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Review article

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NUTRACEUTICALS AS ANTI-ANGIOGENIC AGENTS: HOPES AND REALITY

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Angiogenesis, the formation of new blood vessels from preexisting vascular network is a driving force of organ development in ontogeny, is necessary for ovulation and hair growth, and is prerequisite for proper wound healing. It is also a critical mechanism of numerous diseases, the most important of which are cancer and atherosclerosis. Therefore, modulation of angiogenesis is considered as therapeutic strategies of great importance for human health.
Numerous bioactive plant compounds, often referred to as nutraceuticals are recently tested for the potential clinical applications. Among the most frequently studied are resveratrol, a polyphenol present in red-wine and grape-seed, epigallocatechin-3-gallate (EGCG) from green tea and curcumin from *Curcuma longa*. It is also possible that components of other plants, including the constituents of local food diet may find application for modulation of angiogenesis, provided that their effectiveness will be confirmed in controlled, scientifically validated trials.

Key words: *angiogenesis, vascular endothelial growth factor, cancer, atherosclerosis, polyphenols*

Angiogenesis and diseases

Angiogenesis is the process in which the new blood vessels are formed from pre-existing ones (for reviews see: 1-3). It is indispensable for embryonic development as interruption of angiogenic events blocks the growth of the embryo and results in early mortality. After birth angiogenesis plays both adaptive role enabling the regeneration of the damaged body parts and is also involved in numerous pathological changes. Understanding of the basic

mechanisms of blood vessels formation is necessary for the establishment of the effective therapeutic strategies for amelioration of diseases.

The major angiogenic regulator is vascular endothelial growth factor (VEGF), named also VEGF-A, which is one of the several members of VEGF family (for reviews see: 2,3). The most striking demonstration of its significance in blood vessel formation is the early embryonic heterozygous lethality of knockout of *VEGF* gene. Mice embryos devoid of only one functional allele of *VEGF* die at day 9-11 of pregnancy and do not develop vasculature (4, 5).

In adult organisms physiological angiogenesis is limited and occurs during regeneration of uterine epithelium in menstrual cycle, development of the ovum and formation of corpus luteum and hair growth (3). It is also suggested that spermatogenesis may be dependent on the activity of VEGF (6). Moreover, the production of VEGF and other angiogenic mediators is prerequisite for the reparative processes, such as healing of epidermal and internal wounds, including bone fractures. Proper restoration of the continuity of the tissues requires the coordinated actions of several pro-angiogenic mediators. Final establishment of angiogenic homeostasis after effective repair of injured tissues is executed by decrease in the synthesis and activity of those stimulants, but is also dependent on the production of angiogenic inhibitors (*Table 1*).

Angiogenesis is also an important constituent of several pathological processes. It is estimated that about a hundred of various diseases possess a significant angiogenic component (*Table 2*). Among those diseases of particular interest is angiogenesis in cancer and cardiovascular disorders.

Effect of plant derived compounds on tumor growth and angiogenesis

Food components can influence angiogenesis. On the one side this can contribute to pathological changes. For example, ours (7,8) and others (9-11) studies demonstrated that hypercholesterolemia enhances the synthesis of VEGF. It is thus conceivable that long term changes in lipid blood constituents, which may be the result of improper diet, can influence angiogenesis and add to the pathogenesis of cardiovascular diseases. Whether diet compounds can be used to beneficially modulate angiogenesis is not yet proven, although experimental data suggest that this may be possible.

Various pro- and anti-angiogenic approaches have been recently tested for the potential clinical application (*Table 3*). Among them the search for the compounds derived from plants constitutes the significant part of studies, which are supported by various funding bodies, including the European Commission. *Table 4* shows the examples of various components isolated from different plants which demonstrated the anti-angiogenic activity. It has to be, however, remembered that in a vast majority those are mostly preliminary studies and more hard data are necessary to prove those activities. This can be attained by careful and accurate characterization of the active chemical

Table 1. Mediators stimulating or inhibiting formation of blood vessels (for a review see: 3).

Stimulators	Inhibitors
Growth factors/cytokines : Vascular endothelial growth factors (VEGFA, VEGF-B, VEGF-C, VEGF-D, VEGF-E) Placental growth factor (PlGF) Fibroblasts growth factors (FGF-1, FGF-2, FGF-4) Angiopoietin -1 (Ang-1) Angiopoietin-2 (Ang-2) (acting together with VEGF) Hepatocyte growth factor (HGF) Platelet derived growth factor (PDGF) Transforming growth factor α, β (TGFα, β) Insulin growth factor-1 (IGF- 1) Ephrins and ephrin receptors *Proteases*: Matrix Metalloproteinases (e.g. MMP-2, MMP-9) Aminopeptidases (CD13/aminopeptidase N (APN) Urokinase-type plasminogen activator (uPA) *Transcription factors* hypoxia-inducible factor-1 (HIF-1) *Cytokines/chemokines* IL-8 CXC tumor necrosis factor α (TNFα) *Hormones & others* Kallikrein Factor XIII Angiogenin Integrins Tissue factor (TF) inhibitor of DNA binding 1, 3 (Id1, Id3) *Enzymes:* Thymidine phosphorylase *Other angiogenic mediators* Nitric oxide Hydrogen peroxide Carbon monoxide Prostaglandins	angiopoietin-2 (in the absence of VEGF) Pigment epithelium derived factors tumor necrosis factor α (TNFα) Platelet factor-4 Thrombospondin-1,-2 (TSP-1) *Inhbitors of proteases:* Tissue inhibitors of metalloproteinases (TIMP 1-4) plasminogen activator inhibitor (PAI) Maspin Angiostatin Endostatin Interferon α, β, γ (IFN α, β, γ) IP-10

compounds, elucidation of the molecular mechanisms of their actions, demonstration of the real efficacy by *in vivo* studies on proper animal models of human diseases and finally by demonstration of their safety and effectiveness in clinical trials. As all those studies require at least several years of research, so far experiments with only few compounds have generated sufficient amount of data which would allow their investigation in the clinical

Table 2. Diseases in which angiogenesis is either enhanced or attenuated (after ref.3)

Diseases characterized by abnormal/impaired angiogenesis	**Diseases characterized by excessive angiogenesis**
Cardiovascular diseases: Atherosclerosis: heart and peripheral ischemia Diabetes: impaired collateral growth Restenosis: impaired reendothelialization	Hemangiomas – tumors of the vessels Cancer - growth and metastasis depend on angiogenesis
Non-healing wounds: gastric or oral ulcers Diabetic ulcers Impaired bone fracture healing	Psoriasis Obesity Diabetic retinopathy
Crohn disease (mucosal ischemia)	Atherosclerosis (growth of the plaque)
Osteoporosis	Asthma Nasal polyps
Hair loss Skin pupura Telangiectasia	Rheumatoid arthritis
Nephropathy	Inflammatory bowel disease
Neonatal respiratory distress Pulmonary fibrosis Emphysema	Endometriosis
Nervous system: Alzheimer diseases Amyotrophic lateral sclerosis Diabetic nephropathy	

studies. In this review the potential anti-angiogenic activities of three such compounds are discussed.

Effect of plant polyphenols

As stated in a recent review (12), plants have a long history of use in the treatment of cancer, though many of the claims for the efficacy of such treatments should be viewed with some scepticism. Nevertheless, extensive research suggests that regular consumption of certain fruits and vegetables can reduce the risk of acquiring specific cancers (12). The effect seems to be related to the chemicals present in this food. The predominant data referred to compounds knowns as chemopreventive agents, which include resveratrol, catechins genistein, curcumin, as well as others, such as diallyl sulfide, S-allyl cysteine,

Table 3. Some possible ways of affecting angiogenesis

Enhancement	Mode	Inhibition
VEGF (VEGF-A, VEGF-C) FGFs HGF	***Protein factors***	Anti-VEGF antibodies Soluble VEGF receptors Angiogenic inhibitors (eg. endostatin, angiostatin, interferons)
Gene transfer of VEGF, FGF-2, FGF-4, HGF Other angiogenic mediators	***Gene therapy***	Gene transfer of anti-angiogenic genes (eg. endostatin)
Statins?	***Drugs/small molecular compounds***	Inhbitors of VEGF recepotors Thalidomide HMG-CoA reductase inhibitors (statins)? COX-2 inhibitors
Resveratrol/other polyphenols	***Bioactive food components***	Resveratrol Curcumin EGCG (green tea)

allicin, lycopene, capsaicin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, and eugenol (13,14). Those agents have been suggested to suppress cancer cell proliferation, inhibit growth factor signalling pathways, induce apoptosis, as well as inhibit angiogenesis (14).

Effect of resveratrol on angiogenesis

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*) (13). Resveratrol has been found in various plants, including grapes, berries and peanuts. Besides cardioprotective effects, resveratrol exhibits anticancer properties. Thus, it suppressed proliferation of lymphoid and myeloid cancers, cancers of the breast, prostate, stomach, colon, pancreas, and thyroid; melanoma; head and neck squamous cell carcinoma; ovarian carcinoma; and cervical carcinoma (13). Molecular mechanisms of inhibition of tumor cell proliferation have been partly elucidated and involve suppression of several transcription factors, such as NF-κB, AP-1 and Egr-1, downregulation of the expression of anti-apoptotic genes and activation of caspases (13). As far as angiogenesis is concerned, resveratrol has been shown to downregulate the production of several angiogenic cytokines, including VEGF and interleukin-8 (IL-8) (for a review see: 15).

Protective effect of resveratrol in vascular cells is often ascribed to be related to the scavenging of reactive oxygen species (ROS). Interestingly, ROS, such as

Table 4. Bioactive herbal compounds and their effect on angiogenesis.

Common Name	Chemical name	Origin	Activity	References
Resveratrol	3,5,4'-trihydroxy-trans-stilbene	Grapes, grape fruits	Inhibition of ROS synthesis and ROS-dependent angiogenic events (endothelial cell proliferation, VEGF, bFGF, IL-8 production)	(21,22)
Curcumin	diferuloylmethane	*Curcuma longa*	Inhibition of VEGF, Ang1 and Ang2 Inhibition of VEGF and bFGF induced angiogenesis	(38) (32)
Tea catechin	(-)-epigallocatechin gallate (EGCG (-)-epigallocatechin 3,5-di-O-gallate and epitheaflagallin 3-O-gallate	*Camellia sinensis*	Inhibition of matrix-metalloproteinase (MT1-MMP) and in this way the formation of active MMP-2 Suppression of VE-cadherin and Akt phoshporylation Inhibition of VEGF receptors on endothelial cells Inhibition of VEGF and IL-8 release from keratinocytes Down regulation of c-fos, c-jun and and Ets-1 Other genes, including bFGF and aFGF	(47-49) (44) (43) (52) (45) (50,51)
Flavone	Parent compound of flavonoids	Various plants	Inhibition of Rb phoshporylation Cdk2 and cdk 4 Inhibition of HUVEC proliferation	(66)
Luteolin	Tetrahydroxyflavone	parsley, artichoke, peppers, olive oil, lemons, peppermint, sage, thyme	Inhibition of PI3K and VEGF-induced proliferation of HUVEC	(67)
Genistein	4'5, 7-trihydroxyisoflavone	Family *Leguminosae* (including soy bean)	Inhibition of protein tyrosine kinase Inhibition of VEGF and MMP9	(68) (69)
Apigenin	5,7,4'-trihydroxyflavone	apples, beans, broccoli, cherries, grapes, onions, parsley, tomatoes, tea and wine	Inhibition of HIF-1 and VEGF synthesis	(70)
Ponicidin and oridonin		*Rabdosia rubescens*	Inhibition of Akt and MAPK kinases	(71)
Ginseng	Ginsenosides (triterpene dammarenes)	*Panax ginseng*	Inhibition of angiogenesis (tumor)	(56,62)
Ginseng	Ginsenosides (triterpene dammarenes)	*Radix rubra*	Stimulation of angiogenesis (wound healing)	(56,61)
polysaccharopeptide, PSP	?	edible mushroom *Coriolus versicolor*	Inhibition of tumor angiogenesis Suppression of VEGF	(72)
Polyacetylenes (herbal tea)	?	*Bidens pilosa*	Inhibition of HUVEC proliferation Upregulation of p27(Kip) and p21(Cip1)	(73)

Various: berries extracts	?		Inhibition of VEGF expression in human keratinocytes	(74)
baicalein, epicatechin, berberine, and acteoside	?	Herbal ingredients of Chineese plants	Downregulation of MMPs, (MMP-1,2-9) Inhibition of reactive oxygen species	(75)
		e.g. *Berberis paraspecta, Taxus chinensis*	Anti-angiogenic effect in CAM assay and BAEC proliferation	(76)
		Epimedium sagittatum, Trichosanthes kirilowii and *Dalbergia odorifera*	Pro-angiogenic effect in CAM assay and BAEC proliferation	(76)

hydrogen peroxide, appear also to be the crucial players in angiogenesis (16), inducing the expression of angiogenic mediators, among them VEGF (17,18). ROS are also generated by endothelial cells in response to stimulation with growth factors, such as VEGF (19). Some data indicate that scavenging properties of reseveratrol may contribute to its anti-angiogenic effects (20). In a recent study Lin and co-workers (21) demonstrated that exposure of HUVECs to 1 to 2.5 μM resveratrol significantly blocked VEGF-mediated cell migration and tube formation but not cell proliferation. Resveratrol effectively abrogated VEGF-mediated tyrosine phosphorylation of vascular endothelial (VE)-cadherin and its complex partner, beta-catenin. VEGF stimulated an increase of peroxide which has been shown to be involved in VE-cadherin phosphorylation and this was strongly attenuated by resveratrol (21).

Importantly, those in vitro results have been corroborated by in vivo studies. Brakenhielm et al. (22) demonstrated that resveratrol supplied in drinking water suppresses the growth of new blood vessels in the chick chorioallantoic membrane (CAM) assay and in the mice corneal neovascularization model, directly inhibiting capillary endothelial cell growth. The effect was due to block of both VEGF and bFGF-receptor mediated responses, involving the phosphorylation of MAP kinases. In accordance with the role of angiogenesis in tumor growth, oral administration of resveratrol inhibited the growth of murine fibrosarcoma in mice, and significantly delayed wound healing, the process also dependent on angiogenesis (22).

Mechanisms of the effect of red wine polyphenols on production of VEGF have been studied in details by Oak et al. (23). Short-term and long-term exposure of vascular smooth muscle cells to red wine polyphenolic compounds (RWPC) inhibited VEGF mRNA expression and release of VEGF in response to platelet-derived growth factor AB (PDGF-AB), transforming growth factor-beta1 (TGF-β1) or thrombin. Short-term and long-term treatment of VSMCs with RWPCs markedly reduced PDGF-AB-induced production of reactive oxygen species and phosphorylation of p38 (23), again pointing to the role of ROS and downstream

activation of p38 in induction of VEGF synthesis. By demonstration of such an anti-angiogenic effect of red wine polyphenols the study add to the atheroprotective effects of red wine and suggest that one of its mechanisms may be related to the inhibition of atherosclerotic plaque growth by attenuation of the synthesis of VEGF, which promotes the formation of vasa vasorum. Indeed, other studies indicate that the growth of the atherosclerotic plaque is dependent on the building of new vasculature. The size of the plaque correlates positively with the extent of neovascularization (11) and other inhibitors of angiogenesis, such as TNP-470 or endostatin can diminish the extent of atherosclerosis in apoE knockout mice (24).

As demonstrated by Brakenhielm et al. (22) resveratrol can impede tumor growth by inhibition of angiogenesis. Similarly, Tseng and co-workers have shown that resveratrol at the dose 40 mg/kg/day suppressed the angiogenesis and growth of tumour gliomas in vivo (25). The effect was dependent on the pro-apoptotic effect of resveratrol on tumour cells and attenuation of VEGF production. The authors also showed that resveratrol impaired the proliferation of ECV304 cells, which they described as endothelial. However, it has to be stressed that those cells are of tumour origin and recently it has been proven convincingly that they are identical with bladder cancer cell line T24/83 (26).

Similar effects may be also exerted by resveratrol isolated from other sources, as shown for compounds extracted from roots of *Polygonum cuspidatum*, which prevented tumour growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice (27,28)

To complicate the picture, there is also some evidence that polyphenols may exert pro-angiogenic effects. Accordingly, Sen and co-workers (17,29) have shown that grape seed proanthycyanidin extract (GSPE) containing 5000 ppm resveratrol potently upregulates oxidant and tumor necrosis factor-alpha (TNFα) inducible VEGF expression in human keratinocytes. Their studies have demonstrated that grape seed extract potentiate angiogenesis by enhancing the oxidizing environment of the wound and in this way stimulating VEGF production (29).

Such an effect can be related to stimulatory effect of tannins and proanthocyanidins (condensed tannins) and other on wound healing. Thus, a combination of grape seed proanthocyanidin extract and resveratrol facilitates inducible VEGF expression, a key element supporting wound angiogenesis (17).

Effect of curcumin on angiogenesis

Curcumin is a small-molecular-weight compound isolated from the commonly used spice turmeric. Curcumin down-regulates transcription factors NF-kappa B, AP-1 and Egr-1, inhibits the expression of cyclooxygenase-2, lipooxygenase, inducible nitric oxide synthase, matrix metalloproteinase-9, urokinase-type plasminogen activator, TNFα, chemokines, cell surface adhesion molecules and

cyclin D1; inhibits growth factor receptors (such as epidermal growth factor and human epidermal growth factor receptor-2) activity and blocks the activity of c-Jun N-terminal kinase, protein tyrosine kinases and protein serine/threonine kinases (for references see: 30, 31) .

Effect of curcumin on the growth of cancers has also been investigated. Thus, in animal models, curcumin and its derivatives were shown to inhibit the progression of chemically induced colon and skin cancers (32). As far as angiogenic events are considered, it has been recently demonstrated that blocking of NF-κB and AP-1 by curcumin attenuated IL-8 expression in human breast carcinoma cells but did not affect VEGF production (33).

On the other hand, curcumin was found to completely prevent the induction of VEGF synthesis by microvascular endothelial cells stimulated with advanced glycation end products (34). The effect might be mediated by downregulation of NF-κB and AP-1 activity (34). Curcumin may also affect angiogenesis dependent on other growth factors. In recent study Arbiser et al. reported that curcumin and its derivatives significantly inhibited basic fibroblast growth factor (bFGF)-mediated corneal neovascularization in the mouse (32). However, in that study curcumin had no effect on phorbol ester-stimulated VEGF production (32).

Curcumin can also inhibit the activity of CD13/aminopeptidase N (APN), a membrane-bound, zinc-dependent metalloproteinase that plays a key role in tumour invasion and neovascularization (35). Accordingly, curcumin and other known APN inhibitors strongly inhibited APN-positive tumour cell invasion and bFGF-induced angiogenesis. Because curcumin did not affect the invasion of APN-negative r cells, therefore it seems probable that the anti-invasive activity of curcumin against tumours is attributable to the inhibition of APN (35).

A detailed analysis of gene expression affected by curcumin treatment was performed by Kim and co-workers (36), who studied the effect of demethoxycurcumin (DC), a structural analog of curcumin, isolated from *Curcuma aromatica*, on genetic reprogramming in cultured human umbilical vein endothelial cells (HUVECs) using cDNA microarray analysis. Of 1024 human cancer-focused genes arrayed, 187 genes were up-regulated and 72 genes were down-regulated at least 2-fold by DC. Interestingly, 9 angiogenesis-related genes were down-regulated over 5-fold in response to DC. These data suggest that change of MMP-9 gene expression is a major mediator for angiogenesis inhibition by DC (36).

In accordance with its anti-angiogenic effect demonstrated in vitro, curcumin inhibited also the growth of B16 melanoma cells in mice, attenuating angiogenesis and NO and TNFα production (37). Finally, in one recent study, Gururaj et al. (38) showed that curcumin, when injected intraperitoneally (i.p) into mice, effectively decreased the formation of ascites fluid by 66% in Ehrlich ascites tumor (EAT) bearing mice. The authors suggest that this effect can be due

to anti-angiogenic action, as milimolar concentrations of curcumin inhibited VEGF production by tumor cells (38).

However, the authors of discussed study (38) did not delineate that low concentrations of curcumin (below 1 mM in vitro) in fact induced VEGF expression, while the inhibitory effect was observed only at 1 mM concentration. Meanwhile, curcumin appears to be very poorly absorbed from the intestine. In phase I clinical trials in patients receiving orally very high, i.e. gram doses of curcumin, the serum concentration of this compound was only at the nanomolar range (39-41). This suggests that doses of curcumin required to attain level sufficient to exert pharmacological activity are not feasible in humans and thus curcumin may act only locally.

Green tea catechins

Tea is, besides water, the most widely consumed beverage worldwide. Of several forms of this drink available, most studies examining the usefulness of tea in prevention of cancer focused on green tea. This interest is based on epidemiological evidence, which suggests that people who consume large amounts of green tea have a lower risk of developing various cancers (42). Those studies have been corroborated by experimental data from *in vitro* and *in vivo* experiment on animal models

Green tea extracts contain (-)epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)epicatechin gallate (ECG) and (-)epicatechin (EC) (42). EGCG has been considered as a major acting constituent. In addition to having pro-apoptotic activity on tumor cells, the compounds present in green tea have been shown to inhibit tumor invasion and angiogenesis (*Table 4*).

Indeed, experimental evidence suggests that green tea consumption by mice significantly inhibits angiogenesis (reviewed in 42). Mechanisms of anti-angiogenic effects may involve inhibition of endothelial cell proliferation in response to stimulation with angiogenic growth factors (43). This can be exerted by inhibition of VEGF receptors and suppression of VE-cadherin and Akt phosphorylation (44). Activation of certain transcription factors, such as AP-1, NF-κB and Ets-1 is also blunted (45,46) and the production of metelalloproteinases necessary for endothelial cell migration and invasion is attenuated (47-49). Finally, EGCG can also inhibit the production of VEGF, bFGF and IL-8 (50-52). In human colon cancer cells the attenuation of VEGF production was caused by EGCG-mediated inhibition of Erk-1 and Erk-2 kinases (53).

However, a recent randomised phase II clinical trial in patients with prostate cancer did not demonstrate any improvement in their conditions (54). Out of 42 patients who consumed large amounts of green tea, the level of prostate specific antigen decreased only in one case. Further studies are ongoing (55) and their results will be crucial in establishing the real effectiveness of green tea consumption on tumor growth.

Discussion

The use of herbal products and nutraceuticals is becoming popular and those compounds are often regarded as alternative medicine with the claim that because of their "natural" origin they are inherently safe. It has been estimated that more than 12% of adults in the United States used herbal medicine in 1997 (cited after 56). The number is similar in European countries, while in the developing countries the products of traditional medicine constitute the majority of medicines (57).

No doubts, plants are the source of many bioactive compounds and a lot of them may possess significant biological activity. However, besides enthusiasm which many people uncritically express towards natural products, there are several problems which should be discussed.

First, the non-standardised extracts of plants contain various compounds, which may influence angiogenesis in opposite way. A recent interesting study demonstrated such an effect of extracts of ginseng, the very popular herbal product and commonly used nutraceutical. Ginsgeng is a key component in a traditional Chinese medicine and is sold widely in the West, with the annual sales exceeding a quarter of billion dollars in US only (cited after 56). Sengupta and co-workers have performed the detailed mass spectroscopic analysis of American, Chinese, Korean and Sanqi ginsgeng and revealed the presence of distinct ginsenosides. They represent the active components of ginseng and can be classified on the basis of their structure as panaxdiols and panaxtriols (56). In the ginsengs analysed in the discussed study the most common diol was Rb1, while Rg1 was a predominant triol and different ginsengs demonstrated various ratio of Rg1 and Rb1. Interestingly, those compounds seem to exert opposite effects on angiogenesis. Thus, the dominance of Rg1 leads to angiogenesis, whereas Rb1 inhibits it. A molecular mechanism of actions of those compounds has been further elucidated (56). The treatment of HUVEC with Rg1 induced a strong expression of endothelial nitric oxide synthase (NOSIII), the activity of which is required for VEGF-dependent angiogenesis (58-60). Accordingly, inhibition of NOSIII activity by L-NAME abrogated the pro-angiogenic effect of Rg1, while it failed to exert similar effect on Rb1. Interestingly, the inhibitory effect of Rb1 on angiogenesis (demonstrated by in vivo Matrigel angiogenesis assay and in vivo scaffold implant neovascularization assay) appears to be due to the inhibitory effect of Rb1 on chemoinvasion of endothelial cells, while they proliferation was actually enhanced by Rb1, similarly as by Rg1.

These findings by Sengupta and co-workers explain the ambiguity about the effects of ginseng in vascular pathology, i.e. the reports on stimulation and inhibition of vessel growth, which have been demonstrated in a context of stimulation of wound healing (61) and inhibition of tumor growth (62), respectively. No doubts, this exciting finding open the possibility of

developing new small-molecule based therapeutics. However, as Sengupta and co-workers clearly noted, it emphasizes also a need for regulations standardizing herbal therapy and calls for better control of the quality and constituents of sold products.

Second, there is a common belief, that natural compounds are inherently safe because of their origin. However, it is a truism to state that the activity of those compounds and beneficence, if any, derives from their chemical nature, not the source of origin. As every chemical also those derived from plants may exert various effects and one may expect the interaction with various metabolic pathways. Therefore, there is a strong need that all such compounds have to be carefully tested in context of their safety and interactions with other substances, particularly the drugs taken potentially by consumers.

No doubt, plants may be a source of new, very active molecules of potential medical applications. However, one has to consider also the costs of such analysis. It is known that the so called "hits" detected by high-throughput screening of various compounds are very rare. As an example, in recent analysis demonstrating the potent antilipidemic effect of berberine, a plant derived compound, the extracts of 700 hundreds plants have been tested (63). In another large scale study being performed in India, during five years 78,000 samples has been screened at the cost of nearly $10 million spent on equipment, *in vitro* and animal testing facilities and modelling workstations. Out of those thousands of samples 44 have been identified as potential investigational new drugs (64).

Many observations suggest that compounds isolated from plants being the constituents of local food or traditional medicine may exert potent beneficial effect. However, it is also possible that such discoveries are simply the chance events and that common belief on the action of such compounds has no relevance to the real effect of plant derived compounds (65).

Indeed, the indications for the therapeutic potentials of various plants may be completely different from the confirmed activity of the bioactive plant compounds as was in the case of berberine (63), known to have an effect on diarrhea, but which turn out to be a strong inducer of LDL receptor and thereby can be regarded as a potential drug for dyslipidemia (63). Other examples might be the plant alkaloids, vinblastine or vincristine, which are used for treatment of cancer, but originally were derived from plants used for amelioration of diabetes (12).

Importantly, demonstration of the real effectiveness of plant derived compounds requires rigorous testing and randomized clinical studies. Otherwise, all marketing claims on potential therapeutic effectiveness and health benefits of nutraceuticals have to be recognized as anecdotal and unreliable. Meanwhile, analysis of Chinese, US and European trials of medicinal approaches based on plant derived compounds indicates that they are almost universally flawed (65).

In summary, it seems that various approaches, including the design of targeting drugs, will in the future determine the search for new potential therapeutics. The studies of natural components may not necessarily provide the straightforward clue on their immediate clinical applications. Nevertheless, demonstration of the safety of all such products, which may be important constituents of our food, is of the greatest importance and should not be neglected.

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REFERENCES

1. Dulak J, Loboda A, Zagorska A *et al.* Complex role of heme oxygenase-1 in angiogenesis. *Antioxid Redox Signal* 2004; 6: 858-66.
2. Dulak J, Jozkowicz A. Regulation of vascular endothelial growth factor synthesis by nitric oxide: facts and controversies. *Antioxid Redox Signal* 2003; 5: 123-32.
3. Carmeliet P. Angiogenesis in health and disease. *Nat Med* 2003; 9: 653-60.
4. Carmeliet P, Ferreira V, Breier G *et al.* Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 1996; 380: 435-9.
5. Ferrara N, Carver-Moore K, Chen H *et al.* Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996; 380: 439-42.
6. Huminiecki L, Chan HY, Lui S *et al.* Vascular endothelial growth factor transgenic mice exhibit reduced male fertility and placental rejection. *Mol Hum Reprod* 2001; 7: 255-64.
7. Dulak J, Jozkowicz A, Dichtl W *et al.* Vascular endothelial growth factor synthesis in vascular smooth muscle cells is enhanced by 7-ketocholesterol and lysophosphatidylcholine independently of their effect on nitric oxide generation. *Atherosclerosis* 2001; 159: 325-32.
8. Alber HF, Dulak J, Frick M *et al.* Atorvastatin decreases vascular endothelial growth factor in patients with coronary artery disease. *J Am Coll Cardiol* 2002; 39: 1951-5.
9. Ramos MA, Kuzuya M, Esaki T *et al.* Induction of macrophage VEGF in response to oxidized LDL and VEGF accumulation in human atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 1998; 18: 1188-96.
10. Inoue M, Itoh H, Tanaka T *et al.* Oxidized LDL regulates vascular endothelial growth factor expression in human macrophages and endothelial cells through activation of peroxisome proliferator-activated receptor-gamma. *Arterioscler Thromb Vasc Biol* 2001; 21: 560-6.
11. Inoue M, Itoh H, Ueda M *et al.* Vascular endothelial growth factor (VEGF) expression in human coronary atherosclerotic lesions: possible pathophysiological significance of VEGF in progression of atherosclerosis. *Circulation* 1998; 98: 2108-16.
12. Cragg GM, Newman DJ. Medicinals for the millennia: the historical record. *Ann N Y Acad Sci* 2001; 953: 3-25.
13. Aggarwal BB, Bhardwaj A, Aggarwal RS *et al.* Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res* 2004; 24: 2783-840.

14. Dorai T, Aggarwal BB. Role of chemopreventive agents in cancer therapy. *Cancer Lett* 2004; 215: 129-40.
15. Cao Y, Cao R, Brakenhielm E. Antiangiogenic mechanisms of diet-derived polyphenols. *J Nutr Biochem* 2002; 13: 380-390.
16. Maulik N. Redox signaling of angiogenesis. *Antioxid Redox Signal* 2002; 4: 805-15.
17. Sen CK, Khanna S, Gordillo G *et al.* Oxygen, oxidants, and antioxidants in wound healing: an emerging paradigm. *Ann N Y Acad Sci* 2002; 957: 239-49.
18. Grzenkowicz-Wydra J, Cisowski J, Nakonieczna J *et al.* Gene transfer of CuZn superoxide dismutase enhances the synthesis of vascular endothelial growth factor. *Mol Cell Biochem* 2004; 264: 169-81.
19. Colavitti R, Pani G, Bedogni B *et al.* Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. *J Biol Chem* 2002; 277: 3101-8.
20. Igura K, Ohta T, Kuroda Y *et al.* Resveratrol and quercetin inhibit angiogenesis in vitro. *Cancer Lett* 2001; 171: 11-6.
21. Lin MT, Yen ML, Lin CY *et al.* Inhibition of vascular endothelial growth factor-induced angiogenesis by resveratrol through interruption of Src-dependent vascular endothelial cadherin tyrosine phosphorylation. *Mol Pharmacol* 2003; 64: 1029-36.
22. Brakenhielm E, Cao R, Cao Y. Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. *FASEB J* 2001; 15: 1798-800.
23. Oak MH, Chataigneau M, Keravis T *et al.* Red wine polyphenolic compounds inhibit vascular endothelial growth factor expression in vascular smooth muscle cells by preventing the activation of the p38 mitogen-activated protein kinase pathway. *Arterioscler Thromb Vasc Biol* 2003; 23: 1001-7.
24. Moulton KS, Vakili K, Zurakowski D *et al.* Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. *Proc Natl Acad Sci USA* 2003; 100: 4736-41.
25. Tseng SH, Lin SM, Chen JC *et al.* Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats. *Clin Cancer Res* 2004; 10: 2190-202.
26. Brown J, Reading SJ, Jones S *et al.* Critical evaluation of ECV304 as a human endothelial cell model defined by genetic analysis and functional responses: a comparison with the human bladder cancer derived epithelial cell line T24/83. *Lab Invest* 2000; 80: 37-45.
27. Kimura Y, Okuda H. Resveratrol isolated from Polygonum cuspidatum root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice. *J Nutr* 2001; 131: 1844-9.
28. Kimura Y, Okuda H. Effects of naturally occurring stilbene glucosides from medicinal plants and wine, on tumour growth and lung metastasis in Lewis lung carcinoma-bearing mice. *J Pharm Pharmacol* 2000; 52: 1287-95.
29. Khanna S, Venojarvi M, Roy S *et al.* Dermal wound healing properties of redox-active grape seed proanthocyanidins. *Free Radic Biol Med* 2002; 33: 1089-96.
30. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res* 2003; 23: 363-98.
31. Witek-Zawada B, Koj A. Regulation of expression of stromyelysin-1 by proinflammatory cytokines in mouse brain astrocytes. *J Physiol Pharmacol* 2003; 54: 489-96.
32. Arbiser JL, Klauber N, Rohan R *et al.* Curcumin is an in vivo inhibitor of angiogenesis. *Mol Med* 1998; 4: 376-83.
33. Bobrovnikova-Marjon EV, Marjon PL, Barbash O *et al.* Expression of angiogenic factors vascular endothelial growth factor and interleukin-8/CXCL8 is highly responsive to ambient

glutamine availability: role of nuclear factor-kappaB and activating protein-1. *Cancer Res* 2004; 64: 4858-69.
34. Okamoto T, Yamagishi S, Inagaki Y *et al.* Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. *FASEB J* 2002; 16: 1928-30.
35. Shim JS, Kim JH, Cho HY *et al.* Irreversible inhibition of CD13/aminopeptidase N by the antiangiogenic agent curcumin. *Chem Biol* 2003; 10: 695-704.
36. Kim JH, Shim JS, Lee SK *et al.* Microarray-based analysis of anti-angiogenic activity of demethoxycurcumin on human umbilical vein endothelial cells: crucial involvement of the down-regulation of matrix metalloproteinase. *Jpn J Cancer Res* 2002; 93: 1378-85.
37. Leyon PV, Kuttan G. Studies on the role of some synthetic curcuminoid derivatives in the inhibition of tumour specific angiogenesis. *J Exp Clin Cancer Res* 2003; 22: 77-83.
38. Gururaj AE, Belakavadi M, Venkatesh DA *et al.* Molecular mechanisms of anti-angiogenic effect of curcumin. *Biochem Biophys Res Commun* 2002; 297: 934-42.
39. Cheng AL, Hsu CH, Lin JK *et al.* Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* 2001; 21: 2895-900.
40. Garcea G, Jones DJ, Singh R *et al.* Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *Br J Cancer* 2004; 90: 1011-5.
41. Sharma RA, Euden SA, Platton SL *et al.* Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* 2004; 10: 6847-54.
42. Jung YD, Ellis LM. Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea. *Int J Exp Pathol* 2001; 82: 309-16.
43. Kojima-Yuasa A, Hua JJ, Kennedy DO *et al.* Green tea extract inhibits angiogenesis of human umbilical vein endothelial cells through reduction of expression of VEGF receptors. *Life Sci* 2003; 73: 1299-313.
44. Tang FY, Nguyen N, Meydani M. Green tea catechins inhibit VEGF-induced angiogenesis in vitro through suppression of VE-cadherin phosphorylation and inactivation of Akt molecule. *Int J Cancer* 2003; 106: 871-8.
45. Lai HC, Chao WT, Chen YT *et al.* Effect of EGCG, a major component of green tea, on the expression of Ets-1, c-Fos, and c-Jun during angiogenesis in vitro. *Cancer Lett* 2004; 213: 181-8.
46. Park AM, Dong Z. Signal transduction pathways: targets for green and black tea polyphenols. *J Biochem Mol Biol* 2003; 36: 66-77.
47. Yamakawa S, Asai T, Uchida T *et al.* (-)-Epigallocatechin gallate inhibits membrane-type 1 matrix metalloproteinase, MT1-MMP, and tumor angiogenesis. *Cancer Lett* 2004; 210: 47-55.
48. Fassina G, Vene R, Morini M *et al.* Mechanisms of inhibition of tumor angiogenesis and vascular tumor growth by epigallocatechin-3-gallate. *Clin Cancer Res* 2004; 10: 4865-73.
49. Oku N, Matsukawa M, Yamakawa S *et al.* Inhibitory effect of green tea polyphenols on membrane-type 1 matrix metalloproteinase, MT1-MMP. *Biol Pharm Bull* 2003; 26: 1235-8.
50. Sartippour MR, Heber D, Henning S *et al.* cDNA microarray analysis of endothelial cells in response to green tea reveals a suppressive phenotype. *Int J Oncol* 2004; 25: 193-202.
51. Sartippour MR, Heber D, Zhang L *et al.* Inhibition of fibroblast growth factors by green tea. *Int J Oncol* 2002; 21: 487-91.
52. Trompezinski S, Denis A, Schmitt D *et al.* Comparative effects of polyphenols from green tea (EGCG) and soybean (genistein) on VEGF and IL-8 release from normal human keratinocytes stimulated with the proinflammatory cytokine TNFalpha. *Arch Dermatol Res* 2003; 295: 112-6.
53. Jung YD, Kim MS, Shin BA *et al.* EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. *Br J Cancer* 2001; 84: 844-50.

54. Jatoi A, Ellison N, Burch PA *et al.* A phase II trial of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma. *Cancer* 2003; 97: 1442-6.
55. Greenwald P. Clinical trials in cancer prevention: current results and perspectives for the future. *J Nutr* 2004; 134: 3507S-3512S.
56. Sengupta S, Toh SA, Sellers LA *et al.* Modulating angiogenesis: the yin and the yang in ginseng. *Circulation* 2004; 110: 1219-25.
57. Foster BC, Arnason JT, Briggs CJ. Natural Health Products and Drug Disposition. *Annu Rev Pharmacol Toxicol* 2004.
58. Morbidelli L, Chang CH, Douglas JG *et al.* Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. *Am J Physiol* 1996; 270: H411-5.
59. Dulak J, Jozkowicz A. Nitric oxide and angiogenic activity of endothelial cells: direct or VEGF-dependent effect? *Cardiovasc Res* 2002; 56: 487-8; author reply 489-91.
60. Jozkowicz A, Dulak J, Nigisch A *et al.* Involvement of nitric oxide in angiogenic activities of vascular endothelial growth factor isoforms. *Growth Factors* 2004; 22: 19-28.
61. Morisaki N, Watanabe S, Tezuka M *et al.* Mechanism of angiogenic effects of saponin from ginseng Radix rubra in human umbilical vein endothelial cells. *Br J Pharmacol* 1995; 115: 1188-93.
62. Sato K, Mochizuki M, Saiki I *et al.* Inhibition of tumor angiogenesis and metastasis by a saponin of Panax ginseng, ginsenoside-Rb2. *Biol Pharm Bull* 1994; 17: 635-9.
63. Kong W, Wei J, Abidi P *et al.* Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat Med* 2004; 10: 1344-51.
64. Jayaraman KS. Technology, tradition unite in India's drug discovery scheme. *Nat Med* 2003; 9: 982.
65. Normile D. Asian medicine. The new face of traditional Chinese medicine. *Science* 2003; 299: 188-90.
66. Arakaki N, Toyofuku A, Emoto Y *et al.* Induction of G1 cell cycle arrest in human umbilical vein endothelial cells by flavone's inhibition of the extracellular signal regulated kinase cascade. *Biochem Cell Biol* 2004; 82: 583-8.
67. Bagli E, Stefaniotou M, Morbidelli L *et al.* Luteolin inhibits vascular endothelial growth factor-induced angiogenesis; inhibition of endothelial cell survival and proliferation by targeting phosphatidylinositol 3'-kinase activity. *Cancer Res* 2004; 64: 7936-46.
68. Radzikowski C, Wietrzyk J, Grynkiewicz G *et al.* [Genistein: a soy isoflavone revealing a pleiotropic mechanism of action - clinical implications in the treatment and prevention of cancer]. *Postepy Hig Med Dosw (Online)* 2004; 58: 128-39.
69. Ravindranath MH, Muthugounder S, Presser N *et al.* Anticancer therapeutic potential of soy isoflavone, genistein. *Adv Exp Med Biol* 2004; 546: 121-65.
70. Osada M, Imaoka S, Funae Y. Apigenin suppresses the expression of VEGF, an important factor for angiogenesis, in endothelial cells via degradation of HIF-1alpha protein. *FEBS Lett* 2004; 575: 59-63.
71. Sartippour MR, Seeram NP, Heber D *et al.* Rabdosia rubescens inhibits breast cancer growth and angiogenesis. *Int J Oncol* 2005; 26: 121-7.
72. Ho JC, Konerding MA, Gaumann A *et al.* Fungal polysaccharopeptide inhibits tumor angiogenesis and tumor growth in mice. *Life Sci* 2004; 75: 1343-56.
73. Wu LW, Chiang YM, Chuang HC *et al.* Polyacetylenes function as anti-angiogenic agents. Pharm Res 2004; 21: 2112-9.
74. Roy S, Khanna S, Alessio HM *et al.* Anti-angiogenic property of edible berries. *Free Radic Res* 2002; 36: 1023-31.

75. Wartenberg M, Budde P, De Marees M *et al.* Inhibition of tumor-induced angiogenesis and matrix-metalloproteinase expression in confrontation cultures of embryoid bodies and tumor spheroids by plant ingredients used in traditional chinese medicine. *Lab Invest* 2003; 83: 87-98.
76. Wang S, Zheng Z, Weng Y *et al.* Angiogenesis and anti-angiogenesis activity of Chinese medicinal herbal extracts. *Life Sci* 2004; 74: 2467-78.

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