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REGULAR ARTICLE

PATHOPHYSIOLOGY OF ANGIOGENESIS AND NOVEL ANGIOMODULATORS FROM PLANT SOURCES FOR THERAPEUTIC PURPOSE

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SUMMARY

The formation of a 'tumor –associated vasculature', a process referred to as tumor angiogenesis, is a stromal reaction specific for tumor angiogenesis. Angiogenesis, the development of new blood vessels from the existing vasculature, is important for normal developmental process. Angiogenesis is an essential step for the progression of solid tumors. Uncontrolled angiogenesis is a major contributor to a number of disease states such as cancer, inflammatory disorders, Obesity, asthma, diabetes, multiple sclerosis, AIDS, bacterial infections, autoimmune diseases, endometriosis. It is also considered a key step in tumor growth, invasion and metastasis. Thus anti-angiogenic therapy is one of the most promising approaches to control tumor growth. Angiogenesis is required for proper nourishment and removal of metabolic wastes from tumor sites. Angiogenesis may be a relevant target to inhibit tumor progression. The anti-angiogenic molecules reported to date many are plant compounds, peptides, chemokines, cytokines, nanoparticles, siRNA, Flavonoids and Chalcones, monoclonal antibodies, soluble receptors, to vascular growth factors and growth factor receptors, soluble receptors and fragments derived from extracellular matrix proteins. Therefore, modulation of angiogenesis is considered as therapeutic strategies of great importance for human health.

Numerous bioactive compounds from plant sources and other antiangiogenic compounds are recently tested for their antiangiogenic potential. Those compounds inhibits angiogenesis and metastasis through regulation of multiple signaling pathways. Those antiangiogenic compounds regulate the expression of VEGF, MMPs, EGFR, and inhibit NFkB, PI-3K/Akt, ERK signaling pathways, there by causing strong anti-angiogenic effects. Thus, anti-angiogenic compounds are emerging as leading molecules among the plethora of compounds with anti-angiogenic activity.

This review article describes the latest development using anti-angiogenic inhibitors to down regulate various angiogenic and tumor –associated factors, both in biological assays and in animal disease models. The majority of research efforts are currently focused on understanding gene function, as well as proof-of- concept for antiagiogenesis compounds mediated anti-angiogenesis process.

In this article, we will review some of these molecules particularly from plant based sources and discuss their mechanism of action and their potential therapeutic use as anticancer agents in humans.

Key words: Tumor angiogenesis, Vascular endothelial growth factor, Cancer, Bioactive compounds, Peptides, Antibodies, Nanoparticles, Flavonoids, Chalcones

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1. Introduction to Angiogenesis

The term "angiogenesis" was first used by Hertig in 1935 and refers to the formation of new blood vessels in the placenta (1) Angiogenesis is the process of generating new capillary blood vessels from already existing blood vessels which encompases the

multiple gene products expressed by various cell types and intergrated sequence of events. This uncontrolled process of new blood vessel growth from the pre-existing circulation network is an important pathogenic cause of tumor growth, many blinding ocular conditions and inflammatory diseases (2) Angiogenesis is a multistep process which includes degradation of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs),endothelial cell proliferation, migration, and capillary tube formation. The whole process is tightly regulated by the balance between counteracting angiogenic stimulators and inhibitors (3,4).Angiogenesis can be characterized distinctly as hemangiogenesis (HA:blood neovascularisation) and Lymphangiogenesis (LA:Lymphatic neovascularisation), the later being an important initial step in tumor metastasis and transplant sensitization (5). During recent years, much has been learned about the stimulators and inhibitors of HA and LA and members of the vascular endothelial growth factor (VEGF) family emerged as prime mediators of both process (6). Increased vascularity likely allows for both an increase in tumor growth and a greater likelihood of hematogenous tumor migration. Thus,it has been hypothesized that inhibition of tumor angiogenesis will block tumor growth and reduce the potential for tumor metastasis. This hypothesis has led to an intensive effort to identify the molecular mechanisms of tumor angiogenesis to serve as the basic for novel anti-cancer therapy. VEGF is an endogenous mediator of angiogenesis (7,8).VEGF induces vascular growth *in vivo* and promotes endothelial cell responses that are important angiogenic responses such as increased proliferation, migration and protease production(7,9).Therefore ,inhibition of VEGF activity of disabling VEGF receptor function has been useful to inhibit tumor growth and metastasis in a variety of animal tumor models(10,11).

In the early 1970s, Folkman and co-workers hypothesized that tumor growth is angiogenesis-dependent and an anti-angiogenic strategy might constitute a new therapeutic approach for the treatment of solid tumors (12,13). Now a days much effort has been directed toward discovering of new antiangiogenic agent.A variety of these agents such as Avastin(bevacizumab),thalidomide,TNP-470. Endostatin and angiostatin have been currently undergoing clinical evaluation for their efficacy in anti-angiogenic therapy (14,15). Moreover, Macugen (pepgaptanip sodium), a VEGF165-blocker,is the first anti-angiogenic drug approved by FDA in 2005 (16). Plants contain many active compounds. These are complex chemical cocktails with medicinal properties that modern pharmaceuticals cannot reproduce. A wide range of plants contains compounds with angiogenesis modulating properties

Basic steps of angiogenesis

Since Judah Folkman's seminal article about tumor angiogenesis (17), numerous articles have been published about the various aspects of angiogenesis (18), refer also Angiogenesis: http://www.Kluwere online. com/issn/0969-6970/contents). There are at least ten sequential steps of angiogenesis (Figure 1) Recent studies have shown the importance of leucocytes as providers of cytokines, chemokines and enzymes that are involved in angiogenesis. Angiogenesis stimulators and inhibitors target one or more of these steps.

- Diseased or injured tissues produce and release angiogenic growth factors (proteins) that diffuse into the nearby tissues.

- The angiogenic growth factors bind to specific receptors located on the endothelial cells (EC) of nearby preexisting blood vessels.

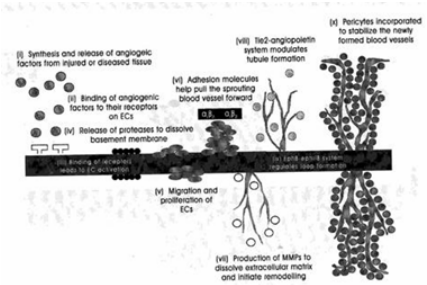
- Once growth factors bind to their receptors, the endothelial cells become activated. Signals are sent from the cell's surface to the nucleus.

- The endothelial cell's machinery begins to produce new molecules including enzymes. These enzymes dissolve tiny holes in the sheath-like covering (basement membrane)

surrounding all existing blood vessels.

- The endothelial cells begin to divide (proliferate) and migrate out through the dissolved holes of the existing vessel towards the diseased tissue (tumor).
- Specialized molecules called adhesion molecules called integrins (avb3, avb5) serve as grappling hooks to help pull the sprouting new blood vessel sprout forward.
- Additional enzymes (matrix metalloproteinases or MMP) are produced to dissolve the tissue in front of the sprouting vessel tip in order to accommodate it. As the vessel extends, the tissue is remolded around the vessel.
- Sprouting endothelial cells roll up to form a blood vessel tube.
- Individual blood vessel tubes connect to form blood vessel loops that can circulate blood.
- Finally, newly formed blood vessel tubes are stabilized by specialized muscle cells (smooth muscle cells, pericytes) that provide structural support. This starts the blood flow.

Fig. 1: The ten sequential steps of angiogenesis



Health and diseases of Angiogenesis

In 1994, The Angiogenesis Foundation (http://www.angio.org) declared angiogenesis a 'common denominator' in the most important diseases of society. In many serious diseases, the body loses control of angiogenesis.

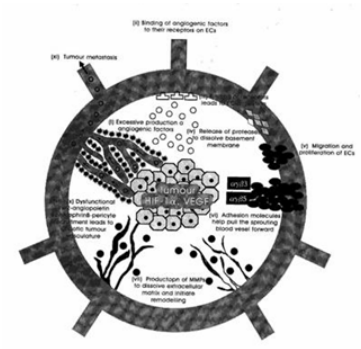
Normal Physiology

In health, angiogenesis is under stringent control by ab 'angiogenic switch (Table1). Selected endogenous positive and negative regulators of angiogenesis) and this rarely occurs in adults except for embryogenesis, placentation, endometrial repair and wound healing (19,20). In the latter case, formation of neovessels is necessary to sustain newly formed granulation tissues,in such a way that the ECs divide with a turnover rate of about 5 days, giving rise to a new microvascular network. During the menstrual cycle,however,angiogenesis occurs in copora lutea and endometrium with rapid growth and regression (21,22). Thus angiogenesis is normally in the quiescent state but is capable of both rapid activation and shutting down.

Pathophysiology of Angiogenesis

Excessive angiogenesis has been defined as a prominent pathological feature of many diseases such as cancer (Figure 2-Tumor angiogenesis and metastasis), rheumatoid arthritis, diabetic retinopathy, psoriasis, atherosclerosis, age related macular degeneration, endometriosis. The excessive angiogenesis occurs when diseased cells produce abnormally large amount of angiogenesis factors (e.g) VEGF-vascular endothelial growth factor, fibroblast growth factor (FGF)-2 and hepatocyte growth factors (23,24). During the early stage of tumorigenesis, tumors are usually not angiogenic, since oxygen can only diffuse to around 150-200 microns from capillaries, solid tumors can only grow to 1-2 mm autonomously at which stage they may exist for months or years without neovascualrisation (i.e. avascular phase). However, once tumor cells switch to the angiogenic phenotype (i.e) vascular phase), an extensive vascular network is constructed through sprouting or non-sprouting angiogenic mechanisms. The large amount of angiogenic factors, mainly VEGF secereted by tumor cells or activated ECS cells form a positive feedback on angiogenesis, leading to tumor growth and subsequent metastasis (25, 26).

Figure 2-Tumor angiogenesis and metastasis



A similar case has also been found in atherosclerosis, in which excessive angiogenesis enhance plaque growth in such a way to sustain perfusion, increase leukocyte exchange, deposition of proatherogenic plasma molecules and finally promote intraplaque hemorrhage (27).

Insufficient Angiogenesis

An insufficient amount of angiogenic stimulators and/or an excess of angiogenic inhibitors can tip the balance resulting inadequate or limited angiogenesis. Inadequate angiogenesis can also cause serious pathological outcomes such as stroke, Alzheimer disease, chronic wound, ulceration, ischemic coronary artery, critical limb ischemia and hypertension, hair loss (28,24). In diseases such as ischemic coronary artery or limb ischemia, the functional blood flow is partially lost in certain organs or limbs poor blood circulation greatly prolongs the recovery period or even results in morbidity or death. A similar phenomenon has been found in the aberrant wound repair in diabetic and gastric ulcer (29,30) excessive/abnormal disease table-angiogenesis.

Tumor angiogenesis as a therapeutic target

Angiogenesis is considered a key step in tumour growth; invasion and metastasis. Tumours remain avasculat and latent for years: However tumor growth can be initiated by neoangiogenesis (25), the idea of blocking tumour growth by the attenuation of angiogenesis was put forward in the early 70's by Folkman (17).

Since the discovery in the early 1970s that tumor progression is angiogenesis – dependent, the concept of angiotherapy has been hypothesized as a therapeutic strategy (31). Ideally, this strategy could be used in the case of insufficient angiogenesis (e.g.heart ischemia) through stimulation of neovessels formation by introducing angiogenic stimulators. An over-burst of uncontrolled angiogenesis (e.g. tumours) could be treated using antiangiogenic inhibitors to shut down their formation of neovessels (32,33,34). After thirty years, this idea became a reality when the first-generation anti-angiogenic drugs called Avastin (bevacizumab) and Macugen (Pegaptanip sodium) were approved by the Food and Drug Administration in the US in 2004 and 2005 respectively for clinical application in cancer patients (35).

Actually, a plethora of anti-angiogenic agents (e.g. TNP-470, thalidomide, Endostatin, and angiostatin) are being tested or undergoing preclinical or clinical trials either alone or combination with conventional therapies (36,37,38). Most of these drugs target the ECs rather than tumor cells, thereby affecting different stages of angiogenesis. Therefore, they are less likely to cause bone marrow suppression, gastrointestinal symptoms or hair loss than conventional tumour therapies.

During the last 12 years, considerable effort has been dedicated to identifying compounds that can be used to either prevent insurgence of primary tumours in subjects at high risk to develop cancer or prevent tumour relapse after surgical removal (39,40,41,42,43).

Currently, ongoing clinical trials are focused to drugs that block matrix breakdown, drugs that inhibit endothelial cells directly, drugs that block activators of angiogenesis, drugs that inhibit endothelial-specific integrin/survival signaling as well as drugs with non-specific mechanism of action (based on National Cancer Institute, clinical database).

On the other hand, the antiangiogenic effect of plant –derived natural compounds, Nanoparticle/Peptides/Flavonoids and chalcones/VEGE Isoforms/siRNA has been

studied intensively only for the last few years.

2. Novel molecular targets of angiogenesis

Eph receptors and ephrins

Eph receptors are the largest family of receptor tyrosine kinase. These receptors and ephrin ligands were originally identified as neuronal-pathfinding molecules. Intriguingly, gene-targeting experiments in mice have identified the Eph B-Ephrin B system as the crucial and rate-limiting determinant of arteriovenous differentiation during embryonic vascular development. Recent work has focused on the roles of the Eph-ephrin system in controlling tumour and vascular functions during tumorigenesis and tumor progression (44). A better understanding of the Eph-ephrin system would lead to the selective targeting in antiangiogenic therapy of the molecules involved.

Anti-angiogenic therapy is based on the normal genetic status of the target vasculature, and therefore is thought unlikely to lead to acquired resistance, however; recent data challenge this concept, using a xenograft murine model of human Wilms' tumour. Huang *et al*. (45) characterised molecular changes in the vasculature during the apparent resumption of xenograft growth after initial inhibition by VEGF blockade, via 'metronome' topotecan chemotherapy and combined agents. They showed that tumours that grow during anti-angiogenic blockade develop as visible clusters surrounding remodeled vessels. These vessels display siginificant increases in diameter, are involved in the active proliferation of vascular mural cells and express PDGF-B,which enhances vascular integrity by stromal cells recruitment. In addition, remodeled vessels express ephrin B2, which is required for the proper assembly of stromal cells into vasculature. Thus, Chronic anti-angiogenesis could lead to enhanced vascular stability, resulting in increased perfusion and recurrent tumour growth. If this phenomenon occurs in human cancers,targeting the EphB-ePhrinB system could be advantageous for treatment.

Histone deacetylase

Histone deacetylase (HDAC) is implicated in the alteration of chromatin assembly and tumorigenesis. Kim *et al.* (46) showed that HDAC is induced under hypoxia and elucidated a role for HDAC in the regulation of hypoxia –induced angiogenesis. The over expression of wild-type HDAC1 down regulates p53 and VON Hippel-Lindau tumour suppressor gene expression, thus stimulating the angiogenesis of human ECs.

TrichostatinA (TSA) is a potent and specific inhibitor of mammalian HDAC that upregulates p53 and von Hippel-Lindau expression and down regulates HiF-1Alpha and VEGF expression. It also blocks angiogenesis *in vitro* and *in vivo*, specifically hypoxia-induced angiogenesis in the Lewis lung carcinoma model. These results indicate that hypoxia enhances HDAC function and that HDAC is closely involved in angiogenesis by suppressing hypoxia-responsive tumour suppressor genes.

Using polymer–assisted solution–phase synthesis an array of HDAC inhibitors was generated. These compounds have considerable potential as anti-proliferative agents. Selected compounds inhibited human Ec proliferation and tube formation in an *in vitro* model of angiogenesis (47). Qian *et al* (48) recently reported that the hydroxamic acid derivative LBH589 induces a wide range of effects on ECs that leads to inhibition of angiogenesis and phosphatidylcholine-3 tumour growth *in vivo*. Thus HDAC inhibitors should be considered as a therapeutic strategy for targeting both the tumour and the endothelial compartment and the clinical development of these agents in combination with other angiogenesis inhibitors is warranted.

Nuclear receptors

The structural differences in angiogenic factors and inhibitors are attributable to differences in receptor-binding affinities, pharmacology and mechanisms of action: for example, ginsenoside Rg1 and ginsenoside Rb1-The pharmacologically active components of Panax Ginseng. Protopanaxatriols such as Rg 1 and Re have pro-angiogenic effects (49, 50).

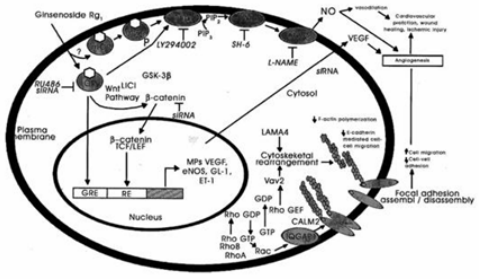
Whereas protopanaxtriols such as Rb1 and Rg3 are antiangiogenic (51,52,53,54). Ginsenoside are triterpene saponins with subtle structural differences in the number of position of the sugar side-chain on a common steroidal backbone (Figure 4). Several studies indicate that the ginsenosides exert their effect by binding to nuclear hormone receptors(55,56,57). Indeed, recent findings demonstrate that ginsenosides can bind to nuclear receptors and exert both genomic and nongenomic effects on HUVECs. Recent studies show mapping of the signalling pathway of Rg1 that leads to the production of nitric oxide (K.W. Leung *et al*., unpublished report). Although ginsenosides such as Rg1, Rb1 are similar molecules, their minor structural differences might enable them to interact differentialy with nuclear receptors, leading to opposing effects on angiogenesis. Further investigations could lead to the development of specific ligands for the modulation of angiogenesis.

Medicinal compound from plant sources

In 1805, morphine was isolated from the opium poppy (Papaver somniferum) by the German Pharmacist Friedrich Serturner. Following the isolation of salicylic acid from the bark of the Willow tree(Salix alba),Felix Hoffmann synthesized aspirin in 1897. Ephedrine was isolated from the Chinese herb Mahuang (Ephedra) in 1887 and became popular with American Physicians in 1924 for its bronchodilating and decongestant properties. Sodium cromoglyate,first used in 1968, is a Khellin derivatives that was isolated from Egyptian Khella seeds (*Ammi visnaga*) by Roger Altounyan. The anti-malarial drug artemisinin was developed in 1972 from the Chinese herb qinghao (sweet wormwood, *Artemisia annua* L.). These examples illustrates the rich history of plant –based medicinal compounds from medicinal plant for therapeutics model in human beings.

Angiogenesis is the growth of neovessels from existing vaculature. Angiogenesis diseases result from new blood vessels growing either excessively (e.g. cancer diabetic retinopathy and psoriasis or insufficiently (e.g chronic wounds and ischaemic heart diseases). To date, the stimulation of angiogenesis using angiogenesis peptides has produced encouraging clinical results in treating coronary artery diseases. Blocking angiogenesis with antibodies/ peptides/ Nanopaticles/ siRNA/Flavonoids/chalcones of angiogenic factors or with enzyme inhibitors is effective for treating malignancy but there is room for improvement (Figure 4). Of particular relevance of this article is the fact that some of the plant derived compound and Peptide, siRNA, Flavonoids, chalcones, Ginsenosides, Nanoparticles antiangiogenic compounds/drugs (e.g. Taxol/ Camtothecin/ curcumin/ Ginestin/ Ginsenosides/ combreatastatin/ Traditional Chinese medicine) are useful for anti-angiogenic therapy. Many herbs are used in the treatment of angiogenic diseases such as cancer, rheumatoid arthritis/chronic wound/heart diseases. Thus it is rational to explore these medicinal compounds from plant and Nano particles/ siRNA/ Flavonoids/ Chalcone/ Peptides/ VEGF isoform as a source of novel angiomodulators. In this article, we review plant based/ Nanoparticles, peptides, siRNA, Flavonoids, chalcone, Chinese traditional medicine etc angiotherapy and discuss the potential future of antiangiogenic therapeutic for treatment of angiogenic diseases.

Fig. 4: Schematic overview of Ginsenoside Rg1 mediated angiogenic action



Angiogenic –modulating compounds from plants

As highlighted by Szymkowski (58) and Lindsay (59), recent genomics focused drug discovery efforts have failed to produce the expected large number of compounds aimed

at "novel' targets. The multifactorial nature of chronic diseases is probably the most significant problem in relation to target discovery. Different countries have distinct herbal traditions,each with their indigenous plants and unique practices.But one claim underlies them all that is the herbs have remarkable properties that make them potentially powerful medicines.In south Africa, *Sutherlandia frutescens* is being developed as new treatment for HIV-AIDS. In Germany medicinal herbs are being broken down into their constituent parts and submitted to rigorous clinical trials.

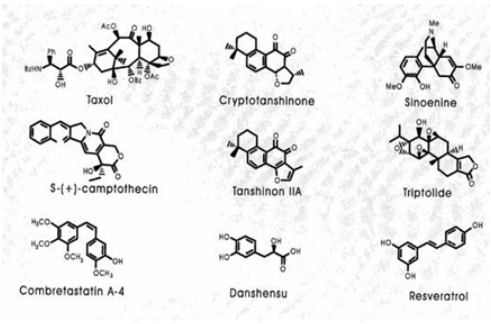
Plants contain many active compounds. These are complex chemical cocktails with medicinal properties that modern pharmaceuticals cannot reproduce. A wide range of plants contains compounds with angiogenesis modulating properties.

3. Effect of plant derived compound on Tumor growth and angiogenesis

Combretastatin

Combretastatin is a microtubule – targeting agent found in the bark of the African bush willow tree (Combretum caffrum), identified in 1987 by Pettit *et al*. (60). Vincent *et al* (61) showed that combretastatin A4 phosphate(CAP4). (Figure- 3) selectively targets ECs, but not smooth muscle cells and induces the regression of unstable nascent tumor neovessels in mice by rapidly disrupting the molecular engagement of the EC-specific junctional molecule vascular endothelial (VE) cadherin *in vitro* and *in vivo*.

Figure- 3-Chemical structures of angiomodulators derived from medicinal plants and functional foods



CAP4 increases EC permeability and inhibits EC migration and capillary tube formation, predominantly through the disruption of the VE-Cadherin-β –catenin-Akt signaling pathway, resulting in rapid vascular collapse and tumor necrosis.CAP4 synergises with low and nontoxic doses of neutralizing monoclonal antibodies to VE cadherin by blocking the assembly of neovessels, there by inhibiting tumour growth.

CAP4 selectively induces the regression of unstable tumor neovessels, in part through the disruption of VE cadherin signaling. A combined treatment of anti-VE cadherin agents in conjuction with microtubule-disrupting agents provides a novel synergistic strategy to disrupt assembly and induce regression of tumour neovessels selectively, with minimal toxicity and without affecting normal stabilized vasculature.

Taxol

In 1962, scientists at the National cancer Institute (http://www.cancer.gov/) determined that an extract from the bark of the pacific yew tree (Taxus brevifolia) possessed anticancer properties. In 1971,the active compound in this extract was identified by Wani *et al*.(62) as Taxol, a complex polyoxygenated diterpene (Figure- 3). Taxol kills proliferating cancer cells by disrupting their microtubule cytoskeletons (63,64,65). Of particular interest is the recent discovery that, at low picomolar concentrations, Taxol is antiangiogenic, inhibiting VEGF production(66) and hypoxia-inducible factor (HIF)-α protein expression (67)The data support a clinical application of continuous ultra-low-dose Taxol to treat cancer(68).

Fortunately, the needles and twigs of the Europena Yew tree(Taxus Baccata) contain 10-deacetyl baccatin III,which is a close relative of Taxol.Because the needles are quickly replenished, harvesting large quantities has little effect on the population of yew trees. To produce sustainable source,10-deacetyl baccatinIII was chemically modified by Bristol Myers Scquibb Pharmaceuticals (http//:www.bms.com/landing/dat/inde.html)and, in 1995, a semi-synthetic version of Taxol-Paclitaxel-was made available to the public.

Camptothecin

Using bioactivity –directed fractionation, Wall *et al*. (69) isolated Camptothecin (Figure- 3) from the Chinese tree *Camtotheca acuminate*.

The use of Camptothecin as an anticancer agent languished for almost 15 years until its unique mode of action for killing tumour cells was determined. Canmptothecin traps topoisomerase I in complex with DNA ,thus preventing DNA replication and resulting in the death of the cancer cell. This discovery rekindled the interest in developing analogues of Camptothecin that are water soluble and retain their anticancer activity. In the mid -1990s, two camptothecin analogues, topotecan, and irinotecan, received FDA approval for use against ovarian, lung, breast and colon cancers (70,71).

In 1999, Clemets *et al*.(72) showed that camptothecin and topotecan inhibit human EC growth *in vitro* in a non-cytotoxic manner and that this inhibition lasts >96 h after drug removal. They also showed that these two compounds,unlike the non-specific cytotoxic agent cisplatin, are effective as TNP-470 (73) at inhibition angiogenic growth in the *in vivo* disc angiogenesis model. Thus, in addition to their tumoricidal activities, camptothecins might have an indirect *in vivo* antitumour effect that is mediated through the inhibition of angiogenesis.

Antiangiogenic effects of functional foods

Food components can influence angiogenesis. On the one side this can contribute to pathological changes. For example (74,75) 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.The antiangiogenic functional foods provide a good strategy for the prevention of angiogenic diseases-(76,77).

4. Conclusion

Angiogenesis inhibitors are likely to change the face of medicine in the next decade. The chemopreventive agents that selectively interfere with particular biochemical alterations occurring in tumor cells or those acting on the highly specialized biology of endothelial cells during neovascularisation deserve special attention.

Most of the currently available bioactive compounds, peptides, proteins, with antiangiogenic activities have been initially developed or used for experimental purposes, and have greatly contributed to the understanding and the modulation of the molecular mechanism of tumor angiogenesis. Several bioactive compounds, proteins (antibodies), peptides, siRNA, nanoparticles, flavonoids and chalcones etc. entered clinical testing as anti-angiogenic drugs, and a few of them are now approved for clinical use (e.g: Taxol, IFN-α, TNF, Bevacizumab).

The discoveries of Bioactive compounds such as Taxol, camptothecin and combrestastatin, Ginsenosides, sinomenine, and triptolide from plants are important milestone in the history of anticancer drugs. These examples of synthetic chemistry leading to more-efficacious analogues and their subsequent clinical use illustrate the power of combining medicinal chemistry and pharmacology.

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