Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer—Part 2

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ABSTRACT
The herbalist has access to hundreds of years of observational data on the anticancer activity of many herbs. Laboratory studies are expanding the clinical knowledge that is already documented in traditional texts. The herbs that are traditionally used for anticancer treatment and that are anti-angiogenic through multiple interdependent processes (including effects on gene expression, signal processing, and enzyme activities) include *Artemisia annua* (Chinese wormwood), *Viscum album* (European mistletoe), *Curcuma longa* (curcumin), *Scutellaria baicalensis* (Chinese skullcap), resveratrol and proanthocyanidin (grape seed extract), *Magnolia officinalis* (Chinese magnolia tree), *Camellia sinensis* (green tea), *Ginkgo biloba*, quercetin, *Poria cocos*, *Zingiber officinalis* (ginger), Panax ginseng, *Rabdosia rubescens* hora (Rabdosia), and Chinese destagnation herbs. Natural health products target molecular pathways other than angiogenesis, including epidermal growth factor receptor, the HER2/neu gene, the cyclo-oxygenase-2 enzyme, the nuclear factor kappa-B transcription factor, the protein kinases, the Bcl-2 protein, and coagulation pathways. Quality assurance of appropriate extracts is essential prior to embarking upon clinical trials. More data are required on dose–response, appropriate combinations, and potential toxicities. Given the multiple effects of these agents, their future use for cancer therapy probably lies in synergistic combinations. During active cancer therapy they should generally be evaluated in combination with chemotherapy and radiation. In this role, they act as modifiers of biologic response or as adaptogens, potentially enhancing the efficacy of the conventional therapies or reducing toxicity. Their effectiveness may be increased when multiple agents are used in optimal combinations. New designs for trials to demonstrate activity in human subjects are required. Although controlled trials may be preferable, smaller studies with appropriate endpoints and surrogate markers for anti-angiogenic response could help to prioritize agents for larger, resource-intensive phase III trials.

KEY WORDS
Angiogenesis, anti-angiogenic, natural health products, herbal medicine, anticancer, clinical trials, integrative, molecular biology

1. INTRODUCTION
The biochemical signalling pathways of angiogenesis form a complex, interconnected web. Inhibition of one part of the web may result in compensation through another pathway. Because botanicals contain a variety of organic chemical complexes, they usually act on multiple targets. A potential advantage of phytochemicals is that they may act through multiple pathways and reduce the development of resistance by cancer cells. This model of pharmacognosy recognizes the advantage of administering the whole plant product to maximize activity. Over-extraction of a specific chemical constituent may remove this therapeutic gain. The challenge for modern pharmacognosy is to ensure that the optimum mixture of chemical constituents is maintained when a product is purified. Usually, such assurance will require a combination of chemical and biologic assays. The additional anticancer properties of some anti-angiogenic botanicals are briefly discussed here. Their properties may affect various biochemical pathways that indirectly influence angiogenesis. Traditional practice has been to combine multiple natural health products, and scientifically, such combination may provide a therapeutic advantage.

2. MULTISTEP ACTIVITY OF PHYTOCHEMICAL COMPLEXES DERIVED FROM HERBS

2.1 Targeting Alternative Angiogenesis Pathways
The adipocytokines—polypeptides produced by adipocytes—have autocrine, paracrine, and endocrine activities, and are associated with obesity, hyperinsulinemia, and chronic vascular disease as
well as with the development of cancer. The adipocytokines include vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), leptin, tumour necrosis factor alpha (TNFα), heparin-binding epidermal growth factor, insulin-like growth factor, and interleukin-6 (IL-6). All can promote angiogenesis.

Curcumin (from turmeric) and epigallocatechin-3 gallate (EGCG, from green tea) can inhibit aminopeptidase-N (CD13), a member of the matrix metalloproteinase family that is implicated in the angiogenic switch process. Curcumin and EGCG can also interfere with the expression of VEGF by suppressing a series of activities that promote angiogenesis. These angiogenic pathways include production of transforming growth factor beta (TGFβ), amplification of cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR), aberrant expression of src, and amplification of nuclear factor kappa-B (NF-κB) signalling. Curcumin, grape seed extract, and green tea constituents may also interfere with endothelial cell function by inhibiting the engagement of specific integrins. These phytochemicals interact at multiple levels to suppress the inflammatory, hyperproliferative and transformative processes that constitute carcinogenesis.

2.2 Targeting EGFR (HER1)

In many human tumours, EGFR is overexpressed. Such overexpression is associated with more aggressive disease, relative resistance to cytotoxic chemotherapy, and a poorer prognosis. Activity of EGFR induces angiogenesis. Blockade of EGFR reduces angiogenesis and cell proliferation. Monoclonal antibodies have been developed to block the receptor or the linked intracellular signalling system.

Epidermal growth factor (EGF) stimulates urokinase-type plasminogen activator (uPA) expression, which can promote angiogenesis. Genistin (an isoflavone constituent of soy) and curcumin (a constituent of turmeric) both inhibit the effects of EGF. In cell cultures, genistin and curcumin inhibit EGF-stimulated urokinase production and phosphorylation of EGFR. Both botanicals also inhibit protein tyrosine kinases, which could stimulate the enhancement of uPA levels induced by TGFβ.

Other natural health products that can block activity of EGFR include resveratrol and quercetin.

2.3 Targeting HER2/neu

The HER2/neu gene (formerly known as c-erbB-2) is amplified in more than 30% of patients with breast cancer. It is linked to highly aggressive tumours with a poorer prognosis. Overexpression of HER2 is also seen in a significant proportion of patients with other cancer types, including non-small-cell lung cancer, ovarian cancer, prostate cancer, and gastric cancer, in which it may predict a worse outcome. Amplification of the HER2 gene correlates with higher levels of angiogenesis.

Herceptin (Genentech, San Francisco, CA, U.S.A.) is a drug that inhibits HER2/neu. It is usually administered adjuncively with cytotoxic chemotherapy. The activity of Herceptin may be further enhanced by oleic acid. Emolin, a natural constituent of Polygonum multiflorum and aloe, inhibits HER2/neu expression and is toxic against cancer cells, but nontoxic for normal cells.

2.4 Targeting Inflammatory Pathways: COX-2 and NF-κB

Prostaglandins are autacoids derived from arachidonic acid via the COX enzymes. They include prostacyclin, thromboxane, and prostaglandin E types 1–3. A role for arachidonic acid-derived prostaglandins in the process of angiogenesis is now established through in vitro assays. Prostaglandin E2 is a potent inducer of angiogenesis. A correlation exists between COX-2 expression and angiogenesis. Neovascularization is blocked by COX-2 antagonists.

The COX-2 and lipoxygenase (LOX-5) products of omega-6 fatty acid metabolism may exert stimulatory effects on cancer progression including angiogenesis. The omega-3 fatty acids and some pharmacologic inhibitors of eicosanoid biosynthesis antagonize these effects. Large amounts of omega-3 fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid] are found in cold-water fish oils. Liquorice contains glycyrrhizic acid and polyphenols that inhibit COX-2, LOX-5, and protein kinase C (PKC) and also downregulate EGFR.

The NF-κB family consists of closely related protein dimers that bind to a common sequence motif in DNA called the κB site. The NF-κB inducible transcription factor is increased in tissue inflammation, cell proliferation, and cancers. Nuclear factor kappa B induces overactivation of the COX enzymes and is associated with increased angiogenesis.

The COX enzymes are expressed in most normal tissues. The COX-1 enzyme synthesizes non-inflammatory prostaglandins such as prostaglandin E1. In contrast, COX-2 is amplified as part of the inflammatory response and produces prostaglandins such as prostaglandin E2, which may induce uncontrolled cell proliferation and carcinogenesis.

Nuclear factor kappa B may be amplified by growth factors, including TGFβ and basic fibroblast growth factor. Besides NF-κB, other transcription factors, such as activator protein-1 (AP-1) and IL-6, can stimulate COX-2 transcription. Activator protein-1 also promotes the metastatic phase of tumour cells.

Angiogenesis mediated by COX-2 also has a role in the progression of pre-neoplastic lesions to the invasive phenotype. Conventional cancer therapies—such as radiation, surgery, and chemotherapy—may...
induce COX-2 amplification as part of the inflammatory response. The significance of this induction is unclear, but it could hypothetically reduce therapeutic gain.

Several phytochemical derivatives are potent inhibitors of NF-κB. These include resveratrol, piceatannol, curcumin, EGCG, 6-gingerol (ginger), ursolic acid (holy basil), and ginseng. Many botanical COX-2 inhibiting agents block the amplified activity of the transcription factor NF-κB without affecting its normal function.

A variety of natural health products can specifically inhibit the COX-2 enzyme and could play a role in reducing tissue toxicity and improving tumour control when used alongside therapies such as radiotherapy, chemotherapy, and surgery (Table 1). A botanical that protects an organism from the adverse effects of an intervention is termed an adaptogen. Panax ginseng and curcumin are adaptogens that inhibit COX-2 and that have anti-angiogenic activity derived through the inactivation of NF-κB.

2.5 Targeting Protein Kinases

Oncogenes that encode protein kinases may contribute to the development of cancer. In normal cells, protein kinases are involved in signals between the cell membrane and the nucleus, regulating progression through the cell cycle. Protein kinases control these processes by activating other messenger proteins that can influence cell proliferation.

Mutated kinase genes have been found in a number of malignancies, including chronic myelogenous leukemia and breast and bladder cancers. The mutated kinases can contribute to the development of cancer. In many tumour cells, protein kinases are permanently turned on, forcing the cell into constant division. Examples of abnormal kinases are the abl, src, and cyclin-dependent kinases. The kinases may be amplified or permanently switched on by mutations in the control regions of their genes. A commonly overproduced kinase in cancer is EGFR.

Numerous phytochemicals are reported to interfere with cell signalling and may reverse the adverse effects of protein kinase overactivity. Some botanicals with COX-2 inhibitory activity target the intracellular signalling molecules. Inhibition of specific protein kinases suppresses angiogenesis.

Many phytochemicals appear to selectively react with the regulatory centre of PKC. Curcumin, vitamin E, green tea (catechins), resveratrol, Ganoderma lucidum, and liquorice can inhibit PKC activity.

2.6 Targeting the Bcl-2 Protein

The signalling protein Bcl-2 plays a key role in the process of controlled cell death called apoptosis, which is necessary to eliminate aged or damaged cells. The Bcl-2 protein is normally found in the mitochondrial membrane, where it regulates the release of cytochrome C. The latter protein can trigger a series of enzymes (caspases) that lead to cell death. High Bcl-2 levels are associated with most types of human cancer and block the release of cytochrome C. They appear to be a contributor to both inherent and acquired resistance to anticancer treatments. The BCL2 and TP53 genes regulate VEGF-mediated angiogenesis.

Curcumin and green tea extract inhibit BCL2 expression. Scutellaria baicalensis contains the phenolic compounds baicalin, baicalein, wogonin, and oroxylin. These constituents inhibit BCL2 overexpression, plus COX2 gene expression and NF-κB activation. Hiberiscus protocatechuic acid is a phenolic compound isolated from the dried flower of Hibiscus sabdariffa L.; it inhibits Bcl-2 activity.

Other inhibitors of Bcl-2 include EPA fish oil, 6-gingerol, grape seed extract, echinocystic acid (a triterpene found in ginseng and other Asian herbs), parthenolide (a sesquiterpene lactone found in feverfew), and betalapachone (a quinone obtained from the bark of the lapacho tree).

2.7 Targeting Coagulation Pathways Associated with Angiogenesis

In some clinical trials, anticoagulation drugs have been associated with a reduction in metastases.
In Chinese medicine, destagnation herbs are traditionally thought to overcome blockages of qi and blood. Laboratory evidence now suggests that these herbs may have anti-angiogenic and anticoagulation properties. A randomized placebo-controlled trial showed that the addition of “destagnation” herbs (including Salvia miltiorrhiza and Angelica sinensis) to radiotherapy doubled both the local control and the survival rate in patients with nasopharyngeal cancer.

3. CONCLUSION

Angiogenesis involves multiple interdependent processes operating at the molecular level. These include gene expression, signal processing, and enzyme activities. Most anti-angiogenic natural health products block new vessel formation at multiple levels.

Lack of standardization of screening assays may be an obstacle to defining the most effective products for clinical use. Over-extraction of constituents may negate some of the potential synergy. Quality assurance of appropriate extracts is essential before embarking upon clinical trials.

Most studies of anti-angiogenic activity are based on in vitro or animal work that cannot be readily extrapolated to humans. Phase I and II studies are required to determine the potential of these substances to improve cytotoxic therapies. Mainly preclinical data exist for most of the naturally derived anti-angiogenic agents. However, because anti-angiogenic agents are mainly cytostatic in nature, the usual paradigm for anticancer drug development, in which tumour response in phase I trials prompts further development, is not always appropriate. More data are required on dose–response, appropriate combinations, and potential toxicities. Given the multiple effects of these agents, their future use for cancer therapy probably lies in synergistic combinations. They may be evaluated alone for the prevention of cancer recurrence following definitive treatment.

To be suitable for long-term chronic use, these agents should possess minimal toxicity and should be orally administered. However, angiogenesis is also essential for healing of injuries. Most compounds that inhibit tumour angiogenesis are likely to inhibit physiologic angiogenesis, leading to potential side effects such as ulceration and bleeding. Studies are required to determine features that distinguish tumour vessels from normal vessels so that a therapeutic gain can be achieved. Some of the differences have already been described, but the doses and scheduling of anti-angiogenic agents appropriate to achieving the optimum therapeutic gain are unclear.

During active cancer therapy anti-angiogenic agents should generally be evaluated in combination with chemotherapy and radiation. In that role, they act as modifiers of the biologic response and as adaptogens, potentially enhancing the efficacy and reducing toxicity of conventional therapies. The combination of diversity in angiogenic factor expression and different phenotypes of endothelial cells within various tumours is a major challenge for the development of effective anti-angiogenic regimens.

Effectiveness may be increased when multiple agents are used in optimal combinations.

Surrogate markers, such as angiogenic cytokines, are necessary to predict anti-angiogenic response. Circulating levels of fibroblast growth factor–2, VEGF, vascular cell adhesion molecule–1, endothelial intercellular adhesion molecule–1, insulin-like growth factor–1, and cytokines such as interleukin-8 may correlate with tumour angiogenesis. In addition, circulating endothelial cells and their progenitors may be a more reliable marker of response to anti-angiogenic therapies. Non-invasive functional imaging, such as positron emission tomography and functional magnetic resonance imaging, may play a role.

Current laboratory evidence suggests a useful role for natural health products in the treatment of cancer. The input of an herbalist, an oncologist, a laboratory scientist, and a clinical trials methodologist to the research effort is essential to distil the wealth of traditional knowledge into a modern framework that can be evaluated scientifically. Information on traditional dose levels is important for designing initial phase I clinical trials for safety and maximum tolerated dose (Table II). However, the traditional model of pharmacognosy may not necessarily use the highest dose. Establishing the maximum tolerated dose in a phase I study may not always be appropriate. Instead, determination of the biologically active dose that may possess less toxicity may be more relevant. Combinations of whole herbs or constituent phytochemicals at lower doses may be important. In addition, a longer period of exposure to the natural health product may be more effective than a short exposure to the highest possible dose level. New designs for trials to demonstrate activity in human subjects are required.

Although controlled trials might be preferred, smaller studies with appropriate endpoints and surrogate markers for anti-angiogenic response could help to prioritize agents for the larger, resource-intensive phase III trials. Because most of the agents are expected to be cytostatic, it is inappropriate to require the standard criteria of measured tumour response. On the other hand, simply confirming stable disease may be misleading. More research on surrogate markers of anti-angiogenic response is obviously necessary before resources can be directed to large-scale clinical trials (Table III).

The further development of natural health products for clinical trials will require a team effort between academic centres, government, and industry. Appropriate financial support and market protection will be necessary to encourage this activity. New ways of supporting evidence-based innovation for natural
TABLE II Dose ranges of some phytochemicals used by an herbalist for angiogenesis inhibition

<table>
<thead>
<tr>
<th>Herb/phytocoeutical</th>
<th>Preventive dose*</th>
<th>Cancer adjuvant dose*</th>
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<tbody>
<tr>
<td>Turmeric (95% curcumin)</td>
<td>500–1000 mg daily</td>
<td>1000–2500 mg 3 times daily</td>
</tr>
<tr>
<td>Green tea (95% phenols; 50% EGCG)</td>
<td>200–500 mg daily</td>
<td>1000–1200 mg 3 times daily</td>
</tr>
<tr>
<td>Grape seed extract (95% proanthocyanidin)</td>
<td>100–200 mg daily</td>
<td>600–1000 mg daily</td>
</tr>
<tr>
<td>Japanese knotweed (20% resveratrol)</td>
<td>30–50 mg daily</td>
<td>300–500 mg daily</td>
</tr>
<tr>
<td>Quercetin with bromelain</td>
<td>500–1500 mg daily</td>
<td>500–1000 mg 3 times daily</td>
</tr>
<tr>
<td>Holy basil and rosemary (2.37% and 1.5% ursolic acid)</td>
<td>10–20 mg day</td>
<td>10–20 mg 3 times daily</td>
</tr>
<tr>
<td>Silibinin (80% silymarin)</td>
<td>200 mg daily</td>
<td>Up to 2000 mg 3 times daily</td>
</tr>
</tbody>
</table>

* Note that these dose ranges have not all been evaluated in clinical pharmacokinetic studies and are not approved by the U.S. Food and Drug Administration or Health Canada at this stage. The Natural Health Products Directorate of Health Canada is in the process of registering quality, efficacy, and dosing data for natural health products.

EGCG = epigallocatechin-3 gallate.

TABLE III Potential surrogate blood tests for monitoring angiogenesis and its response to therapies 109–116

<table>
<thead>
<tr>
<th>Circulating vascular molecules</th>
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<tbody>
<tr>
<td>Vascular endothelial growth factor</td>
<td></td>
<td></td>
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<tr>
<td>Fibroblast growth factor –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor –1</td>
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<td></td>
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<tr>
<td>Vascular adhesion molecule –1</td>
<td></td>
<td></td>
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<tr>
<td>Endothelial intercellular adhesion molecule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix metalloproteinase –9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circulating cells</td>
<td></td>
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<tr>
<td>Circulating endothelial cells</td>
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<td></td>
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<tr>
<td>Circulating endothelial cell progenitors (CD34+ peripheral blood mononuclear cells)</td>
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</table>

health products are necessary, without necessarily separating and isolating all the constituents of a natural health product simply to allow patent registration. Commercial protection for companies that provide quality assurance and clinical trials evidence may be necessary. Another model includes government support for evaluation, with some profit being returned to the government when a product is commercialized. Part of the funding for clinical studies of the AE-941 shark cartilage derivative (Neovastat: Æterna Zentaris, Quebec, QC, Canada) came from Technology Partnerships Canada, a research support program of the federal government of Canada 118.

Teamwork between the oncologist, the herbalist, the laboratory scientist, and the research methodologist is important for studying anti-angiogenic herbs and other natural health products. As clinical trials introduce these products into the clinic, more definitive evidence of efficacy will be provided and more cancer patients may potentially experience improved outcomes.

4. REFERENCES


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NATURAL HEALTH PRODUCTS FOR ANGIOGENESIS INHIBITION


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