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Antiangiogenic effect of selected phytochemicals

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Angiogenesis, the formation of new blood vessels from a preexisting vascular network is considered a key step in tumour growth, invasion, and metastasis. Recent studies show that several natural compounds inhibit angiogenesis and nowadays numerous bioactive plant compounds are tested for their antiangiogenic potential. This review examines current knowledge regarding the antiangiogenic potential of several phytochemicals, including polyphenols resveratrol and curcumin as well as miscellaneous compounds from garlic, *Hypericum perforatum*, *Panax ginseng*, *Coptis chinensis* and *Rheum palmatum*.

1. Introduction

Angiogenesis, the development of new blood vessels, is a fundamental physiological process that promotes embryonic development, tissue repair and fertility (Coultas et al. 2005). Under pathological conditions, tumors stimulate angiogenesis, the formation of blood vessels, and take oxygen and nutrition for rapid growth. Furthermore, tumors utilize blood vessels and lymph vessels for metastasis to other tissues and lymph nodes (Hanahan and Folkman 1996; Fujita et al. 2008).

Tumors can remain avascular and latent for years and activation of angiogenesis is a necessary condition for tumor development (Bergers and Benjamin 2003). It is known that without blood supply the dimensions of a tumor nodule cannot exceed 2–3 mm³ due to hypoxia leading to death of tumor cells (Folkman 1995). Since a close relationship between tumor growth and angiogenesis had been clarified, various antiangiogenic inhibitors for use in cancer treatment have been studied. The best-known anti-angiogenic agents of this class are the vascular endothelial growth factor (VEGF) inhibitors including bevacizumab that was approved for use as part of combination therapy with fluorouracil-based regimens for metastatic colorectal cancer (Shih and Lindley 2006).

During the last 15 years, substantial effort has been dedicated to identifying compounds that can be used to either prevent insurgence of primary tumors in subjects at high risk to develop cancer or prevent tumor relapse after surgical removal (Brem et al. 1993; Beckeen et al. 2000; Eder et al. 2002; Cox et al. 2006; Kulke et al. 2006). At present, ongoing clinical trials are focused on drugs with different mechanisms of action such as blockade of matrix breakdown, direct inhibition of endothelial cells, blockade of activators of angiogenesis, inhibition of endothelial-specific integrin/survival signaling as well as drugs with non-specific mechanism of action.

2. Antiangiogenic effects of phytochemicals

Several hypotheses have been suggested to explain beneficial effects of increased consumption of vegetables and fruits on

human health. An attractive hypothesis is that vegetables and fruits contain compounds that have protective effects, independent of those of known nutrients and micronutrients. Current interest is focused on beneficial health effects of dietary polyphenols because these compounds have many biological activities (for review, see Magrone et al. 2008; Pan and Ho 2008; Khan et al. 2009).

As stated in our recent review (Mojžiš et al. 2008), flavonoids and their metabolic precursors, chalcones, possess very promising antiangiogenic properties. However, other non-flavonoid polyphenols, such as resveratrol and curcumin, may also significantly contribute to the antiangiogenic effect of polyphenol-rich plants.

2.1. Resveratrol

One of the most widely investigated plant-derived bioactive compound is resveratrol (*trans*-3,5,4'-trihydroxystilbene). It was first isolated in 1940 as a constituent of the roots of white hellebore (*Veratrum grandiflorum*) (Aggarwal et al. 2004). Resveratrol has been found in grapes, berries, peanuts and various other fruits. Similarly to other polyphenolic compounds, it has been shown to affect tumorigenesis and tumor growth (Nobili et al. 2009; Zhou et al. 2005; Fuggetta et al. 2006). Recently, resveratrol has been found to inhibit angiogenesis, but the mechanisms of its antiangiogenic activity has not been fully understood.

In 2001, Kimura and Okuda tested the effect of resveratrol on the growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice. They found that resveratrol, at doses of 2.5 and 10.0 mg/kg, significantly reduced tumor volume, its weight and metastasis to the lung in these animals. Resveratrol also inhibited tumor-induced neovascularization at the same doses in an *in vivo* model. Moreover, resveratrol significantly inhibited the *in vitro* formation of capillary-like tube formation by human umbilical vein endothelial cell (HUVECs).

Later, it was documented that resveratrol (40.0 mg/kg/day) exerted significant antitumor effects on experimentally-induced gliomas. Immunohistochemical analyses showed that the gliomas from resveratrol-treated rats had lower microvessel densities than did control rats (Tseng et al. 2004; Chen et al. 2006). Furthermore, the antiangiogenic effect of resveratrol was examined also on chick chorioallantoic membrane (CAM) model of angiogenesis. Mousa et al. (2005) found potent inhibitory effects of resveratrol on fibroblast growth factor-2 (FGF2) induced angiogenesis and tumor growth. Resveratrol in a concentration-dependent manner also significantly promoted apoptosis in FGF2-stimulated endothelial cells by increasing p53 protein production. Although the mechanisms of antiangiogenic activity of resveratrol are not clear, some mechanisms have been proposed.

Hypoxia-inducible factor-1 α (HIF-1 α) is overexpressed in many human cancers and their metastases and is closely associated with a more aggressive tumor phenotype. Cao et al. (2004) investigated the effect of resveratrol on HIF-1 α and VEGF expression in human ovarian cancer cells. They found that although resveratrol did not affect HIF-1 α mRNA levels, it did significantly inhibit both basal-level and growth factor-induced HIF-1 α protein expression in the cells. Resveratrol also greatly inhibited VEGF expression. It was demonstrated that resveratrol inhibited HIF-1 α and VEGF expression through multiple mechanisms. Resveratrol inhibited Akt and mitogen-activated protein kinase activation, inhibited insulin-like growth factor 1-induced HIF-1 α and induced substantial HIF-1 α protein degradation through the proteasome pathway. These results were confirmed by Zhang and co-workers (2005) in human tongue squamous cell carcinomas and hepatoma cells. They documented a significant inhibition of both basal level and hypoxia-induced HIF-1 α protein accumulation in cancer cells. Pretreatment of cancer cells with resveratrol significantly reduced hypoxia-induced VEGF promoter activities and VEGF expression. In addition, resveratrol markedly inhibited hypoxia-mediated activation of extracellular signal-regulated kinase 1/2 (Erk 1/2) and Akt, leading to a marked decrease in hypoxia-induced HIF-1 α protein accumulation and VEGF transcriptional activation.

Later, Park and co-authors (2007) investigated the effect of resveratrol on lysophosphatidic acid (LPA) and hypoxia-induced HIF-1 α and VEGF expression. Their results showed that LPA treatment under hypoxia increased HIF-1 α protein level followed by increased expression of VEGF protein and mRNA. These increases in HIF-1 α and VEGF expression were dramatically attenuated by resveratrol. The authors suggested that the underlying mechanism of inhibition of HIF-1 α expression by resveratrol seems to be associated with both inactivation of p42/p44 MAPK and p70S6K as well as enhanced degradation of HIF-1 α protein, resulting in profound decrease in VEGF expression and cell migration.

Resveratrol treatment also inhibited endothelial cell attachment to basement membrane components fibronectin and laminin, and displays similar effect on cell chemotaxis. In addition, resveratrol was found to be an angiogenesis inhibitor as documented in the rat aorta matrix culture model (Cao et al. 2005) or bovine aorta endothelial (BAE) cells (Igura et al. 2001).

There are two isomers of resveratrol found in grapes and red wine – cis and trans stereoisomers but the mechanism of their antiangiogenic activity has not been fully elucidated. To get novel insights about the antiangiogenic activity of resveratrol, Balleri and co-workers (2008) compared cis- and trans-resveratrol stereoisomers for their effect on the angiogenesis process. *trans*-Resveratrol inhibited endothelial cell proliferation and the repair of mechanically wounded endothelial cell monolayers. Also, it prevented endothelial cell sprouting in fibrin gel, collagen gel invasion, and morphogenesis on

Matrigel. *In vivo*, *trans*-resveratrol inhibited vascularization of the chick embryo area vasculosa and murine melanoma B16 tumor growth and neovascularization. In all of the assays, *cis*-resveratrol exerted a limited effect. Furthermore, the authors found that *trans*-resveratrol but not *cis*-resveratrol inhibited α 5 β 3 integrin-dependent endothelial cell adhesion and the recruitment of enhanced green fluorescent protein-tagged β 3 integrin in focal adhesion contacts.

Reactive oxygen species (ROS) play a crucial role in vascular angiogenesis. Both *in vitro* and *in vivo* studies indicate that angiogenic response in vascular tissue is triggered by ROS signaling in a highly coordinated manner (Maulik 2002). Some data indicate that scavenging properties of resveratrol may contribute to its anti-angiogenic effects. In a recent study, Lin and co-workers (2003) investigated the mechanisms by which resveratrol inhibits VEGF-induced angiogenic effects in HUVECs. Exposure of HUVECs to 1 to 2.5 μ M resveratrol significantly blocked VEGF-mediated migration and tube formation but not cell proliferation. At the same concentrations, resveratrol effectively abrogated VEGF-mediated tyrosine phosphorylation of vascular endothelial (VE)-cadherin and its complex partner, beta-catenin. Src kinase assay showed that VEGF-induced endogenous Src kinase activation was strongly inhibited by 1.0 and 2.5 μ M resveratrol. Reactive oxygen species have been shown to be involved in VE-cadherin phosphorylation and its related functions. Detailed analysis showed that VEGF caused an evident increase in peroxide which was strongly attenuated by resveratrol. These data suggest that resveratrol inhibition of VEGF-induced angiogenesis was mediated by disruption of ROS-dependent Src kinase activation and the subsequent VE-cadherin tyrosine phosphorylation.

Encouraging results of *in vitro/in vivo* studies of antiangiogenic effects of resveratrol led to the synthesis of novel derivatives of resveratrol. Kimura et al. (2008) examined the antiangiogenic effects of 21 synthetic and/or natural stilbenes. Among these stilbenes, 2,3-, 3,4-, and 4,4'-dihydroxystilbene inhibited pro-MMP-9 production in colon 26 (C-26) cells, VEGF-induced HUVECs migration and VEGF-induced angiogenesis at micromolar concentrations. Resveratrol inhibited the pro-MMP-9 production and VEGF-induced angiogenesis only at higher concentrations. The three above mentioned dihydroxystilbenes also inhibited the tumor-induced neovascularization in C-26-packed chamber-bearing mice and the tumor growth in colon 26-bearing mice. Finally, the three dihydroxystilbenes inhibited VEGF-induced VEGF-receptor (VEGFR-2) phosphorylation.

2.2. Curcumin

Curcumin (diferuloylmethane), a polyphenol derived from the plant *Curcuma longa* (commonly called turmeric), has emerged as one of the most powerful chemopreventive and anticancer agents (Singh and Khar 2006). Extensive research over the last 50 years has indicated that this compound can both prevent and treat cancer. The anticancer potential of curcumin stems from its ability to suppress proliferation of a wide variety of tumor cells, down-regulate transcription factors NF-kappa B, AP-1 and Egr-1; down-regulate the expression of COX2, LOX, NOS, MMP-9, uPA, TNF, chemokines, cell surface adhesion molecules and cyclin D1; down-regulate growth factor receptors (such as EGFR and HER2); and inhibit the activity of c-Jun N-terminal kinase, protein tyrosine kinases and protein serine/threonine kinases. In several systems, curcumin has been described as a potent antioxidant and anti-inflammatory agent (Aggarwal et al. 2003; Lee et al. 2007). Evidence has also been provided to suggest that curcumin can suppress tumor angiogenesis.

Thirteen years ago, Singh and co-workers (1996) studied the effect of curcumin on the proliferation and cell cycle progression of HUVECs. Curcumin inhibited the DNA synthesis of HUVECs as revealed by [3 H]thymidine incorporation in a dose-dependent manner. The growth of HUVECs was also inhibited. Moreover, the addition of curcumin to HUVECs resulted in an accumulation of the cells in early S-phase.

Since then dozens of articles on the antiangiogenic effects of curcumin have been published.

Thaloor et al. (1998) studied the effect of curcumin on endothelial cell migration, attachment, and tube formation on Matrigel. Curcumin treatment resulted in an inhibition of tube formation as well as it caused the preformed tubes to break down. Moreover, it inhibited angiogenesis in a s.c. Matrigel plug model in mice. To study the molecular mechanisms of anti-angiogenic effect of curcumin, Gururaj et al. (2002) used both cancer and endothelial cells. Curcumin proved to be a potent angioinhibitory compound as demonstrated by the inhibition of angiogenesis in two different models: in peritoneal angiogenesis and CAM. The angioinhibitory effect of curcumin *in vivo* was corroborated with the results on down-regulation of the expression of proangiogenic genes in endothelial cells by curcumin. Their results clearly indicated an inhibition of VEGF, angiopoietin 1 and 2 gene expression in Ehrlich ascites tumor cells, and KDR (kinase domain region) gene expression in HUVECs.

Later, Shims et al. (2003) studied the effect of curcumin on CD13/aminopeptidase N (APN). APN is a membrane-bound, zinc-dependent metalloproteinase that plays a key role in tumor invasion and angiogenesis. Curcumin directly interacted with APN in both *in vitro* and *in vivo* conditions. Moreover, curcumin strongly inhibited APN-positive tumor cell invasion and basic fibroblast growth factor-induced angiogenesis. The antiangiogenic effect of curcumin have been also associated with other molecular events.

Hypoxia-inducible factor-1 (HIF-1) plays a key role in cell survival and angiogenesis in hypoxic tumors. Upregulation of the HIF-1 system is observed in many common cancers and occurs by a multiplicity of genetic and environmental mechanisms. The effects of curcumin on HIF-1 activity and expression have been examined in cancer cell lines and in xenografted tumors. It was found that curcumin inhibits HIF-1 activity and that this in turn down-regulates genes targeted by HIF-1. In mice bearing Hep3B hepatoma, curcumin retarded tumor growth and suppressed HIF-1 and VEGF in tumors (Choi et al. 2006). Similar results were also obtained by Bae et al. (2006). They found that curcumin significantly decreased HIF-1 α protein levels in HepG2 hepatocellular carcinoma cells. Moreover, curcumin suppressed the transcriptional activity of HIF-1 under hypoxia leading to a decrease in the expression of VEGF, a major HIF-1 target angiogenic factor. Curcumin also blocked hypoxia-stimulated angiogenesis *in vitro* and down-regulated HIF-1 α and VEGF expression in vascular endothelial cells.

Cyclooxygenases (COX) are the key rate-limiting enzymes in the conversion of arachidonic acid to prostaglandins. There are at least 2 isoforms of COX: COX-1 and COX-2. During the last decade, an enormous body of evidence has accrued showing up regulation of COX-2 expression in many human cancers including: gastrointestinal and gynaecological malignancies and tumours of the breast, prostate and lung (reviewed in Dannenberg and Subbaramaiah 2003; Kanaoka et al. 2007). One of the mechanisms by which COX-2 acts as a tumor promoter is through stimulating angiogenesis. Cyclooxygenase-2 modulates angiogenesis by increasing the production of angiogenic factors, such as vascular endothelial growth factor (Tsuji et al. 1998).

Recently, Lev-Avri and co-workers clearly documented the modulatory effect of curcumin on COX-2. Two cancer cell lines, HT29 cells (expressing COX-2) and SW480 (deficient

of COX-2), were exposed to curcumin for 72 h. There was a significant difference between curcumin effect on HT29 (IC₅₀ = 15 μ mol/l) and SW480 (IC₅₀ = 40 μ mol/l) cells. Similarly, induction of apoptosis was higher in HT29 cells. Western blot analysis and PGE₂ immunoassay showed that curcumin inhibited COX-2 protein activity and expression in a dose-dependent manner. The authors concluded that inhibition of cell survival and induction of apoptosis by curcumin in colorectal adenocarcinoma cell lines was associated with the inhibition of PGE₂ synthesis and down-regulation of COX-2 (Lev-Avri et al. 2006).

These results were also confirmed by the work of Su et al. (2006). They studied the effect of curcumin on several cancer markers in human colon cancer cells and found that curcumin decreased expression of NF-kappaB/p65, COX-2 as well as MMP-2.

Recently, Yoysungnoen et al. (2006) investigated the *in vivo* effects of curcumin on the level of angiogenic biomarkers, COX-2 and VEGF. Inoculation of hepatocellular carcinoma cells (HepG2) to nude mice led to a significant increase in neocapillary density (NCD) after 7 and 14 days. The increased NCD on day 7 and 14 were significantly attenuated by daily treatment with curcumin solution (3000 mg/kg BW). The curcumin treatment also reduced the tumor-induced over-expression of COX-2 and serum VEGF. These results indicated that curcumin could inhibit tumor angiogenesis and this mechanism might be mediated through reduction of COX-2 and VEGF. Later, they also found that curcumin (CUR) and its derivative tetrahydrocurcumin (THC) significantly decreased capillary vascularity in male BALB/c nude mice inoculated with human HepG2 cells. Tetrahydrocurcumin expressed its anti-angiogenesis without any cytotoxic activities to HepG2 cells, even at the highest doses. The authors suggested that the antiangiogenic properties of CUR and THC represent a common potential mechanism for their anticancer actions (Yoysungnoen et al. 2008).

Curcumin is a promising anticancer agent and a number of curcumin analogues have been prepared and evaluated as potential anticancer as well as antiangiogenic compounds.

Woo and co-workers (2005) synthesized various curcumin derivatives with asymmetric units possessing alkyl amide, chloro-substituted benzamide, or heteroaromatic amide moieties. These synthetic molecules showed stronger antiangiogenic activity than their mother molecule, curcumin, without exhibiting any cytotoxic activity against HUVECs. Their antiangiogenic activity was evaluated by the proliferation and tube formation inhibitory activity on the HUVEC.

Other synthetic curcuminoid derivatives (tetrahydrocurcumin, salicyl curcumin and curcuminIII) reduced the number of tumour directed capillaries induced by injecting B16F-10 melanoma cells on the ventral side of C57BL/6 mice. Treatment with these curcuminoids reduced the serum NO as well as TNF-alpha levels (Leyon and Kuttan 2003).

In another study authors evaluated the antiangiogenic activity of a structural analog of curcumin, demethoxycurcumin (DC). They investigated the effect of DC on genetic reprogramming in cultured HUVECs using cDNA microarray analysis. Interestingly, 9 angiogenesis-related genes were down-regulated over 5-fold in response to DC, suggesting that the genetic reprogramming was crucially involved in antiangiogenesis by the compound. To verify the results obtained from cDNA microarray analysis, MMP-9 was investigated using gelatin zymography. Demethoxycurcumin potently inhibited the expression of MMP-9, but showed no direct effect on its activity. These data show that gene expressional change of MMP-9 is a major mediator for angiogenesis inhibition by DC (Kim et al. 2002).

Very promising results with curcumin analogues were also obtained by Shim et al. (2002). They synthesized a novel

curcumin derivative, named hydrazinocurcumin (HC). In *in vitro* experiments HC potently inhibited bovine aortic endothelial cells (BAECs) migration as well as tube formation at nanomolar concentrations without cytotoxicity. These results were confirmed also by *in vivo* experiments on chorioallantoic membrane from growing chick embryo.

2.3. Miscellaneous compounds

Many studies provide compelling evidence that *garlic* and its organic allyl sulfur components are effective inhibitors of the cancer process. These studies reveal that the benefits of garlic are not limited to a specific species, to a particular tissue, or to a specific carcinogen (see rev. Milner 2001; Khanum et al. 2004). Alliin, a compound derived from garlic, demonstrated a dose-dependent inhibition of fibroblast growth factor-2 (FGF2)-induced human endothelial cell tube formation and angiogenesis in the chick CAM. Additionally, alliin demonstrated a potent inhibition of VEGF-induced angiogenesis in the CAM model. Alliin significantly inhibited both FGF2 and VEGF secretion from human fibrosarcoma cells in a concentration-dependent manner (Mousa and Mousa 2005).

Recently, it was shown that diallyl trisulfide, a cancer-chemopreventive constituent of garlic, has the ability to inhibit angiogenic features of human endothelial cells. Survival of HUVECs was reduced significantly in the presence of diallyl trisulfide in a concentration-dependent manner, with an IC_{50} of approximately 4 μ mol/l. This effect was associated with the induction of apoptosis characterized by the accumulation of sub-diploid cells, DNA fragmentation, and cleavage of caspase-3 and poly-(ADP-ribose)-polymerase. Furthermore, diallyl trisulfide inhibited the formation of capillary-like tube structure and migration by HUVECs in association with the suppression of VEGF secretion and VEGF receptor-2 protein level and the inactivation of Akt kinase (Xiao et al. 2006).

The antiangiogenic effect of garlic was also confirmed by Matsuura and co-authors (2005) who investigated the effect of aged garlic extract (AGE) on endothelial cells. They found that AGE suppressed endothelial cell motility, proliferation, and tube formation.

Hypericum perforatum, also known as St. John's wort, is commonly used as a treatment for depression and anxiety disorders. Moreover, many articles documented anticancer effects of the active compound from *H. perforatum* in the last ten years (e.g. Mirossay et al. 1999; Agostinis et al. 2002; Roscetti et al. 2004; Sackova et al. 2005; Sarisky et al. 2005; Kiesslich et al. 2006). The „classical” compound isolated from *H. perforatum*, hypericin, also possesses antiangiogenic effects.

Hendrickx and co-workers (2005) studied the effect of hypericin on bladder cancer cells. They found that inhibition of phospholipase A₂ significantly protects cells from the hypericin-induced apoptosis and attenuates the activation of p38 MAPK. Importantly, they found that inhibition of p38 α MAPK blocks the release of vascular endothelial growth factor and suppresses tumor-promoted endothelial cell migration, a key step in angiogenesis.

Our results also documented antiangiogenic effects of hypericin. Photoactivated hypericin in non-toxic concentrations blocked migration of endothelial cells and inhibited matrix metalloproteinases as well as *in vitro* capillary-like tube formation (Stupakova et al. 2009). An antiangiogenic effect of hypericin was also observed in animal experiments (Lavie et al. 2005; Yee et al. 2005).

A promising antiangiogenic effect was also documented in hyperforin, another bio-active compound of *H. perforatum*. Recently, Martinez-Poveda and co-workers (2005) clearly

showed that hyperforin inhibited angiogenesis *in vitro* as well as *in vivo*. Hyperforin treatment of endothelial cells resulted in strong inhibitory effects. Capillary tube formation on Matrigel was abrogated completely by the addition of hyperforin at the low micromolar range. It also exhibited a clear inhibitory effect on the invasive capabilities of endothelial cells. Zymography showed that hyperforin treatment produced a complete inhibition of urokinase and a marked inhibition of MMP-2. The antiangiogenic effect of hyperforin was also observed in the CAM assay. Similarly, Schempp et al. (2005) have shown that hyperforin blocked angiogenesis *in vitro* as well as *in vivo* by a direct non-toxic effect on endothelial cells.

Hyperforin can also inhibit the ability B-cell lymphocytic leukemia to secrete MMP-9. It acts through decreasing the production of the latent 92 kDa pro-enzyme. The authors also showed that hyperforin impaired the VEGF release by the leukemic cells. Moreover, hyperforin was found to prevent the formation of microtubules by human bone marrow endothelial cells cultured on Matrigel (Quiney et al. 2006). Blocking effect of hyperforin on MMP-9 activity was recently confirmed by Dell'aica et al. (2007).

Panax ginseng is one of the most commonly used and widely researched species of ginseng. This species has been an important herbal remedy in traditional Eastern medicine for thousands of years (Mahady et al. 2000). It is used as a general tonic or adaptogen in chronically ill patients and is frequently featured in traditional medicine prescriptions from China, Japan, and Korea used by cancer patients. The putative active compounds are the ginsenosides, of which there are more than two dozens. These compounds are found in both *Panax ginseng* and other *Panax* species that are used in herbal medicine. Furthermore, it seems that some ginsenosides possess also antiangiogenic effect.

Sato et al. (1994) studied the effect of ginsenoside-Rb2 extracted from *Panax ginseng* on the angiogenesis and metastasis produced by B16-BL6 melanoma cells in syngeneic mice. Ginsenoside-Rb2 caused a marked inhibition of both neovascularization and tumor growth after intra-tumoral or oral administration.

Recently, another ginsenoside (Rg3) was found to inhibit the expression of certain angiogenesis factors. Under *in vitro* conditions, Rg3 significantly decreased the expression of VEGF in cancer cells as well as in HUVECs. In lung cancer cells Rg3 also decreased MMP-2 expression (Chen et al. 2005). Later, Yue et al. (2006) found that Rg3 at nanomolar concentrations inhibited the proliferation of HUVECs and suppressed the capillary tube formation by HUVECs on the Matrigel. Moreover, Rg3 attenuated VEGF-induced chemoinvasion of HUVECs and *ex vivo* microvascular sprouting in rat aortic ring. In addition, Rg3 remarkably abolished the basic fibroblast growth factor-induced angiogenesis in an *in vivo* Matrigel plug assay as well as decreased gelatinolytic activities of MMP-2 and MMP-9.

Berberine is one of the major alkaloids in Huanglian (*Coptis chinensis*), a widely used herb in traditional Chinese medicine for the treatment of inflammation-related diseases. On the other hand, the information regarding berberine's anticancer activities has been limited. It was reported that berberine could inhibit the transcriptional activity of cyclooxygenase-2, inhibit the activity of activator protein 1, interleukin-1 beta or tumor necrosis factor-alpha (Fukuda et al. 1999a,b; Lee et al. 2007). There are also some indication that berberine may modulate process of angiogenesis.

Lin et al. (2004) showed that berberine could directly inhibit *in vitro* HUVEC tube formation and migration. Moreover, they observed that berberine prevented the expression of VEGF and HIF-1 α by gastric adenocarcinoma cell line SC-M1 under hypoxic conditions. However, overexpression of HIF-1 α in SC-M1 cells dramatically reversed the inhibitory effect of berberine

on SC-M1-induced *in vitro* HUVEC migration. These data indicated that HIF-1 α repression can be a critical step in the inhibitory effect of berberine on tumor-induced angiogenesis. Emodin is an anthraquinone derivative isolated from the rhizomes of *Rheum palmatum*. Extract of *R. palmatum* has been used since ancient times as a strong laxative or as an anti-inflammatory agent. Moreover, it has been found that emodin suppresses bacterial and tumor growth and also possesses vasodilatory effect (Huang et al. 1991, 2004). Recently, it was reported that emodin may suppress some important angiogenic processes.

Kwak and co-authors (2006) studied the effects of emodin on VEGF-induced angiogenesis, both *in vitro* and *in vivo*. Emodin in a dose-dependent manner inhibited proliferation, migration and tube formation of HUVECs stimulated with VEGF. It also suppressed bFGF-induced proliferation and migration of HUVECs and VEGF-induced tube formation of human dermal microvascular endothelial cells. Furthermore, emodin induced cell cycle arrest of HUVECs in the G0/G1 phase. Emodin also inhibited basal secretion of MMP-2. *In vivo*, it strongly suppressed angiogenesis in the chicken CAM and VEGF-induced angiogenesis of the Matrigel plug in mice.

These results were supported by Kaneshiro et al. (2006) who documented a modifying effect of emodin on the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2. As the authors suggest, suppression of the phosphorylation of ERK1/2 may be associated with emodin-induced inhibition of endothelial cell proliferation, migration, tube formation as well as MMP-9 expression.

Recently, the effect of emodin on VEGF-receptor (VEGFR) phosphorylation was studied. Emodin caused a dose-dependent inhibition of VEGFR phosphorylation in HCT116 cell (colon cancer cells). Flow cytometric analysis also showed that, upon treatment with emodin, the cycle was blocked at the G2/M phase and apoptosis of HCT116 cells was increased (Lu et al. 2008).

3. Conclusions

Many plants are known to prevent the development and progression of chronic diseases. Although the mechanisms of their antitumor effect is not yet fully understood, it has been reported that many natural compounds can act as angiogenesis inhibitors. Phytochemicals have been shown to target and inhibit several key events of the angiogenic process such as proliferation and migration of endothelial cells as well as the expression of some pro-angiogenic factors. Nowadays, numerous bioactive plant compounds are tested for the potential clinical applications. However, although the antiangiogenic effects of natural compounds under experimental conditions are suggestive, further studies are needed to gain a full understanding of the effects of these compounds on tumor angiogenesis in human.

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