



## Decursin and decursinol inhibit VEGF-induced angiogenesis by blocking the activation of extracellular signal-regulated kinase and c-Jun N-terminal kinase

Seung Hwa Son<sup>a,b,1</sup>, Mi-Jeong Kim<sup>a,b,1</sup>, Won-Yoon Chung<sup>a,b</sup>, Ju-Ah Son<sup>a,b</sup>, Yeong Shik Kim<sup>c,d</sup>, Young-Choong Kim<sup>c</sup>, Sam Sik Kang<sup>c,d</sup>, Sang-Kook Lee<sup>e</sup>, Kwang-Kyun Park<sup>a,b,\*</sup>

<sup>a</sup> Department of Oral Biology, Oral Cancer Research Institute, Oral Science Research Institute, and Brain Korea 21 Project, Yonsei University College of Dentistry, Seoul 120-752, Republic of Korea

<sup>b</sup> Department of Applied Life Science, The Graduate School, Yonsei University, Seoul 120-749, Republic of Korea

<sup>c</sup> College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea

<sup>d</sup> Natural Products Research Institute, Seoul National University, Seoul 151-742, Republic of Korea

<sup>e</sup> College of Pharmacy, Ewha Womans University, Seoul 120-750, Republic of Korea

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### ABSTRACT

The root of *Angelica gigas* Nakai contains two major coumarins, which have been previously identified as decursin and decursinol. Decursin has been demonstrated to exhibit potent anti-cancer activity both *in vitro* and *in vivo*. In this study, we found that decursin and decursinol at non-cytotoxic doses inhibited the VEGF-induced proliferation, migration, and capillary-tube formation of HUVECs. Moreover, decursin and decursinol suppressed microvessel formation on chorioallantoic membranes in fertilized eggs and into mouse Matrigel plugs. The oral administration of decursin and decursinol also reduced VEGF-induced angiogenesis in Matrigel. Furthermore, decursin and decursinol reduced the phosphorylation of ERK and JNK, but not p38 MAPK, in VEGF-stimulated HUVECs. Taken together, our results reveal that decursin and decursinol inhibit VEGF-induced angiogenesis by reducing the activation of ERK and JNK in HUVECs, and possess potent *in vivo* anti-angiogenic activity, coupled with the advantage of oral dosing. Thus, these compounds may have the potential for the treatment of cancers dependent on VEGF-induced vascularization.

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### 1. Introduction

Angiogenesis is a process by which endothelial cells divide and migrate to form new capillaries from pre-existing blood vessels [1]. This acquisition of capillaries can be observed under normal conditions such as embryonic vascular development and wound healing, as well as in disease states including ocular neovascularization in diabetes, arthritis, duodenal ulcers, and cancer [2]. In a cancerous

state, an adequate oxygen and nutritional supply is crucial for an expansion of solid cancers beyond the size of 1–2 mm [3,4]. The newly formed capillaries are thin-walled and leaky, and thus provide access to the circulation for groups of cancer cells. An accumulating body of evidence now shows that the blockade of tumoral angiogenesis represents a promising strategy for cancer therapy [5,6].

One of the most essential factors regulating angiogenesis is the vascular endothelial growth factor (VEGF). VEGF is expressed and secreted by hypoxia, as well as genetic mutations in oncogenes and tumor suppressor genes in tumors [5–8]. VEGF activity is mediated by VEGF receptor (VEGFR)-1 (Flt-1, 180 kDa) and VEGFR-2 (KDR/Flk-1, 200 kDa), both of which are almost exclusively located on endothelial cells [9]. The binding of VEGF to VEGFRs and

\* Corresponding author. Address: Department of Oral Biology, Yonsei University College of Dentistry, Seoul 120-752, Republic of Korea. Tel.: +82 2 2228 3056; fax: +82 2 364 7113.

E-mail address: [biochelab@yuhs.ac](mailto:biochelab@yuhs.ac) (K.-K. Park).

<sup>1</sup> Seung Hwa Son and Mi-Jeong Kim contributed equally to this work.

the subsequent ligand-induced dimerization of these receptors stimulate their intrinsic tyrosine kinase activity, and trigger key angiogenic responses of endothelial cells, including proliferation, migration, differentiation, and protection from apoptosis, via a number of signaling cascades [10–15]. Thus, a variety of angiogenesis inhibitors have been developed to prevent vascular endothelial cells from responding to tumor cell-derived VEGF, or to block the expression and secretion of VEGF from tumor cells, as well as VEGFRs on endothelial cells [5].

Angiogenesis is also a common and key target of chemopreventive molecules, where they most likely suppress the angiogenic switch in premalignant tumors [16,17]. Preventative anti-angiogenic strategies may prove particularly useful in patients at high risk for developing cancer or cancer recurrence, or the development of metastases. To achieve these strategies, it is necessary to isolate and identify anti-angiogenic or angio-preventive compounds with low or no toxicity, as long-term treatment is required for the inhibition of tumor growth. Natural products have performed a crucial function in the discovery and development of new anti-angiogenic and angio-preventive agents. Particularly, plant-derived compounds and dietary substances with chemopreventive activity, including curcumin [18,19], epigallocatechin gallate [20,21] and genistein [22,23], have been previously reported to interfere with the angiogenic process via the inhibition of the recruitment and/or activation of endothelial cells.

The root of *Angelica gigas* Nakai (Umbelliferae) is one of the Korean medicinal herbs used to treat female afflictions and anemia [24,25], and harbors pyranocoumarin compounds as major active principles, including decursin [26], its isomer decursinol angelate [27] and decursinol [28]. Decursin and decursinol angelate have been reported to inhibit the growth of androgen-independent human prostate cancer cells [29,30], as well as estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 breast cancer cells [31], whereas decursinol without the side chain has been demonstrated to exert far less profound effects that have been noted with decursin [29,31]. Decursin and decursinol angelate also function as cytotoxic and tumor-suppressing protein kinase C activators, and evidence anti-leukemic activity in human K562 erythroleukemia and U937 myeloleukemia cells [32].

In this study, we evaluated the anti-angiogenic potential of decursin and decursinol. We determined that both decursin and decursinol significantly inhibited VEGF-induced angiogenesis by suppressing the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in human umbilical vein endothelial cells (HUVEC). The *in vitro* anti-angiogenic activity of these compounds was also verified in fertilized eggs and mice.

## 2. Materials and methods

### 2.1. Materials

Collagenase type II was obtained from BD Transduction Laboratories (San Diego, CA), and the endothelial cell basal medium (EBM)-2, EBM-2 supplemented with 10 ng/mL hEGF, 5 ng/mL hFGF-1, 1 µg/mL hydrocortisone, 50 µg/mL

gentamicin, 50 ng/mL amphotericin B, and 10 µg/mL heparin (EGM-2), and fetal bovine serum (FBS) were purchased from Lonza (Walkersville, MD). Decursin and decursinol were generously provided by Professor Young Choong Kim, a co-author, and the purity of the compounds was over 98% by HPLC analysis. Recombinant human VEGF was acquired from Upstate Biotechnology (Lake Placid, NY). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), gelatin, heparin, Drabkin's reagent kit 525, and a primary antibody of  $\beta$ -actin were purchased from Sigma-Aldrich (St. Louis, MO). PD 98059 (an ERK inhibitor), SB 203580 (a p38 MAPK inhibitor) and SP 600125 (a JNK inhibitor) were purchased from Calbiochem (La Jolla, CA). Primary antibodies of ERK, JNK and p-JNK were purchased from Cell Signaling Technology (Danvers, MA). Those of p38, p-p38 and p-ERK, and horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

### 2.2. Cell culture

Freshly delivered umbilical cords were obtained from natural births, and HUVECs were isolated from human umbilical cord veins via collagenase digestion, as described previously [33]. The cells were cultured in EGM-2 supplemented with 20% FBS at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>. Confluent cultures between the third and eighth passages were employed in the functional assays.

### 2.3. Animals

Male C57BL/6 mice (approximately 7 weeks of age) were supplied by Orient Co. (Seoul, Korea). The mice were provided with a standard laboratory diet (Daejong Inc., Seoul, Korea) and water *ad libitum*, and were maintained at a temperature of 22 ± 2 °C with a 12 h light/dark cycle. Animal studies were conducted after the experimental protocols had been approved by the animal ethics committee of the Yonsei University College of Dentistry.

### 2.4. Endothelial cell viability assay

Cell viability was assessed via MTT assay, based on the cleavage of the yellow tetrazolium salt (MTT) by metabolically active cells, resulting in the formation of purple formazan crystals. HUVECs ( $5 \times 10^3$  cells) were seeded into each well of a 96-well plate with EGM-2 containing 20% FBS. The cells were cultured in fresh 2% FBS-EBM-2 containing various concentrations of each test compound at 37 °C, for 3 days without VEGF and for 24 h with VEGF (20 ng/ml), respectively. An MTT solution (5 mg/ml) was added to each well, and the wells were incubated for 4 h at 37 °C. The medium was removed carefully, and the formazan product was dissolved in DMSO. The absorbance was measured at 570 nm using an ELISA reader (Bio-Rad Benchmark, Hercules, CA).

### 2.5. 5-Bromo-2'-deoxyuridine (BrdU) incorporation assay

DNA synthesis was assessed via the BrdU labeling method with commercially available kits (Roche Diagnostics,

Mannheim, Germany). In brief, HUVECs ( $5 \times 10^3$  cells) were seeded into each well of a 96-well plate with EGM-2 containing 20% FBS, then incubated overnight at 37 °C in an atmosphere of 5% CO<sub>2</sub>. The cells were cultured for 24 h in fresh 2% FBS-EBM-2 with each compound at various concentrations and VEGF (20 ng/ml). BrdU (10 μM/well) was added and incubated for an additional 4 h. The quantity of BrdU incorporated into the newly synthesized DNA was assessed in accordance with the manufacturer's protocol. Absorbance was measured at 450 nm using an ELISA reader.

#### 2.6. Endothelial cell migration assay

The chemotactic motility of HUVECs was evaluated using Transwell (Corning Costar, Cambridge, MA) with 6.5 mm-diameter polycarbonate filters (8 μm pore size). In brief, the lower surface of the filter was coated with gelatin (10 μg). EBM-2 containing 2% FBS and VEGF (20 ng/ml) was placed in the lower wells. The cells were pretreated with various concentrations of decursin or decursinol for 1 h at room temperature. The cell suspension ( $1 \times 10^5$  cells/100 μl) was loaded into each upper well, then incubated for 7 h at 37 °C. The nonmigrating cells on the upper surface of the filter were removed by wiping with a cotton swab. The gelatin membrane, with cells that had migrated to the lower surface of the filter, was fixed with methanol and stained with hematoxylin and eosin. The migrated cells were then counted under an optical microscope at  $\times 200$  magnification.

#### 2.7. Tube formation assay

250 μl of growth factor-reduced Matrigel (Collaborative Biomedical Products, Bedford, MA) was added to a 24-well plate and polymerized for 30 min at 37 °C. After the HUVECs at subconfluence were cultured overnight in 2% FBS-EBM-2 medium, the cells ( $1 \times 10^5$  cells) were seeded onto each well of the Matrigel-precoated 24-well plates, then incubated in 2% FBS-EBM-2 with various concentrations of each test compound or specific inhibitors of ERK (10 μM), p38 MAPK (10 μM) and JNK (5 μM) in the presence of VEGF (20 ng/ml) at 37 °C, respectively. Eight hours later, the endothelial cells-derived tube-like structure was visualized under an inverted microscope and photographed at a magnification of  $\times 40$ . Furthermore, tube-like structure formation was quantified by calculating the tube length, and expressed using untreated wells as 100%

#### 2.8. Chorioallantoic membrane (CAM) assay

Fertilized chicken eggs (Pulmuone Co, Seoul, Korea) were maintained in a humidified incubator at 37 °C. On day 3, 2 ml of albumin was removed and a 1-cm square window was made. On day 6, Thermanox coverslips (Nunc, Roskilde, Denmark) with decursin or decursinol were placed on the CAM of individual embryos for 2 days at 37 °C. A 10% fat emulsion was injected into the CAM, such that the vascular network of the CAM stood out against the white background of lipids. The presence of an avascular zone ( $\geq 4$  mm diameter) was observed and scored positive for the inhibition of angiogenesis. The anti-angiogenic

activity was expressed as the percentage of tested eggs evidencing a positive response.

#### 2.9. Mouse Matrigel plug assay

Seven-week-old male C57BL/6 mice were assigned randomly to six groups, each group containing five mice: the groups were subcutaneously injected with Matrigel mixture (600 μl) containing heparin (20 units) and VEGF (100 ng/ml) with or without each test compound (5 and 10 μM). The control group received Matrigel containing 0.01% DMSO without VEGF and test compounds. In the experiment to assess bioavailability of these compounds, mice were subcutaneously injected with Matrigel mixture containing heparin and VEGF, and administered by oral gavage [0.075 and 0.15 mg/kg body weight (BW)] with decursin or decursinol once daily. The control group received PBS containing 0.01% DMSO without test compounds. Seven days later, the mice were sacrificed and the plugs were collected. In order to quantify the formation of a vessel in the Matrigel plug, the quantity of hemoglobin (Hb) was measured using Drabkin's reagent kit 525. The results are expressed in g of hemoglobin/dL.

#### 2.10. Protein extraction and Western blot analysis

The HUVECs were treated for 24 h with decursin or decursinol at the indicated concentrations. The cell lysates were prepared using lysis buffer [50 mM Tris-HCl (pH 7.9), 5 mM EDTA, 0.1% SDS, 10% glycerol, 0.2% Triton X-100, 5 μg/ml aprotinin, 1 mM PMSF, and a protease inhibitor cocktail tablet]. The protein concentrations were determined using a BCA kit (Pierce, Rockford, IL). Each protein lysate (30 μg) was separated via SDS-PAGE, then transferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA). The blots were incubated overnight with primary antibodies against total/phospho ERK1/2, JNK, or p38 at 4 °C, followed by 1 h of incubation with horseradish peroxidase-conjugated secondary antibodies. The targeted proteins were visualized using an enhanced chemiluminescence detection system (Amersham Life Science, Little Chalfone, UK).

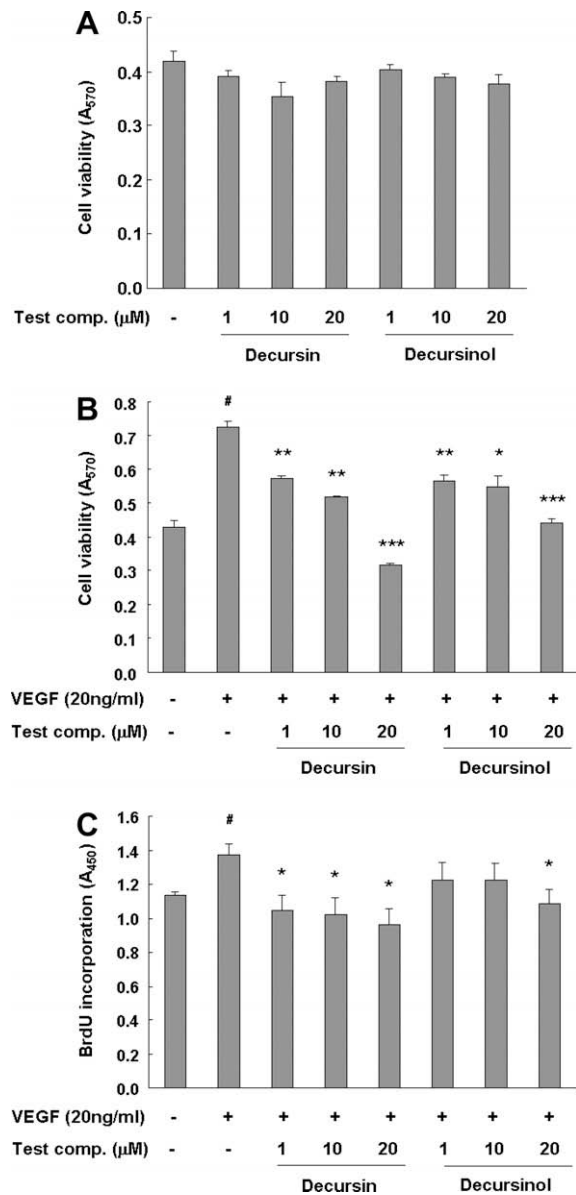
#### 2.11. Statistical analysis

The data were expressed as the means  $\pm$  SD. The statistically significant differences between the groups was determined via 1-way ANOVA followed by the Student's *t* test, and considered to be present at  $P < 0.05$ .

### 3. Results

#### 3.1. Effects of decursin and decursinol on VEGF-induced proliferation in HUVECs

In an effort to determine the anti-angiogenic activity of decursin and decursinol, we first assessed their effects on VEGF-induced proliferation in HUVECs. Three days of treatment with decursin and decursinol ranged from 1 μM to 20 μM did not significantly affect cell viability in the absence of VEGF (Fig. 1A). When HUVECs were treated for 24 h with various concentrations of decursin or decursinol and VEGF (20 ng/ml), both compounds significantly reduced the VEGF-induced cell viability (Fig. 1B) and DNA synthesis in a dose-dependent manner (Fig. 1C). These results dem-

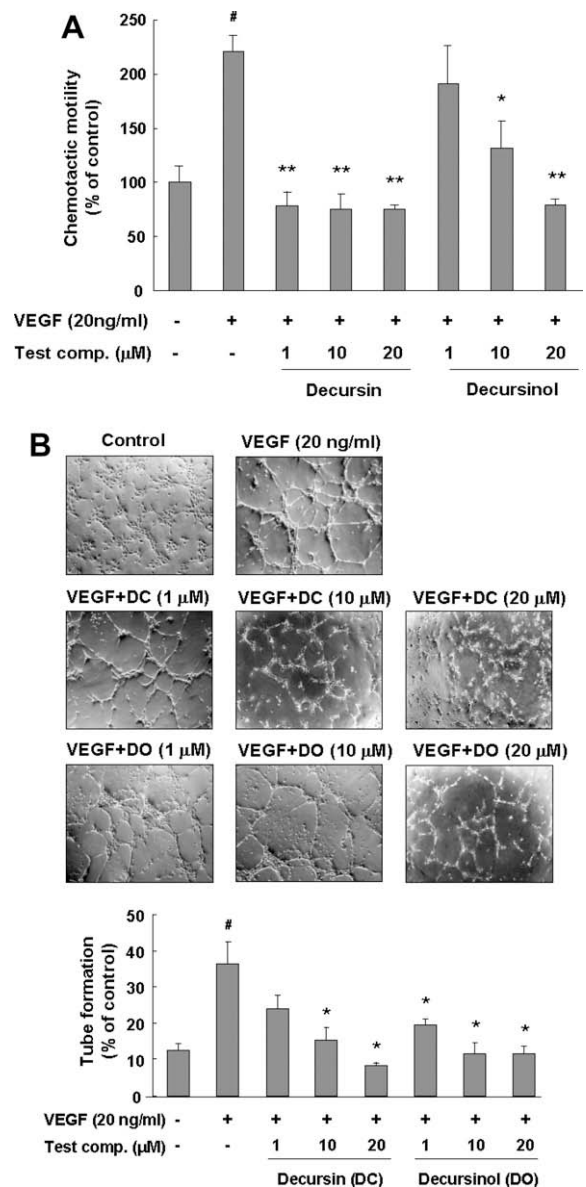


**Fig. 1.** Decursin and decursinol inhibited the VEGF-induced proliferation of HUVECs. (A) HUVECs ( $5 \times 10^5$  cells/well) were cultured for 3 days in 2% FBS-EBM-2 containing decursin or decursinol (1, 10 or 20 μM) in the absence of VEGF (20 ng/ml). (B, C) HUVECs were treated for 24 h with decursin or decursinol in the presence of VEGF (20 ng/ml). Cell viability and DNA synthesis in proliferating cells were measured via a MTT assay and a BrdU incorporation assay, respectively. The values were expressed as the means  $\pm$  SD. <sup>#</sup> $P < 0.05$  versus unstimulated cells, <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$ , <sup>\*\*\*</sup> $P < 0.001$  versus VEGF-stimulated cells.

onstrated that decursin and decursinol at non-cytotoxic doses inhibit VEGF-induced proliferation of HUVECs.

### 3.2. Effects of decursin and decursinol on VEGF-induced migration and tube formation in HUVECs

We then assessed the effects of decursin and decursinol on VEGF-induced chemotactic motility and tube formation in HUVECs. VEGF (20 ng/ml) significantly increased cell migration by 2.2-fold, but decursin at 1 μM or decursinol at 20 μM completely inhibited the VEGF-induced



**Fig. 2.** Decursin and decursinol inhibited VEGF-induced migration and tube formation. (A) The chemotactic motility of HUVECs was assayed using Transwell. Cells were pretreated with 2% FBS-EBM-2 containing decursin or decursinol (1, 10 or 20 μM) for 1 h at room temperature. The cell suspension ( $1 \times 10^5$  cells/100 μl) was loaded into gelatin-coated upper wells, and incubated for 7 h in 2% FBS-EBM-2 medium with VEGF (20 ng/ml) in the lower wells. Chemotaxis was quantified by counting the cells that had migrated to the lower surface of the filter with optical microscopy at a magnification of  $\times 200$  after staining with hematoxylin and eosin. The values were expressed as the means  $\pm$  SD. <sup>#</sup> $P < 0.005$  versus unstimulated cells, <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.001$  versus VEGF-stimulated cells. (B) HUVECs ( $1 \times 10^5$  cells/well) were seeded onto Matrigel-precoated 24-well plates, then incubated in 2% FBS-EBM-2 with various concentrations of each compound and VEGF (20 ng/ml) at 37 °C. Eight hours later, the tube network formed by endothelial cells was visualized under an inverted microscope and quantified by calculating the tube length ( $\times 40$  magnification).

migration of HUVECs (Fig. 2A). In addition, decursin and decursinol dose-dependently blocked capillary-like tube formation in VEGF-treated HUVEC (Fig. 2B). These results show that decursin and decursinol could

inhibit VEGF-induced angiogenesis by blocking cell migration and tube formation in VEGF-treated HUVECs.

### 3.3. Effects of decursin and decursinol on *in vivo* angiogenesis

In order to further verify the anti-angiogenic activity of decursin and decursinol, we conducted a CAM assay in fertilized eggs and a Matrigel plug assay in mice. Avascular zones, which are reflective of anti-angiogenic activity, were observed in decursin- or decursinol-treated CAMs in fertilized eggs, whereas microvessels were normally produced in CAMs lacking these compounds (Fig. 3A). VEGF-mediated angiogenesis was inhibited in Matrigel plugs containing decursin or decursinol together with VEGF as compared to plugs containing only VEGF (Fig. 3B). When the Hb contents in Matrigel plugs were measured to quantify the newly formed vessels, the VEGF-induced increase in the Hb contents of plugs was dramatically inhibited by decursinol rather than by decursin at 5  $\mu\text{M}$ , and to almost control level by both compounds at 10  $\mu\text{M}$ . The oral administration of decursin or decursinol, following subcutaneous injection of Matrigel mixture containing heparin and VEGF, also reduced VEGF-induced angiogenesis (Fig. 3C). These results show that decursin and decursinol exhibit potent *in vivo* anti-angiogenic activity.

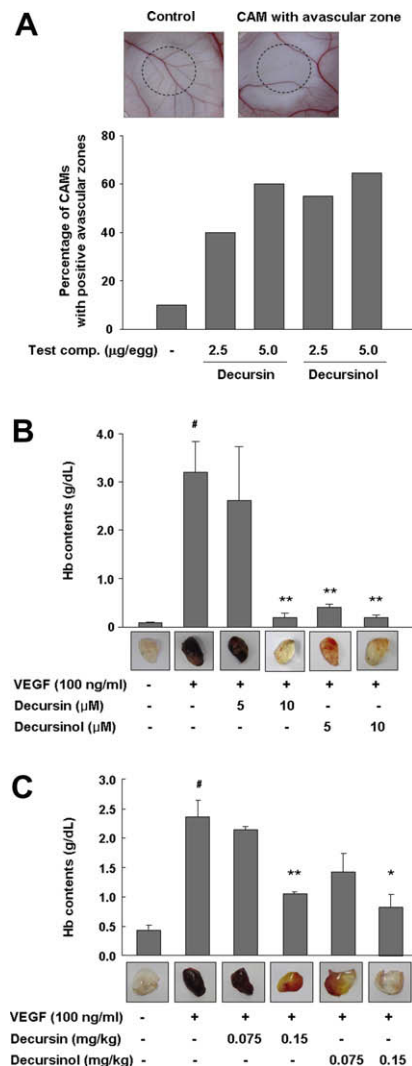
### 3.4. Effects of decursin and decursinol on VEGF-induced expression of mitogen-activated protein kinases (MAPKs) in HUVECs

We attempted to determine whether the anti-angiogenic activity of decursin and decursinol are mediated through the modulation of MAPKs. MAPKs are activated by the binding of VEGF to VEGF receptors, and consequently regulate the VEGF-induced proliferation and migration of HUVECs [34–37]. In HUVECs, specific inhibitors of ERK, p38 MAPK, and JNK reduced VEGF-stimulated tube formation (Fig. 4A). VEGF resulted in the activation of all three MAPKs, but decursin and decursinol markedly inhibited the VEGF-induced phosphorylation of ERK and JNK, but not p38 MAPK (Fig. 4B).

## 4. Discussion

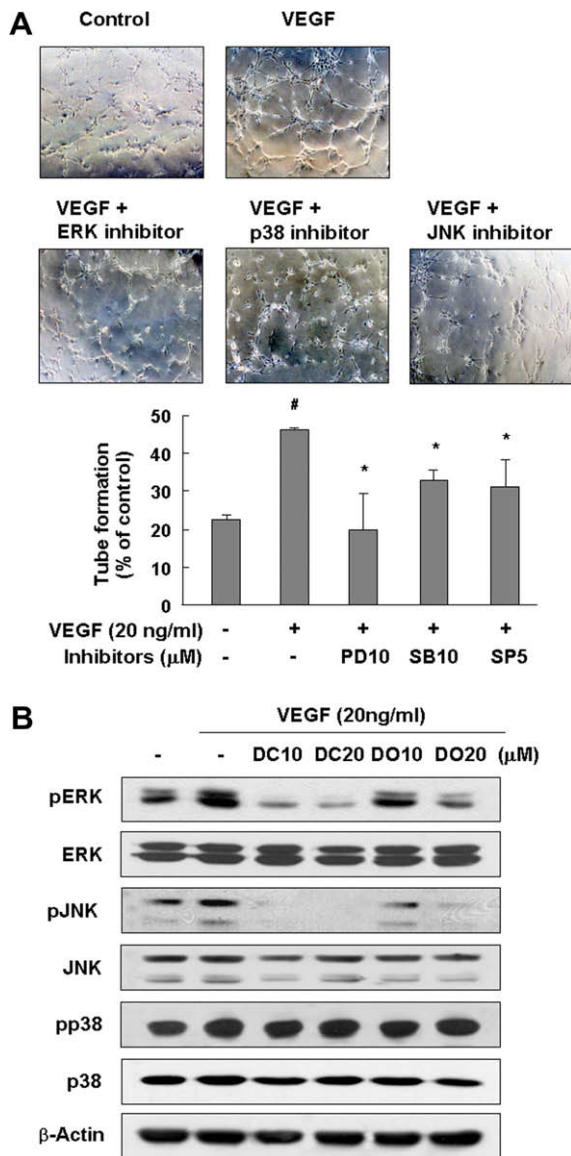
Cancer cells begin to promote angiogenesis early in tumorigenesis to expand their growth, and the newly formed vessels allow tumor cells to metastasize to distant target organs. Thus, the inhibition of angiogenesis could prevent tumor progression and metastasis, thereby turning cancer into a manageable chronic disease [16,17,38], and may also constitute a promising therapeutic approach for the treatment of patients afflicted with tumors [6]. Moreover, slowly growing tumors, which are more difficult to treat with chemotherapy, tend to respond well to anti-angiogenic therapy, whereas rapidly growing tumors require higher doses of angiogenesis inhibitors. The combination of a low-dose chemotherapeutic agent and an angiogenesis inhibitor can induce full and sustained regressions of large established tumors without host toxicity or drug resistance [39,40]. On the basis of these factors, it is necessary to develop nontoxic antiangiogenic agents for clinical application, and many plant-derived compounds have been determined to harbor anti-angiogenic potential, in addition to chemopreventive effects [18–23,41]. In the present study to evaluate the potential of decursin and decursinol as new nontoxic antiangiogenic agents, we investigated their inhibitory effects on VEGF-induced angiogenesis both *in vitro* and *in vivo*.

Several angiogenesis inhibitors have been developed to target the vascular endothelial cells that support tumor growth, rather than cancer cells specifically, as these endothelial cells are genetically stable, nontransformed cells and are thus generally less prone to develop drug resis-



**Fig. 3.** Decursin and decursinol inhibited *in vivo* angiogenesis. (A) Fertilized chicken eggs were incubated for 3 days before a 1-cm square window was made. On day 6, Thermanox coverslips with each test compound were placed on the CAM of individual embryos for 2 days at 37 °C. The presence of avascular zones ( $\geq 4$  mm diameter) was observed and scored positive for inhibition of angiogenesis. The anti-angiogenic activity of each compound is given as the percentage of tested eggs presenting an avascular zone. (B) Male C57BL/c mice ( $n = 5$ ) were subcutaneously injected with Matrigel mixture (600  $\mu\text{l}$ ) including decursin or decursinol (5 or 10  $\mu\text{M}$ ) together with VEGF (100 ng/ml). (C) The other mice were orally administered decursin or decursinol after the subcutaneous injection of Matrigel with VEGF (100 ng/ml). Seven days later, the plugs were collected and the amount of hemoglobin in the plugs was measured using Drabkin's reagent kit 525. The values were expressed as the means  $\pm$  SD.  $\#P < 0.01$  versus Matrigel alone,  $^*P < 0.05$ ,  $^{**}P < 0.01$  versus VEGF-treated Matrigel.

tance [5]. These inhibitors block a multistep angiogenic process of vascular endothelial cells induced by pro-angiogenic proteins, including VEGF, basic fibroblast growth factor, interleukin-8, and platelet-derived growth factor. We determined that decursin and decursinol at non-cytotoxic doses inhibited VEGF-induced proliferation, migration, and tube formation in HUVECs. Furthermore, decursin and decursinol markedly inhibited the formation of micro-



**Fig. 4.** Decursin and decursinol inhibited VEGF-induced phosphorylation of ERK and JNK in HUVECs. (A) HUVECs were treated with PD 98059 (an ERK inhibitor, 10 μM), SB 203580 (a p38 MAPK inhibitor, 10 μM) or SP 600125 (a JNK inhibitor, 5 μM) in the presence of VEGF (20 ng/ml) at 37 °C for 8 h. Tube formation was visualized and quantified under an inverted microscope (×40 magnification). (B) HUVECs were treated with decursin (DC) or decursinol (DO) (10 or 20 μM) for 24 h. Protein extract (30 μg) was resolved by 10% SDS-PAGE, and total/phospho ERK1/2, JNK, and p38 MAPK were detected by Western blotting.

vessels on the CAM of fertilized eggs, and VEGF-induced angiogenesis in Matrigels injected subcutaneously into mice, in both cases in which these compounds were included in Matrigel together with VEGF or orally administered. Decursin was found to be more potent in the cell-based experiments, but was less effective than decursinol in the *in vivo* experiments. The results of several studies have indicated that decursinol, in which the (CH<sub>3</sub>)<sub>2</sub>-C=CH-COO-side chain of decursin is replaced with -OH,

evidences significantly lower anti-cancer activity or no effect in human cancer cells [29,32,42]. However, decursin and decursinol exhibited excellent anti-angiogenic activity in endothelial cells and in an animal model. This is the first report to show that decursinol, as well as decursin, exhibits anti-angiogenic activities *in vitro* and *in vivo*.

MAPK signaling is one of the critical molecular events for growth, survival, and migration in the VEGF-induced angiogenesis of vascular endothelial cells. VEGF activates three MAPKs—namely, ERK, JNK, and p38 MAPK [34,35,37,43]. ERK activation results in an increased proliferation of endothelial cells [35,37], whereas p38 MAPK activation triggers actin-based cell motility [34–36]. JNK performs a significant role in both the proliferation and migration responses [37]. We verified that VEGF-stimulated tube formation was blocked by treatment with each specific inhibitor of ERK, p38, and JNK at a non-cytotoxic dose in the HUVECs. Decursin and decursinol attenuated the VEGF-induced phosphorylation of ERK and JNK, but not p38 MAPK, in HUVECs. Our data support the notion that three MAPKs mediate VEGF-induced tube formation, but decursin and decursinol inhibit VEGF-induced angiogenesis via an induced reduction in ERK and JNK activation.

Collectively, our results show that decursin and decursinol isolated from Korean *A. gigas* root are potent VEGF-induced angiogenesis inhibitors, both *in vitro* and *in vivo*. Decursin and decursinol provide the advantage of oral dosing, and lack some of the shortcomings of large proteins (difficulty of manufacture in large quantities, cost, immunogenic potential, and need for parental administration) due to their low molecular weights. Therefore, these compounds may be more appropriate for prolonged treatments, not only in cancers dependent on VEGF-induced vascularization but also in other diseases in which VEGF-mediated angiogenesis performs a crucial role in pathogenesis. Decursin and decursinol may be useful in the prevention of cancer growth and metastasis.

### Conflict of interest

None of the authors has any potential conflicts of interest.

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### Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.canlet.2009.02.012](https://doi.org/10.1016/j.canlet.2009.02.012).

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