Angiogenic Effects of the Extracts from Chinese Herbs: Angelica and ChuanXiong

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Abstract: Angelica and ChuanXiong are used to cure ischemic heart disease in China. Previous studies found that these two herbs could increase myocardial blood flow, oxygen-supply and keep myocardial oxygen balance, etc. However, the mechanisms of angiogenic effects of these two herbs are not well-known. The purpose of this study was to assess the effects of Angelica and ChuanXiong on vascular endothelial growth factor (VEGF) expression in rat myocardial infarction, on endothelial cell proliferation and quantity of vessels on chick embryo chorioallantoic membrane (CAM). In this study, rats were divided randomly into either pre-treatment or acute-treatment group and sacrificed at the end of the treatments. VEGF expression using Western blot analysis was significantly increased in the groups pre-treated with ChuanXiong and Angelica when compared to the control group (p < 0.05). There was significant increase in VEGF expression in the rats treated acutely with Angelica (p < 0.05). In the contrary, the rats treated with ChuanXiong showed a decrease in VEGF expression when compared to the acute-treatment control group (p < 0.05). Similar results were observed in immunohistochemistry of VEGF expression in the myocardia. Our study also demonstrated that these two herbs significantly enhanced endothelial cell proliferation (p < 0.05) and revascularity in CAM (p < 0.05). The data showed that Angelica and ChuanXiong could affect VEGF expression in rat myocardial infarction, promote endothelial cell proliferation and stimulate quantity of vessels on CAM model. The results suggest that Angelica and ChuanXiong have angiogenic effects, and may provide some mechanisms for the treatment of myocardial infarction and peripheral ischemia.

Keywords: Angiogenesis; Endothelial Cell; Chicken Chorioallantoic Membrane; Myocardial Infarction; Angelica; Chuanxiong.
Introduction

Angiogenic cytokine therapy has been widely regarded as an attractive approach for treating ischemic heart disease and enhancing arterioprotective functions of the endothelium (Khurana et al., 2005). Successful preclinical studies paved the way for the first clinical trials, with single growth factors given as recombinant proteins or genes. However, various studies suggested that neovascularization contributes to the growth of atherosclerotic lesions and is a key factor in plaque destabilization leading to rupture. The potential risk and clinical safety should be considered for therapeutic angiogenesis since clinical results have not matched the initial promise. Clinical studies also failed to show evidence of angiogenic cytokines contributing to accelerated atherosclerosis, although documentation of this complication would not be obvious (Imran et al., 2004).

Many studies had focused on the role of vascular endothelial growth factor (VEGF) as a key mediator for ischemia-driven angiogenesis. VEGF induced sprouting and capillary growth toward the ischemic tissue and matches the vascular density according to physiological increase in oxygen consumption (Zachary and Gliki, 2001). VEGF and other angiogenic cytokines could promote revascularization in diverse animal models of ischemic cardiovascular disease (Celletti et al., 2001; Moulton et al., 1999; Heeschen et al., 2001); however the data from clinical trials so far have been inconclusive. Several studies (Celletti et al., 2001; Moulton et al., 1999; Heeschen et al., 2001) suggested that VEGF and other angiogenic factors could promote atherosclerosis in certain animal models and potentially destabilize coronary plaques by promoting intralesion angiogenesis, which made proponents of therapeutic angiogenesis seemed more problematic.

In recent years, bioactive compounds from natural sources had been used as regulatory agents. Many investigators had been searching for inhibitors and promoters of angiogenesis from plants. The extracts of Ginkgo-biloba, Aloe vera, Angelica sinensis, Dalbergia odorifera, Epimedium sagittatum, Patrinia villosa and Trichosanthes kirilowii enhanced angiogenesis in vivo (Juarez et al., 2000; Choi et al., 2002; Wang et al., 2004). On the other hand, the extract from Chrysobalanus icaco methanol (Alves De Paulo et al., 2000), fern Polypodium leucotomos (Gonzalez et al., 2000), Torilis japonica (Kim et al., 2000), Cassia garrettiana heartwood (Kimura et al., 2000), Agaricus blazei (Takaku et al., 2001), Pulsatilla koreana (Kim et al., 2002), green tea (Jung et al., 2001), grapes (Brakenhielm et al., 2001), ginseng saponins and some related triterpenoid compounds (Shibata, 2001) had anti-angiogenic activities either in vitro or in vivo. Some traditional Chinese medicinal herbs are effective in curing ischemic disease. However, their mechanisms have not been tested. This report described the angiogenic activity of Angelica and ChuanXiong via assaying vascular endothelial growth factor (VEGF) expression in rat myocardial infarction, endothelial cell proliferation and quantifies vessels on chick embryo chorioallantoic membrane (CAM) model.
Materials and Methods

Preparation of Crude Plant Extracts

The dried medicinal herbs used in this study were from Pharmacy of Traditional Chinese Medicine in Xi-Jing Hospital, which were purchased from local herbal company (it is the certified drug supplier of China). Radix Angelicae sinensis (AS) was collected from Gansu province and Rhizoma chuanxiong (CX) was collected from Sichuan province, China. The dried herb (100 g) was extracted with 1000 ml boiling distilled water under reflux for 2 hours. After filtration to remove insoluble material, the extract was stored at −20ºC until use. During the assay, 1 g dry herb/ml and 0.5 g dry herb/ml were prepared using purified water or DMSO and sterilized by passing through a 0.22 µm filter. The identity of AS and CX were confirmed by Prof. Yang Xizhong (Pharmacy of Traditional Chinese Medicine, Xi-Jing Hospital, The Fourth Military Medical University).

Animals Groups and Treatment Procedure

Male Sprague Dawley rats (180–220 g) were supplied by Animal Center of the Fourth Military Medical University. Myocardial infarction was induced in 30 rats as described previously (Zhu et al., 2005). Briefly, thoractomy was performed by invasion of the intercostal muscles between the 4th and 5th ribs. The heart was exposed and maintained by using a retractor. The left anterior descending coronary artery was occluded 3 mm distally from where it branches off the aorta using a 5-0 polypropylene thread. The thorax and chest were then closed and the rats were recovered with ventilation for 1 hour.

Rats were divided randomly into pre-treatment and acute-treatment groups. Three rats were fed regular diet for 2 weeks as normal control. The pre-treatment group (15 rats) was fed regular diet and the herbal extracts for 2 weeks. After 2 weeks, they were led to acutely lacking blood in myocardium for 2 hours. The acute-treatment group (15 rats) were made myocardial infarction model and then fed regular diet and the herbal extracts for 2 weeks. All rats were sacrificed at the end of the treatments (Table 1). All surgical procedures and protocols were performed in accordance with the Ethical Principles in Animal Research set forth by the Fourth Military Medical University for Animal Experimentation.

Heart Tissue Preparation

Fourteen days after treatment, the rats were anesthetized with sodium pentobarbital (3.3 ml/kg, intraperitoneal) and the thorax was opened. The rats were submitted to transcardiac perfusion with saline solution. The heart was quickly excised. All surrounding fat and connective tissue were removed. The left ventricle (LV) and the ischemic LV were harvested. In ischemic LV a transverse slice of approximately 2–3 mm tissue was taken in
the center of the infarction. The tissue in the midline between the base of the heart and the apex from sham rats were used as a control. A portion of the myocardial specimens were fixed in 4% paraformaldehyde and embedded in paraffin for histological analysis. The rest of the myocardium were snap frozen in liquid nitrogen and stored at −80°C until use.

**Western Blot Analysis**

The heart tissues were thawed, minced and homogenized in extraction buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 100 µg/ml PMSF, 2 µg/ml leupeptin) with a Dounce homogenizer. All procedures were carried out at 4°C. The homogenate was incubated on ice for 30 min, and centrifuged at 12,000 g for 15 min at 4°C. The supernatant was used for Western Blotting. Protein concentrations were determined by the BCA assay using bovine serum albumin as a standard according to the manufacturer’s instruction (Pierce, Rockford, IL). Proteins (50 µg) were separated on a 12% SDS-PAGE and transferred to PVDF membranes. The membranes were incubated with blocking buffer (Tris buffered saline containing 0.1% Tween-20 and 5% non-fat milk) for 1 hour at room temperature, followed by incubation with anti-VEGF mice polyclonal antibody (1:1000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. α-tubulin (Invitrogen, CA) was used as a loading control. The membranes were then washed and incubated with secondary antibody (1:5000 dilutions, Amersham Life Science, Amersham, UK). VEGF was detected by ECL staining following the protocols of the manufacturer (Pierce, Rockford, IL).

**Immunohistochemistry**

Longitudinally oriented transmural sections were prepared from the middle part of the left ventricle. Samples for immunohistochemical analysis were fixed in 7.5% formaldehyde (in PBS, pH 7.2). Tissue embedded in paraffin wax was cut into 3- to 5-µm sections, dried at

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**Table 1. Group and Treatment Procedures of Animals**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rat</th>
<th>Diet and Treatment</th>
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</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3</td>
<td>Regular diet, 14 days</td>
</tr>
<tr>
<td><strong>Precautionary groups:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (P-Control)</td>
<td>3</td>
<td>Regular diet (RD), 14 days + ischemia for 2 hours in myocardium</td>
</tr>
<tr>
<td>Chuanxiong high dose (CXH)</td>
<td>3</td>
<td>RD + CX 4.5 kg BW/day for 14 days + ischemia for 2 hours</td>
</tr>
<tr>
<td>Chuanxiong low dose (CXL)</td>
<td>3</td>
<td>RD + CX 1.5 kg BW/day for 14 days + ischemia for 2 hours</td>
</tr>
<tr>
<td>Angelicae high dose (ASH)</td>
<td>3</td>
<td>RD + AS 4.5 kg BW/day for 14 days + ischemia for 2 hours</td>
</tr>
<tr>
<td>Angelicae low dose (ASL)</td>
<td>3</td>
<td>RD + AS 1.5 kg BW/day for 14 days + ischemia for 2 hours</td>
</tr>
<tr>
<td><strong>Therapeutic groups:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (T-Control)</td>
<td>3</td>
<td>Ischemia in myocardium + regular diet (RD) for 14 days</td>
</tr>
<tr>
<td>Chuanxiong high dose (CXH)</td>
<td>3</td>
<td>Ischemia + RD + CX 4.5 kg BW/day for 14 days</td>
</tr>
<tr>
<td>Chuanxiong low dose (CXL)</td>
<td>3</td>
<td>Ischemia + RD + CX 1.5 kg BW/day for 14 days</td>
</tr>
<tr>
<td>Angelicae high dose (ASH)</td>
<td>3</td>
<td>Ischemia + RD + AS 4.5 kg BW/day for 14 days</td>
</tr>
<tr>
<td>Angelicae low dose (ASL)</td>
<td>3</td>
<td>Ischemia + RD + AS 1.5 kg BW/day for 14 days</td>
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</tbody>
</table>
55°C for 2 hours, and then deparaffinized in xylene for 10 min, followed by dehydration through graded alcohols. Later, tissue proteolysis was performed with an autoclave. Sections were then immersed in PBS (pH 7.6) and incubated with the antibody VEGF Ab-6 (1:100) for 2 hours. The slices were washed and incubated with secondary antibody (1:500) for 90 min at room temperature.

**Isolation and Culture of Rat Cardiac Microvascular Endothelial Cells (CMECs)**

Male Sprague-Dawley rats (4 weeks old) were anesthetized with ether. Hearts were rapidly excised and placed in a dish containing Hank’s buffer (136.75 mM NaCl, 5.37 mM KCl, 1.26 mM CaCl₂, 0.81 mM MgSO₄·7H₂O, 0.37 mM Na₂HPO₄·2H₂O, 0.44 mM KH₂PO₄, 4.17 mM NaHCO₃, and 5.55 mM glucose, saturated with 95% O₂-5% CO₂, pH 7.4 at 37°C). Hearts were initially perfused with Hank’s buffer for 5–10 min through the ascending aorta to remove blood cells. After removing the connective tissue, arteries, right ventricle, all valvular tissues, the remaining left ventricular tissue was washed with Hank’s buffer. Left ventricle was immersed in 70% ethanol for 30 sec and then washed with calcium-free Hank’s buffer. The outer part of the left ventricle free wall and septum were dissected away. The remaining left ventricular tissue was finely minced in 0.3% collagenase (Invitrogen Life Technologies, CA) and incubated for 30 min at 37°C in a shaking water bath. Trypsin (0.02%, Invitrogen Life Technologies, CA) was added and the minced tissue was incubated for another 30 min. Dissociated cells were filtered through a 200-µm mesh filter, washed with calcium-free Hank’s buffer and centrifuged at 1000 rpm for 5 min. Cells were resuspended in M131 (Cytotech, North Zealand, Denmark) supplemented with microvascular growth supplement (MVGS) and attachment factor (AF). Four hours later, the attached cells were washed with M131 and cultured in M131 supplemented with MVGS and AF under 5% CO₂-95% O₂ at 37°C (Nishida et al., 1993). The cells were used between passages 1 and 3.

**Culture of Human Umbilical Vein Endothelial Cells (HUVECs)**

HUVECs were purchased from American Tissue Culture Collection (ATCC, No CRL-1998). Cells were cultured in medium 199 (Invitrogen Life Technologies, CA) containing 10% heat-inactivated fetal bovine serum (FBS) (Sijiqing Company, China), supplemented with 2 mM glutamine, 1.5 g/L sodium carbonate, 100 kU/L penicillin, and 100 mg/L streptomycin at 37°C under 5% CO₂. All experiments were performed using cells between passage 6 and 9.

**Cell Proliferation Assay**

Endothelial cells were plated in 96-well plates at a density of 1–5 × 10⁴ cells per well in 100 µl M131 or M199 medium. After overnight culture, the cells were washed with PBS and cultured with serum free M131 or M199 medium supplemented with Chinese
herbs or recombinant human VEGF\textsubscript{165} (BD Bioscience, San Jose, CA). After 72 hours of incubation at 37\(^\circ\)C, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays were performed according to the manufacture protocol. Bio-Rad model 550 plate reader (Bio-Rad) was used to determine the number of living cells in triplicate.

**Chicken Chorioallantoic Membrane (CAM) Assay**

The chicken chorioallantoic membrane (CAM) assay was performed according to Li et al. (2001). Briefly, after incubation at 37\(^\circ\)C for 3 days in a humidified atmosphere, fertilized eggs were opened and the shell membrane was taken off to expose CAM. A thermanox coverslip loaded with the herbal extract (20 µl) was applied to the CAM surface the following day. The chick embryo was returned to the incubator. Three days later, an appropriate volume of methanol and acetone mixture (1:1) was injected using a 33-gauge needle into the 7-day-old embryo chorioallantois. The CAM was cut out from eggs and the number of vessels was observed and vessels radially converging toward the center were counted under a microscope. Eggs treated by vehicle or VEGF\textsubscript{165} were used as the negative or positive controls, respectively. At least 10 eggs were used for each group.

**Statistics Analysis**

All results were expressed as mean ± SEM. Statistical analysis was performed by One-Way ANOVA (Single factor) and Dunnett T3 (multiple factors) assay with SPSS software. The difference is significant when p < 0.05.

**Results**

*The Extracts of Angelica and ChuanXiong Increase VEGF Protein Expression in Post-Myocardial Infarction Rats*

VEGF expression was significantly (p < 0.05) increased in the pre-treatment group treated with ChuanXiong (1.5 and 4.5 kg BW/day) and Angelica (4.5 kg BW/day); there was no difference in the group treated with Angelica (1.5 kg BW/day), compare to the pre-treatment control (P-control) (Fig. 1A). In the acute-treatment groups, there was significant (p < 0.05) increase in VEGF expression in the rats treated with Angelica (1.5 and 4.5 kg BW/day). On the contrary, the rats treated with ChuanXiong (1.5 and 4.5 kg BW/day) showed a decrease in VEGF expression compared to the acute-treatment control group (p < 0.05) (Fig. 1B). Similar results were observed in immunohistochemistry of VEGF expression in these myocardia (Figs. 1C and 1D).

*The Extracts of Angelica and ChuanXiong Increase Endothelial Cells Proliferation*

The effects of ChuanXiong, Angelica and hVEGF\textsubscript{165} on endothelial cell proliferation are shown in Table 2. hVEGF\textsubscript{165} significantly increased the proliferation of CMEC by
ANGIOGENIC EFFECTS OF ANGELICA AND CHUANXIONG

Figure 1. The effects of the extracts of Angelicae and ChuanXiong on VEGF protein expression in the ischemic myocardium. All the rats were fed regular diet and/or the extracts (4.5 kg and 1.5 kg/BW/day, respectively) for 14 days as described in the methods. (A) VEGF protein expression in the myocardium of precautionary (P) group using Western blotting. (B) VEGF protein expression in the myocardium of therapeutic (T) group using Western blotting. (C) VEGF protein expression in the myocardium of precautionary (P) group using immunohistochemistry. (D) VEGF protein expression in the myocardium of therapeutic group using immunohistochemistry. CXh: ChuanXiong high concentration (4.5 kg/BW/day), CXl: ChuanXiong low concentration (1.5 kg BW/day), ASh: Angelicae sinensis high concentration (4.5 kg/BW/day), ASl: Angelicae sinensis low concentration (1.5 kg/BW/day). *p < 0.05, compared to the control.
Table 2. The Effects of the Extracts of Angelicae and ChuanXiong on Angiogenesis Activity of CMECs and HUVECs

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration (µg/ml)</th>
<th>CMEC OD Value</th>
<th>HUVEC OD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>—</td>
<td>0.52 ± 0.03</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>hVEGF165 (Positive control)</td>
<td>10 × 10⁻³</td>
<td>0.77 ± 0.05*</td>
<td>0.99 ± 0.05*</td>
</tr>
<tr>
<td>Chuanxiong</td>
<td>100</td>
<td>0.64 ± 0.03*</td>
<td>0.85 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.52 ± 0.02</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.50 ± 0.04</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Angelicae</td>
<td>150</td>
<td>0.62 ± 0.05*</td>
<td>1.21 ± 0.15*</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.56 ± 0.06</td>
<td>1.08 ± 0.12*</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.49 ± 0.01</td>
<td>0.91 ± 0.07*</td>
</tr>
</tbody>
</table>

Endothelial cells were plated in 96-well plates at a density of 1–5 × 10⁴ cells per well. After overnight culture, the medium was replaced with free-serum medium supplemented with Chinese herbs or recombinant human hVEGF₁₆₅. MTT assays were performed to determine the number of living cells (n = 6). The absorbance (OD value) was measured at 540 nm in triplicate. *p < 0.05, compared with normal control.

48% (p < 0.05), and that of HUVEC by 43% (p < 0.05) when compared to the controls. Chuan Xiong (100 µg/ml) significantly increased the proliferation of CMEC (23%, p < 0.05) and HUVEC (23%, p < 0.05) when compared to the controls. Table 2 also shows that Angelica significantly increased proliferation of CMEC and of HUVEC (p < 0.05).

The Extracts of Angelica and ChuanXiong Increase Capillary Density in CAM

CAM was formed when a fertilized egg was incubated at 37ºC for 3 days. The neovascularization in CAM was observed on the fourth day. The hVEGF₁₆₅ or tested
ANGIOGENIC EFFECTS OF ANGELICA AND CHUANXIONG

Compounds (the extracts of ChuanXiong and Angelica from 10 to 100 µg/ml/egg) were added on CAM for another 72 hours. As shown in Fig. 2A and B, in the presence of hVEGF165 (10^{-3} µg/µl/egg) as positive control, and CX and AS 100 or 50 or 10 µg/ml/egg treatment. B showed CX and AS 50 or 10 µg/ml/egg treatment. *p < 0.05, compared to the normal control (saline).

**Discussion**

Traditional Chinese Medicine (TCM) plays an important role in Chinese modern medicine field. During long-term clinical practices, we have successfully developed some herbs, for example, ChuanXiong and Angelica (also known as Danggui), to treat cardiovascular diseases. The theory of TCM suggests that the functions of ChuanXiong act as Huoxue Qi and Qufeng painkillers, and Angelica as Huoxue Yangxue and detumescent painkillers. In cardiovascular system, Angelica and ChuanXiong also have cardioprotective effects.
Studies (Hu et al., 2004; Wu et al., 2001; Wang et al., 2003; Tian and Fan, 1997) showed that Chuanxiong tetramethylpyrazine extracted from ChuanXiong (or known as Ligustrazine) and ferulic acid extracted from Angelica and ChuanXiong significantly dilated coronary artery, increased myocardial blood flow, increased myocardial oxygen-supply and kept myocardial oxygen balance. In addition, Ligustrazine could increase the anti-anoxia ability of animals to reduce their myocardial oxygen consumption (Hu and Hu, 2004; Tian and Fan, 1997). Ferulic acid had antithrombotic effect in vitro, shortened the length and reduced the weight of thrombosis (Wu et al., 2001; Wang et al., 2003). Angelica had angioplasty, antithrombotic and anti-atherogenic pharmacological effects on cardiovascular diseases (Wu et al., 2001; Wang et al., 2003).

It was reported that Angelica could promote the growth of endothelial cells, increase the cell population in S phase and VEGF expression in endothelial cells (Lei et al., 2003). Angelica also had certain effect on the genesis and development of neogenetic vascularization in ischemic myocardium (Lei et al., 2003). Angelica crude extract increased the proliferation of gastric epithelial cells through modulation of several proliferation-related genes, EGF, ODC, and c-Myc (Ye et al., 2001a and b; Ye et al., 2003). Angelica significantly promoted the recovery rate of immune system in mice (Shang et al., 2003; Tsai et al., 2005) and increased the number of blood vessel in both the gastric and duodenal tissues (Hui et al., 2006). Chuanxiong-pathalide A pretreatment protected the endothelial function from injury caused by ischemia and reperfusion (Gao et al., 2005). In the current study, VEGF expression was significantly increased or decreased, respectively, in the pre-treatment and acute-treatment group treated with ChuanXiong and Angelica, compare to the control. Our study also demonstrated that these two herbs significantly enhanced endothelial cell proliferation and stimulated revascularity in CAM. These studies (Lei et al., 2003; Ye et al., 2001; 2003; Shang et al., 2003; Tsai et al., 2005; Hui et al., 2006; Gao et al., 2005) and our observations suggested that the extracts of ChuanXiong and Angelica might be potential substitutes or mediators of angiogenic cytokines to treat ischemic diseases. However, different concentrations of Angelica and ChuanXiong had different effects on VEGF protein expression in ischemic myocardium and endothelial cell proliferation (Fig. 1, Table 2). At the present time, the active ingredients of Angelica and ChuanXiong remain unclear. Some components such as ligustrazine, ferulic acid and others had been identified from the extracts of Angelica and ChuanXiong. However, the components of these extracts are very complex. Clinical studies showed that different concentrations of herbs may have different effects on different tissue and organism. Consequently, it needs to determine the active components of Angelica and ChuanXiong and their pharmacological effects on cardiovascular system. As potential substitutes or mediators for angiogenic cytokines to treat ischemic diseases, further studies are required to understand the mechanism of action and active substances in their extracts.

Angiogenesis plays an important role in numerous diseases. The main role of VEGF in vessel growth makes this protein a tantalizing target for either stimulation or inhibition of angiogenesis. VEGF has endothelial cell-specific mitogenic activity and stimulates angiogenesis in vivo and in vitro (Ng et al., 2006). This observation suggests that endothelial cells are the primary targets of therapeutic angiogenesis of VEGF. Previous
study showed that VEGF promoted endothelial cells proliferation (Khurana et al., 2005). In agreement with this observation, we found hVEGF_{165} significantly increased proliferation of both CMEC and HUVEC (Table 2). In rat myocardial infarction model, VEGF protein expression in two-hour ischemic myocardium was decreased compared to the normal control, and there was no difference in 14-day ischemic myocardium when compared to the control. It was reported that VEGF expression is stimulated by hypoxia, and the levels of this growth factor are substantially increased in ischemic myocardium (Banai et al., 1994; Matsunaga et al., 2000). VEGF expression increased during the early occlusion days and waned as collateral flow and the severity of ischemia was ameliorated by the increase in collateral conductance (Matsunaga et al., 2000). Interestingly, in patients with dilated cardiomyopathy, capillary density is reduced compared with noncardiomyopathy patients, and the expression of hVEGF_{165} and the kinase domain receptor was reduced (Abraham et al., 2000). Matsunaga et al. (2003) reported that myocardial capillary density was augmented by myocardial ischemia, but after the development of collaterals and restoration of flow to the ischemic zone, capillary density returned to control levels. The change in capillary density paralleled with VEGF expression. These results suggested that VEGF could recover to the control levels after development of collaterals and restoration of flow to the ischemic zone. The study showed that Angelica and ChuanXiong could not only change VEGF protein expression in ischemic myocardium, but also stimulate endothelial cell proliferation and enhanced capillary density in CAM. Therefore, Angelica and ChuanXiong could either be a potential stimulator or inhibitor of angiogenesis.

In conclusion, the extracts of Angelica and ChuanXiong could change VEGF protein expression significantly in ischemic myocardium. This effect may be related to enhance proliferation of endothelial cells and quantity of blood vessels. Further study of the effects of Angelica and ChuanXiong on other growth factors may provide insights into the mechanisms on angiogenesis.

Acknowledgments

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