC and EC did not affect *c-jun* mRNA expression. On the other hand, however, *c-fos* mRNA expression induced by Ang II was not affected by 50 μM EGCG, ECG, EGC and EC. These results suggest that the inhibitory effect of EGCG on Ang II-induced VSMC hypertrophy may be partly mediated through down-regulation of *c-jun*.

DISCUSSION

Vascular growth (hyperplasia and hypertrophy) is a crucial determinant of cardiovascular diseases such as atherosclerosis, hypertension and restenosis. Ang II, the dominant effector of the renin-angiotensin system, induces hypertrophy in VSMC (Berk et al, 1989), and plays an important role in various cardiovascular diseases associated with VSMC growth (Berk & Corson, 1997). Accordingly, inhibition of VSMC growth by Ang II is prerequisite for prevention of cardiovascular diseases.

Flavonoids are ubiquitously present in foods of plant origin and are believed to ameliorate vascular disorders (Middleton et al, 2000). Consumption of green tea might prevent the incidence of atherosclerosis in humans (Tijburg et al, 1997). We have previously demonstrated that flavan-3-ol compound, EGCG, selectively inhibited the platelet-derived growth factor (PDGF)-BB-induced intracellular

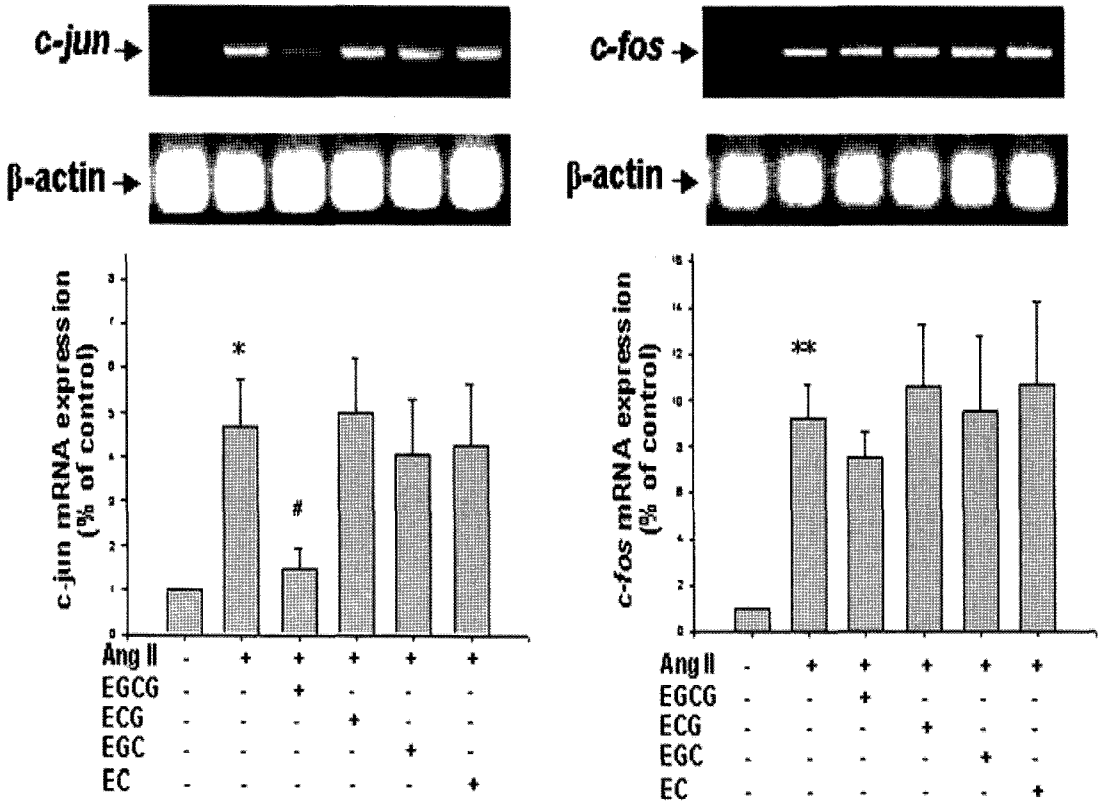

Fig. 5. Effect of EGCG, ECG, EGC, and EC on Ang II-induced *c-jun* and *c-fos* mRNA expression in VSMC. *C-jun* and *c-fos* mRNA expression were analyzed by RT-PCR as described in the *Methods* section. PCR products were resolved by 0.8% agarose gel electrophoresis and visualized by ET-Br staining, and the bands were then photographed under UV illumination. Averaged data were quantified by densitometry and expressed as percentage increase in *c-jun* or *c-fos* mRNA expression observed in unstimulated cells, which was defined as 100% (control). Values are means ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ compared with control; $^{\#}P<0.05$ compared with values obtained during Ang II stimulated alone (n=3).

signaling transduction pathway and proliferation in VSMC (Ahn et al, 1999; Sachinidis et al, 2002; Weber et al, 2004). Moreover, EGCG selectively inhibited c-Jun N-terminal kinase activation, which resulted in the suppression of VSMC hypertrophy (Zheng et al, 2004). EGC, ECG, and EC are structural analogues of EGCG, which are isolated from green tea. In this study, we compared the effect of other catechins on Ang II-induced VSMC hypertrophy with that of EGCG.

In our results, Ang II significantly increased [^{3}H]-leucine incorporation into VSMC, and pretreatment of VSMC with EGCG or ECG remarkably inhibited the Ang II-induced [^{3}H]-leucine incorporation in a dose-dependent manner (Fig. 2). On the other hand, structural analogue of EGCG, EGC or EC did not inhibit the Ang II-induced [^{3}H]-leucine incorporation (Fig. 2). EGCG, ECG, EGC, and EC did not bring any changes in cell number either (data not shown). Therefore, both EGCG and ECG which containing the galloyl group in the position 3 of the catechin structure can attenuate the protein synthesis increased by Ang II, thereby preventing VSMC hypertrophy.

Because the MAPK families, including JNK, ERK 1/2 and p38 MAPK, have been implicated in VSMC hypertrophy (Servant et al, 1996; Ushio-Fukai et al, 1998; Yoshizumi et al, 2001; Zheng et al, 2004), we attempted to clarify the effect of Ang II on MAPKs activity in VSMC. One hundred nM Ang II increased c-Jun phosphorylation (Fig. 3) as well as ERK1/2 and p38 MAPK phosphorylation in VSMC (Fig. 4), in agreement with the results of Yoshizumi et al, 2002. As shown in Fig. 2, 50 μM EGCG and ECG significantly inhibited c-Jun phosphorylation induced by Ang II in VSMC, whereas 50 μM EGC or EC did not affect it. EGCG has recently been shown to inhibit JNK activity in basic fibroblast growth factor (bFGF)-stimulated VSMC (Hwang et al, 2002). In the present study, 50 μM EGCG, ECG, EGC or EC could not affect ERK 1/2 and p38 MAPK phosphorylation induced by Ang II in VSMC (Fig. 4). Therefore, the regulation of JNK activity seems to be important for EGCG and ECG to inhibit VSMC hypertrophy.

AP-1, consisting of either Jun homodimers or Jun/Fos heterodimers, binds to the TPA response element to modulate various gene expressions, including those mediating growth, inflammation, and differentiation (Karin 1995). Ang II has been shown to induce AP-1 activation in VSMC (Takeuchi et al, 1990). MAPKs contribute to the increase of AP-1 activity and abundance (Gille et al, 1992; Smeal et al, 1994; Musti et al, 1997). In this study, we investigated the effect of catechin on the *c-jun* and *c-fos* gene transcription induced by Ang II in VSMC. One hundred nM Ang II increased both *c-jun* and *c-fos* mRNA expressions in VSMC (Fig. 5), and 50 μM EGCG dose-dependently inhibited *c-jun* mRNA expression without influencing *c-fos* mRNA expression. On the other hand, 50 μM EGC, ECG, and EC did not affect *c-jun* or *c- fos* mRNA expression induced by Ang II (Fig. 5). Therefore, the mechanism(s) by which EGCG inhibits VSMC hypertrophy induced by Ang II appear to involve suppression of not only JNK activity but also *c-jun* transcription, which affects AP-1 activity and abundance. In contrast to EGCG, ECG did not affect *c-jun* transcription, but inhibited only JNK activity.

Ang II increases H_2O_2 and p38 MAPK activity, which plays a role in VSMC hypertrophy (Ushio-Fukai et al, 1998). Moreover, Akt/PKB activation, following generation of reactive oxygen species (ROS), plays a role in VSMC hypertrophy induced by Ang II (Ushio-Fukai et al, 1999). On the other hand, EGCG and EGC have been shown to exhibit ROS scavenging activity (Nakagawa and Yokozawa 2002), and EGCG was found to be more effective radical scavenger than EGC in aqueous phase (Salah et al, 1995). Therefore, the inhibition of VSMC hypertrophy by EGCG and ECG may result from their anti-oxidant activity. However, EGC and EC did not inhibit VSMC hypertrophy even at 50 μM concentration, although EGCG and ECG significantly inhibited VSMC hypertrophy induced by Ang II. Therefore, the mechanism(s) other than anti-oxidant activity might also contributes to the inhibitory effect of EGCG and ECG on Ang II-induced VSMC hypertrophy.

In conclusion, EGCG and ECG specifically inhibited VSMC hypertrophy induced by Ang II, and this inhibitory effect may partly result from the inhibition of JNK activation. However, EGC and EC did not affect the MAPK signal transduction pathway and VSMC hypertrophy induced by Ang II. Therefore, the galloyl group in the position 3 of the catechin structure may be important for the inhibition of VSMC hypertrophy. Finally, these findings may partly explain the preventive effects of green tea catechin on cardiovascular diseases observed in epidemiological studies.

ACKNOWLEDGMENT

This work was supported by grants from the Research Center for Bioresource and Health (RCBH), and Sama Pharmaceutical Company, South Korea.

REFERENCES

Ahn HY, Hadizadeh KR, Seul C, Yun YP, Vetter H, Sachinidis A. Epigallocatechin-3 gallate selectively inhibits the PDGF-BB-induced intracellular signaling transduction pathway in vascular smooth muscle cells and inhibits transformation of *sis* -transfected NIH 3T3 fibroblasts and human glioblastoma cells (A172). *Mol Biol Cell* 10: 1093–1104, 1999

Benelli R, Vene R, Bisacchi D, Garbisa S, Albini A. Anti-invasive effects of green tea polyphenol epigallocatechin-3-gallate (EGCG), a natural inhibitor of metallo and serine proteases. *Biol Chem* 383: 101–105, 2002

Berk BC, Corson MA. Angiotensin II signal transduction in vascular smooth muscle. *Circ Res* 80: 607–616, 1997

Berk BC, Vekshtein V, Gordon HM, Tsuda T. Angiotensin II-stimulated protein synthesis in cultured smooth muscle cells. *Hypertension* 13: 305–314, 1989

Black MJ, Adams MA, Bobik A, Campbell JH, Campbell GR. Vascular smooth muscle polyploidy in the development and regression of hypertension. *Clin Exp Pharmacol Physiol* 15: 345–348, 1988

Eguchi S, Iwasaki H, Ueno H, Frank GD, Motley ED, Eguchi K, Marumo F, Hirata Y, Inagami T. Intracellular signaling of angiotensin II-induced P70 S6 kinase phosphorylation at Ser^{411} in vascular smooth muscle cells. Possible requirement of epidermal growth factor receptor, Ras, extracellular signal- regulated kinase and Akt. *J Biol Chem* 274: 36843–36851, 1999

Geisterfer AA, Peach MJ, Owens GK. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. *Circ Res* 62: 749–756, 1988

Giasson E, Meloche S. Role of P70 S6 protein kinase in angiotensin II-induced protein synthesis in vascular smooth muscle cells. *J Biol Chem* 270: 5225–5231, 1995

Gille H, Sharrocks A, Shaw P. Phosphorylation of p62TCF by MAP kinases stimulates ternary complex formation at c-Fos promoter. *Nature* 358: 414–417, 1992

Hixon ML, Muro-Cacho C, Wagner MW, Obejero-Paz C, Mille E, Fujio Y, Kureishi Y, Hassold T. Walsh K, Gualberto A. Akt/PKB upregulation leads to vascular smooth muscle cell hypertrophy and polyploidization. *J Clin Invest* 106: 1011–1020, 2000

Hwang KC, Lee KH, Jang Y, Yun YP, Chung KH. Epigallocatechin-3-gallate inhibits basic fibroblast growth factor- induced intracellular signaling transduction pathway in rat aortic smooth muscle cells. *J Cardiovasc Pharmacol* 39: 271–277, 2002

Imai K, Nakachi K. Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. *Br Med J* 310: 693–696, 1995

Kallunki T, Deng T, Hibi M, Karin M. c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions. *Cell* 87: 929–939, 1996

Kang WS, Chung KH, Chung JH, Lee JY, Park JB, Zhang YH, Yoo HS, Yun YP. Antiplatelet activity of green tea catechins is mediated by inhibition of cytoplasmic calcium increase. *J Cardiovascular Pharmacol* 38: 875–884, 2001

Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* 270: 16483–16486, 1995

McKay S, de Jongste JC, Saxena PR, Sharma HS. Angiotensin II induces hypertrophy of human airway smooth muscle cells: expression of transcription factors and transforming growth factor-beta1. *Am J Respir Cell Mol Biol* 18: 823–833, 1998

Middleton Jr E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacol Rev* 52: 673–751, 2000

Musti AM, Treier M, Bohmann D. Reduced ubiquitin-dependent degradation of c-Jun after phosphorylation by MAP kinases. *Science* 275: 400–402, 1997

Nagai K, Jiang MH, Hada J, Nagata T, Yajima Y, Yamamoto S, Nishizaki T. (-)-Epigallocatechin gallate protects against NO stress-induced neuronal damage after ischemia by acting as an anti-oxidant. *Brain Res* 956: 319–322, 2002

Nakagawa T, Yokozawa T. Direct scavenging of nitric oxide and superoxide by green tea. *Food Chem Toxicol* 40: 1745–1750, 2002

Owens GK, Schwartz SM. Alterations in vascular smooth muscle mass in the spontaneously hypertensive rat. Role of cellular hypertrophy, hyperploidy, and hyperplasia. *Circ Res* 51: 280–289, 1982

Owens GK, Schwartz SM. Vascular smooth muscle cell hypertrophy and hyperploidy in the Goldblatt hypertensive rat. *Circ Res* 53: 491–501, 1983

Pulverer BJ, Kyriakis JM, Avruch J, Nikolakaki E, Woodgett JR. Phosphorylation of c-jun mediated by MAP kinases. *Nature* 353: 67–674, 1991

Rivera VM, Greenberg ME. Growth factor-induced gene expression: the ups and downs of c-fos regulation. *New Biol* 2: 751–758, 1990

Sachinids A, Skach RA, Seul C, Ko Y, Hescheler J, Ahn HY, Fingerle J. Inhibition of the PDGF -receptor tyrosine phosohorylation and its downstream intracellular signal transduction pathway in rat and human vascular smooth muscle cells by different catechins. *FASEB J* 16: 893–895, 2002

Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C. Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch Biochem Biophys* 322: 339–346, 1995

Santana-Rios G, Orner GA, Amantana A, Provost C, Wu SY, Dashwood RH. Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay. *Mutat Res* 495: 61–74, 2001

Servant MJ, Giasson E, Meloche S. Inhibition of growth factor-induced protein synthesis by a selective MEK inhibitor in aortic smooth muscle cells. *J Biol Chem* 271: 16047–16052, 1996

Smeal T, Hibi M, Kanin M. Altering the specificity of signal transduction cascades:positive regulation of c-Jun transcriptional activity by protein kinase A. *EMBO* 3: 6006–6010, 1994

Takahashi T, Kawahara Y, Okuda M, Ueno H, Takeshita A, Yokoyama M. Angiotensin II stimulates mitogen-activated protein kinases and protein synthesis by a Ras-independent pathway in vascular smooth muscle cells. *J Biol Chem* 272: 16018–16022, 1997

Takahashi T, Taniguchi T, Konishi H, Kikkawa U, Ishikawa Y, Yokoyama M. Activation of Akt/protein kinase B after stimulation with angiotensin II in vascular smooth muscle cell. *Am J Physiol* 276: H1927–H1934, 1999

Takeuchi K, Nakamura N, Cook NS, Pratt RE, Dzau VJ. Angiotensin II can regulate gene expression by the AP-1 binding sequence via a protein kinase C-dependent pathway. *Biochem Biophys Res Commun* 172: 1189–1194, 1990

Tijburg LB, Mattern T, Folts JD, Weisgerber UM, Katan MB. Tea flavonoids and cardiovascular diseases. *Crit Rev Food Sci* 37: 771–785, 1997

Ushio-Fukai M, Alexander RW, Akers M, Griendling KK. p38 mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. Role in vascular smooth muscle cell hypertrophy. *J Biol Chem* 273: 15022–15029, 1998

Ushio-Fukai M, Alexander RW, Akers M, Yin Q, Fujio Y, Walsh K, Griendling KK. Reactive oxygen species mediate the activation of Akt/protein kinase by angiotensin II in vascular smooth muscle cells. *J Bio Che* 274: 22699–22704, 1999

Weber AA, Neuhaus T, Skach RA, Hescheler J, Ahn HY, Schror K, Ko Y, Sachinidis A. Mechanisms of the inhibitory effects of epigallocatechin-3 gallate on platelet-derived growth factor-BB-induced cell signaling and mitogenesis. *FASEB J* 18: 128–130, 2004

Yamaguchi K, Honda M, Ikigai H, Hara Y, Shimamura T. Inhibitory effects of (-)-epigallocatechin gallate on the life cycle of human immunodeficiency virus type I (HIV-1). *Antiviral Res* 53: 19–34, 2002

Yamane T, Takahashi T, Kuwata K, Oya K, Inagake M, Kitao Y, Suganuma M, Fujiki H. Inhibition of N-methyl-N'-nitro-N-nitrosoguanidine-induced carcinogenesis by (-)-epigallocatechin gallate in the rat glandular stomach. *Cancer Res* 55: 2081–2084, 1995

Yoshizumi M, Tsuchiya K, Kirima K, Kyaw M, Suzaki Y, Tamaki T. Quercetin inhibits Shc- and phosphatidylinositol 3-kinase-mediated c-Jun N-terminal kinase activation by angiotensin II in cultured rat aortic smooth muscle cells. *Mol Pharmacol* 60: 656–665, 2001

Yoshizumi M, Tsuchiya K, Suzaki Y, Kirima K, Kyaw M, Moon JH, Terao J, Tamaki T. Quercetin glucuronide prevents VSMC hypertrophy by angiotensin II via the inhibition of JNK and AP-1 signaling pathway. *Biochem Biophys Res Commun* 293: 1458–1465, 2002

Zheng Y, Song HJ, Kim CH, Kim HS, Kim EG, Sachinidis A, Ahn HY. Inhibitory effect of epigallocatechin 3-O-gallate on vascular smooth muscle cell hypertrophy induced by angiotensin II. *J Cardiovasc Pharmacol* 43: 200–208, 2004