Vol 2|Issue 1| 2012 |1-12.

e-ISSN 2248 – 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

CURRENT RESEARCH AND CLINICAL MANAGEMENT OF ANGIOGENESIS

[*]A. Saravana Kumar, [1]S. Kavimani, [2]K.N. Jayaveera

[*]Research Scholar**,** Jawaharlal Nehru Technological University, Anantapur**,** Andhra Pradesh, India- 515002**.**
[1]Professor & Head, Department of Pharmacology, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry - 605 006.
[2] Professor & Head, Department of Chemistry, Jawaharlal Nehru Engineering College, Jawaharlal Nehru Technological University**,** Anantapur**,** Andhra Pradesh, India- 515002**.**

ABSTRACT

One of the proposed benefits of targeted therapies is reduced toxicity and improved quality of life. In this review article, we summarize the clinical management of angiogenesis associated diseases such as diabetic retinopathy, cancer and psoriasis and their toxic side effects. We end by discussing the future prospects for the clinical use of vascular targeting in treatment of diabetic retinopathy, cancer and psoriasis.

Keywords: Angiogenesis, Diabetic Retinopathy, Cancer and Psoriasis.

INTRODUCTION

In recent years several therapeutic approaches aimed at inhibiting the different steps of angiogenic process have been used as single agent strategy or in addition to conventional treatments for Diabetic Retinopathy, Cancer and Psoriasis. Angiogenesis is a complex process and there is now evidence that it is initially linked to cell proliferation and apoptosis, thus representing a critical step for tumor formation and progression. Targeting of angiogenesis is a major therapeutic avenue and a large variety of new agents are under development. Although the results of some clinical trials have been disappointing, a better understanding of the correct timing for the use of anti-angiogenic drugs, the selection of patients that can really benefit from these treatments and the combination with cooperating conventional therapies will very likely place these agents among the most advanced strategies for treats Diabetic Retinopathy, Cancer and Psoriasis [1].

Corresponding Author

A. Saravana Kumar
Email:- sarganjune1@gmail.com

CLINICAL MANAGEMENT

I.Pharmacological Management

Antiangiogenic drugs are stopping neovascularization in wet macular degeneration. A substance in the body called Vascular Endothelial Growth Factor (VEGF) is responsible for the growth of new blood vessels. It promotes this growth by stimulating the endothelial cells, which form the walls of the vessels and transport nutrients and oxygen to the tissues. Evidence shows that when the retinal pigment epithelial (RPE) cells begin to wither from lack of nutrition (a condition called "ischemia"), the VEGF goes into action to create new vessels. This process is called "neovascularization," and it acts as a restorative function in other parts of the body. In the retina, however, the vessels do not form properly, and leaking results. This leakage causes scarring in the macula and eventual loss of central vision. Antiangiogenic drugs prevent the VEGF from binding with the receptors on the surface of the endothelial cells. In most cases, the drugs are injected into the vitreous of the eyeball, then pass into the subretinal space, where the vessels proliferate. Neovascularization is then blocked, preventing bleeding into the retina.

This is continually-updated information on all anti-angiogenic drugs for wet MD:

1. Macugen (pegaptanib sodium)
2. Lucentis (ranibizumab)
3. VEGF Trap-Eye (Eylea)
4. Avastin (bevacizumab)
5. Endostatin
6. Pazopanib

1) *Macugen (pegaptanib sodium*)

1.1. *General information*

Macugen (pegaptanib) is a selective vascular endothelial growth factor (VEGF) antagonist. Macugen is indicated for the treatment of neovascular (wet) age-related macular degeneration. According to the present product label, Macugen (0.3 mg) should be administered once every six weeks by ntravitreous injection into the eye to be treated.

There are 15 million people in the United States living with some form of AMD, with more than 1.6 million experiencing the active blood vessel growth and blood vessel leakage associated with eovascular AMD.

1.2. *Mechanism of action:*

Pegaptanib is a selective vascular endothelial growth factor (VEGF) antagonist. VEGF is a secreted protein that selectively binds and activates its receptors located primarily on the surface of vascular endothelial cells. VEGF induces angiogenesis, and increases vascular permeability and inflammation, all of which are thought to contribute to the progression of the neovascular (wet) form of age-related macular degeneration (AMD), a leading cause of blindness

1.3. *Side effects*

Adverse events associated with the use of Macugen may include (but are not limited to) the following:

- Ocular Discomfort
- Eye Pain
- Endophthalmitis
- Reduced Visual Acuity
- Visual Disturbance
- Corneal Edema
- Blurred Vision
- Dizziness[49 to 51]

2) *ranibizumab* (trade name **Lucentis**) is a monoclonal antibody fragment (Fab) derived from the same parent mouse antibody as bevacizumab (Avastin). It is much smaller than the parent molecule and has been affinity matured to provide stronger binding to VEGF-A. It is an anti-angiogenic that has been approved to treat the "wet" type of age-related macular degeneration (ARMD), a common form of age-related vision loss.

Some investigators believe that bevacizumab at an average cost of $42 a dose (in the U.S.) is as effective as ranibizumab at an average cost of $1,593 a dose.

2.1. *Mechanisam of action***:** Ranibizumab binds to and inhibits a number of subtypes of vascular endothelial growth factor A (VEGF-A). VEGF may trigger the growth of new vessels, which may leak blood and fluid into the eye. These leaky blood vessels may contribute to macular edema and choroidal neovascularization, resulting in the wet type of ARMD. By blocking VEGF-A in the eye, ranibizumab may prevent and reverse vision loss caused by wet macular degeneration

2.2. *Side effects***:** The most common side effects in clinical trials were conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation.

Although there is a theoretical risk for arterial thromboembolic events in patients receiving VEGF-inhibitors by intravitreal injection, the observed incidence rate was low (< 4%) and similar to that seen in patients randomized to placebo. Serious adverse events related to the injection procedure occurred with an incidence rate of less than 1% and included endophthalmitis, retinal detachment, and traumatic cataracts. Other serious ocular adverse events observed among ranibizumab-treated patients (incidence rate < 1%) included intraocular inflammation and blindness [2-4].

3*) VEGF trap-eye (eylea*):

The vascular endothelial growth factor (VEGF) family is a group of molecules that direct both normal and pathological processes in the body. Certain members of the family and their receptors have been implicated in the angiogenesis underlying pathological disease processes, including the development of eye diseases such as neovascular (wet) age-related macular degeneration (AMD). Despite recent therapeutic advances, wet AMD continues to be debilitating and rapidly progressive, often having a dramatic impact on the lives of patients, as well as their caregivers. It is the leading cause of vision loss in adults aged 50 years and older in many regions, including North America and Europe.

Endothelial growth and angiogenesis, caused by vascular endothelial growth factors, hypoxia, wound healing, and inflammation, are believed to contribute to wet AMD development. Blocking VEGF activities has become a mainstay therapy for treating eye diseases that have angiogenesis at their etiological core. Although highly effective at delaying disease progression, current therapies do not always prevent loss of visual acuity or consistently improve lost vision. Moreover, treatment often requires frequent office visits and Intravitreal injections.

There are 2 primary receptors in the VEGF family, VEGFR1 and VEGFR2. VEGFR2 is best characterized in terms of its role in angiogenesis: VEGF-A binds to VEGFR2 to stimulate angiogenesis. Although its precise role continues to be investigated, recent research suggests a role for PlGF in promoting pathologic angiogenesis as well. PlGF binds to the VEGFR1, potentially stimulating angiogenesis directly through a signaling pathway through this receptor. Alternatively, the role of PlGF may be to compete with VEGF-A for VEGFR1, allowing VEGF-A to bind to VEGFR2 and stimulate angiogenesis. PlGF may also serve as a chemo attractant for inflammatory cells stimulating the VEGFR1 on monocytes, increasing their migration and stimulating production of VEGF and other inflammatory mediators. A third receptor, VEGFR3, regulates the development of lymphatic endothelial cells [5].

3.1. VEGF trap-eye design:

Regeneron's VEGF Trap-Eye is a cytokine trap — a soluble fusion protein currently being evaluated to treat retinal diseases. Traps include 2 extracellular cytokine receptor domains and a human Fc region of immunoglobulin G (IgG). Because VEGF Trap-Eye is biologically engineered, specific properties of different naturally occurring component molecules were selected for their therapeutic potential. VEGF Trap-Eye includes specific extracellular components of VEGF receptors 1 and 2 fused to the constant region (Fc) of IgG1. As seen in Figures 1 and 2, this results in 2 identical arms, each constructed from select pieces of both VEGFR1 and VEGFR2. These components were selected based on their high affinity for both VEGF-A and PlGF. VEGF Trap-Eye contains all-human amino acid sequences, giving it a low potential for immunogenicity in humans.VEGF Trap-Eye binds both VEGF-A and PlGF, and it does so with higher affinity than they do to their native receptors. VEGF Trap-Eye uniquely binds both ends of activated dimerized VEGF or PlGF between its arms, preventing it from binding to the native receptors or cross-linking, which is possible due to the binding properties of monoclonal antibodies. VEGF Trap-Eye has been developed exclusively for ophthalmic use. The ophthalmic formulation is specifically purified and formulated as an iso-osmotic solution to avoid irritation of the eye.

3.2. VEGF trap-eye mechanism of action:

VEGF Trap-Eye was developed in an effort to address the unmet need for wet AMD treatments that produce sustainable improvements in visual acuity and/or reduced injection frequency. Based on its unique binding properties (which are distinct from VEGF antibodies/antibody fragments), predictive modeling studies indicate that VEGF Trap-Eye should have a longer duration of activity than currently available treatments. Each arm of VEGF Trap-Eye binds to the binding interface on each pole of the active VEGF or PlGF dimer. This forms a stable and inert 1:1 complex with the growth factor,uniquely binding the dimer on both sides. The Trap is aptly named, since the molecule isolates (or traps) the dimer, forming inert complexes with the growth factor that do not interact with more than 1 VEGF Trap molecule. This blocks and effectively arrests the VEGF angiogenesis cascade. It also prevents the creation of multimeric complexes that might aggregate and cause immune responses in body tissues. VEGF Trap-Eye binds VEGF more tightly than native receptors, blocking cell-surface receptor activation.VEGF antibodies and their fragments bind with lower affinity, allowing VEGF dimers to occasionally interact with other molecules, including their receptors [6,7].

4) *Avastin* (bevacizumab)

Avastin is a therapeutic antibody that is believed to work by targeting and inhibiting the function of a natural protein called "vascular endothelial growth factor" (VEGF) that stimulates new blood vessel formation, a process known as angiogenesis. Researchers have shown in preclinical models that anti-VEGF agents like Avastin may work by causing the following changes to occur in the blood vessels.

Working of Avastin:

Avastin specifically binds and blocks VEGF and this precise mode of action helps control tumour growth and metastases.Avastin combats the tumour in three key ways

4.1. Avastin causes the regression of blood vessels:

Avastin causes the small blood vessels around the tumour to regress helping to stop the supply of oxygen and nutrients the tumour needs for development.

4.2. Avastin 'normalises' existing blood vessels:

Tumour vasculature is often immature and prone to leakage. This abnormal permeability may impede the delivery of anticancer treatments, such as chemotherapy. Avastin may cause a normalisation of the chaotic tumour vasculature network, thereby maximising the effectiveness of the overall treatment
strategy.

4.3. Avastin stops the growth of new blood vessels:

Avastin inhibits the growth of new blood vessels further limiting blood supply to the tumour.

Avastin has proven survival benefits across multiple tumour types. Over half a million patients have been treated with Avastin so far. A comprehensive clinical programme with more than 450 clinical trials is investigating the use of Avastin in various tumour types (including colorectal, breast, lung, brain, gastric, ovarian, prostate and others) and different settings (advanced or early stage disease).

5) ***Endostatin:***

Endostatin is a broad spectrum angiogenesis inhibitor and may interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and vascular endothelial growth factor (VEGF). Endostatin suppresses angiogenesis through many pathways affecting both cell viability and movement. Endostatin represses cell cycle control and anti-apoptosis genes in proliferating endothelial cells, resulting in cell death. Endostatin blocks pro-angiogenic gene expression controlled by c-Jun N terminal kinase (JNK) by interfering with TNFα activation of JNK. It reduces the growth of new cells by inhibiting cyclin D1. As a result, cells arrest during G1 phase and enter apoptosis. Alteration of FGF signal transduction by endostatin inhibits the migration of endothelial cells through disruption of cell-matrix adhesions, cell-cell adhesions, and cytoskeletal reorganization. By binding integrin α5β1 on endothelia cells it inhibits the signaling pathways of Ras and Raf kinases and decreases ERK-1 and p38 activity. Endostatin binding and clustering of integrins causes co-localization with caveolin-1 and activates non-receptor tyrosine kinases of the Src family involved in the regulation of cell proliferation, differentiation, and mobility. Other receptor interactions include the VEGF-R2/KDR/Flk-1 receptor on human umbilical vein endothelial cells [8-10].

Endostatin may prevent activity from certain metalloproteinase. Several studies have focused on the downstream effects of endostatin reception. These studies have estimated that endostatin may significantly affect 12% of genes used by human endothelial cells. Although endostatin signaling may affect this vast number of genes, the downstream affects appear surprisingly limited. Endostatin reception seems to only affect angiogenesis that arrives from pathogenic sources, such as tumors. Processes associated with angiogenesis, such as wound healing and reproduction, are seemingly not affected by endostatin. The result is possible because pathogenic-derived angiogenesis usually involves signaling through integrins, which are directly affected by endostatin.

6) ***Pazopanib:***

Researchers state that topical administration of pazopanib causes regression of already established CNV in patients with neovascular AMD. A 28 day safety study in February 2010 revealed no serious adverse events in the eye related to the drug. However, some mild to moderate symptoms were reported in some patients. A phase 3 trial is planned. (anonymous 28 to 30)

Pazopanib is the third vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor (TKI) with IC50 values of 10, 30 and 47 nM for VEGFR-1, VEGFR-2, and VEGFR-3, respectively. Meanwhile pazopanib is the sixth targeted therapeutic drug that has been approved for the treatment of advanced or metastatic renal cell carcinoma (RCC) by US FDA. However, what is its mechanism of action? It has been demonstrated that its primary mechanism of action in RCC is to take advantage of its antiangiogenic properties via prevention of the two intracellular tyrosine kinases including vascular endothelial growth factor receptor (VEGFR) and platelet-dereived growth factor receptor (PDGFR).

Chemically, pazopanib is an indazolypyrimidine that competitively binds to the intracellular side of tyrosine kinase receptors with adenosine triphosphate (ATP). According to the adenine ring of ATP, a part of the pazopanib structure was imitatively designed. The component of the pazopanib structure generates hydrogen bonds with the tyrosine kinase receptor. Subsequently, pazopanib inhibits ATP-induced activation. Pazopanib inhibits not only the intracellular tyrosine kinase portion of the VEGFR subtypes including VEGFR-1, VEGFR-2 and VEGFR-3, but also the two PDGFR subtypes such as PDGFR-alpha and PDGFR-beta, on endothelial cells. This leads to suppression of anigogenesis via blocking signaling transduction pathways implicated in the proliferation, survival and vascular permeability of cells, as well as their migration. In addition, pazopanib represses various of other tyrosine kinase receptors, such as c-kit, fibroblast growth factor receptor (FGFR)-1 and FGFR-3, as well as interleukin (IL)-2 receptor inducible T-cell kinase (Lck) and transmembrane glycoprotein receptor tyrosine kinase (c-Fms) [10].

II. NON PHARMACOLOGICAL MANAGEMENT

Non-pharmacological or natural therapies are things you can do or think about that help decrease your pain. These therapies do not involve taking medicines, but work along with your medicines. People have used "natural" ways to help with pain and healing from the very beginning of time.

- A long time ago, the Chinese learned that putting special needles in areas of the body could decrease pain. Music has also a very important part of healing the sick over time. Scientists are learning that common things like music, laughter, exercise and good smells cause our brains to make special chemicals. These special chemicals may help us to feel less pain.
- Also, being tense and upset causes pain to become worse. When you are tense, your muscles get tight which decreases blood flow in your body. Your heart beats faster and your blood pressure goes higher. Your breathing also gets faster and more shallow. Your brain begins to make chemicals, including ones that may cause pain. This stress and upset cycle causes you more pain. Certain ways to relax help loosen muscles. This breaks the whole cycle and may decrease your pain.

- There are many different ways you can work with your mind and your body to help decrease your pain. A natural therapy that works for you may not work well for another person. This is because people are all different. It may take a little time and practice to find a therapy that works well for you. And, you may find that using several therapies together works even better. Taking a warm bath with scented oil and playing soft music

PAIN CONTROL IMPORTANCE

Pain can affect your appetite (ability or desire to eat), how well you sleep, your energy and your ability to do things. Pain can also affect your mood (how you feel about things) and relationships with others. Using natural therapies with your medicines to help your pain may let you feel more in control of your life. This control may help you suffer less, find hope, and even heal faster.

Care

Almost all types of pain, including cancer pain, can be controlled with medicine and are helped by using natural therapies. It may be hard to get your pain to go away completely. But, it is possible to lower your pain level so you can live and be comfortable doing everyday things. Work with your caregiver to find out what pain control treatments and therapies are best for you. Always tell your caregiver if the pain gets worse. Ask your caregiver if you want more information on any of the following non-drug pain control treatments.

Natural non-drug pain control therapies and methods

- *Acupuncture (AH-q-punk-sher)* is based on the belief that life forces or energy move through the body in specific paths. These paths are called meridians (mer-IH-d-uns). With acupuncture, a needle is put into the meridian that runs to the area where you have pain. This needle blocks the meridian which stops or decreases the pain.
- *Aromatherapy (uh-ro-muh-THAIR-uh-p)* is a way of using good smells to help you relax and decrease pain. Candles, massage oils, scented bubble baths and even baking cookies are all ways that smells are used. Scientists are learning that good smells may change your mood and help you relax. It may also help your brain makes special chemicals like endorphins (n-DOOR-fins). Endorphins are a natural body chemical like morphine that decrease pain.
- *Biofeedback* teaches your body to respond in a different way to the stress of being in pain. Teaching your body to relax helps make the pain less. Caregivers may use a biofeedback machine so that you know right away when your body is relaxed. But, often you may not need any machines. Learn to take your pulse. Then take it while making your mind think about "slowing down" your pulse. This can work with breathing, temperature, and blood pressure too.
- *Breathing exercises* are another physical way to help your body relax. Teaching your body to relax helps make the pain less. Breathing in and out very slowly is all you do. Women have used breathing exercise for many years to decrease the pain of childbirth. A fun way to practice breathing slowly is to blow soap bubbles. You know you are doing a good job when you get very large bubbles. Remember to practice when you are not having pain. This helps it work better when you are having pain.
- *Distraction (dih-STRAK-shun)* teaches you to focus your attention on something other than pain. Try playing cards or games, watching TV, or taking a walk. You can also visit with friends, paint, pet animals, and write out your feelings. Using planned activities helps to manage the boredom that chronic pain and illness can cause. It may also cause you to relax and keep you from thinking about the pain.
- *Environment (your surroundings***) -** Being in a quiet place may make it easier for you to deal with the pain. Avoiding bright lights or loud noisy places can also help control your pain. Making sure your home is not too hot or too cold may also decrease pain.
- *Guided imagery (IH-mij-ree***)** teaches you to put pictures in your mind that will make the pain less intense. With guided imagery, you learn how to change the way your body senses and responds to pain. Imagine floating in the clouds or remembering favorite place. Guided imagery seems to especially help people with chronic lower back pain.
- *Heat and cold* can help decrease pain. Some types of pain improve best using heat while other types of pain improve most with cold. Caregivers will tell you if hot and/or cold packs will help your pain. Also, remember that a long warm bath may help calm you and let your muscles relax. A cool shower on a very hot summer day may do the same thing.
- *Laughter* - It has been said that "10 minutes of belly laughter gives 2 hours of pain-free sleep!" Laughter helps you breathe deeper and your stomach digest (break down) food. It lowers blood pressure and may cause your brain to make endorphins. Laughter can also help change your moods. It helps you relax and let go of stress, anger, fear, depression, and hopelessness. These are all parts of chronic pain.
- *Massage* is often used to help a person become more relaxed. Have someone gently massage your back, shoulders, and neck. Massage can be even more effective if you also use guided imagery, breathing exercises, or music.
- *Music* It does not matter whether you listen to it, sing, hum or play an instrument. Music increases blood flow to the brain and helps you take in more air. Scientists are learning that it increases energy and helps change your mood. Music also may cause your brain to make special chemicals like endorphins. Endorphins are a natural body chemical like morphine that decrease pain. People who use music often say it decreases their need of medicines for pain and anxiety.

- *Physical therapy* can be helpful with pain that was caused by not moving one part of your body. Stretching the muscles and making them stronger around the injured area can help the pain go away.
- *Radiation* can be used to decrease the size of a cancer tumor that is pressing on nerves and causing pain. Radiation can also help decrease bone pain.
- *Self-hypnosis* is a way to change your level of awareness. This means that by focusing your attention you can move away from your pain. You make yourself open to suggestions like ignoring the pain or seeing the pain in a positive way. It is not known exactly how hypnosis helps pain. But, hypnosis can give long-lasting relief of pain without affecting your normal activities. Self-hypnosis gives you better control of your body. You may feel less hopeless and helpless because you are doing something to decrease the pain.
- *Spinal cord stimulation* is a nerve stimulation technique that is similar to TENS. The difference is that in SCS an electrode (a metal wire) is put near the spinal cord during surgery. SCS also uses mild, safe electrical signals to help control pain.
- *Tens* is short for transcutaneous (trans-q-TAIN-e-us) electrical nerve stimulation (stih-mew-LA-shun). A TENS unit is a portable, pocket-sized, battery-powered device which attaches to the skin. The TENS unit uses mild, safe electrical signals to help control pain.
- *Touch energy therapies* come from very old beliefs that life forces or energy move through the body in specific paths. Touch therapies believe disease may cause these paths to become blocked. The therapies use touch to help unblock these paths, and allow the energy to flow normally. Unblocking the paths may help you relax and decrease pain [11].

PREVENTION

1.POLYPHENOLIC COMPOUNDS

Flavonoids:

Flavonoids are the most abundant polyphenols in our diet. They are natural estrogenic compounds derived from soybeans, tea, fruits, and vegetables and have been proposed to act as chemopreventive agents in Asian populations. Soy products reduced angiogenesis, increased apoptosis, and slightly reduced proliferation in transplantable murine bladder cancer in vivo. To determine whether prevention might be associated with dietary-derived angiogenesis inhibitors, Fotsis and coauthors fractionated urine samples of healthy human subjects consuming a plant-based diet and examined the fractions for their abilities to inhibit the proliferation of vascular endothelial cells .The most potent fractions contained several isoflavones, including genistein, which was the most effective inhibitor of endothelial cell proliferation and in vitro angiogenesis.

The isoflavone genistein is a potent inhibitor of tyrosine kinases and, along with flavonoids such as kaempferol and apigenin, is an inhibitor of topoisomerases I and II, enzymes crucial to cellular proliferation Shao and co-workers demonstrated that genistein inhibited invasion in vitro of MCF-7 and MDA-MB-231 breast carcinoma cells by down-regulating MMP-9 and up-regulating the tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), and inhibited angiogenesis by decreasing vessel density and levels of VEGF and TGF-ß1 These in vitro findings were confirmed in vivo in nude mouse xenografts . Data in the literature report increased angiogenesis in the bone marrow of patients with acute myeloid leukemia and B cell chronic lymphoblastic leukemia. When used in the clinic, treatment of therapy refractory B-lineage acute lymphoblastic leukemia with B43-genistein elicited objective responses at nontoxic dose levels.

Flavonoids are not the only active compounds with chemopreventive properties found in soy products. Several protease inhibitors derived from soybeans, including the Bowman-Birk serine protease inhibitor, possess anticarcinogenic and anti-inflammatory activity .As will be discussed later, by inhibiting basement membrane invasion, protease inhibitors could synergize with the antiangiogenic potential of soy isoflavones. Silymarin, another naturally occurring antioxidant flavonoid, exhibits anticancer effects against several epithelial cancers and has shown potential as an antiangiogenic agent in studies using HUVEC and human prostate or breast cancer epithelial cells. The inhibitory effects of silymarin on COX-2 and IL-1α should be explored to develop preventive strategies against those cancers in which these molecular targets play one of the causative roles, such as nonmelanoma skin, colon, and breast cancers in humans.

There is a strong possibility that prostatic intraepithelial neoplasia lesions that switch to the angiogenic phenotype eventually progress to cancer . In vitro studies demonstrated that genistein, silymarin, and epigallocatechin-3-gallate (EGCG) inhibit mitogenic signaling pathway(s) and alter cell cycle regulators, albeit at different levels, leading to growth inhibition and death of advanced and androgen-independent prostate carcinoma cells. Reduction in serum prostate-specific antigen (PSA) levels has been proposed as an end point biomarker for hormone refractory human prostate cancer intervention. However, PSA itself may represent an endogenous antiangiogenesis molecule that can produce angiostatin.

2. NSAIDS

NSAIDs are effective colon cancer chemopreventive agents that might also be useful in preventing other types of cancer. Recent reports indicate

that NSAIDs inhibit tube formation by endothelial cells in in vitro models of angiogenesis. The mitogen/cytokine-inducible cyclooxygenase isoenzyme COX-2, involved in prostaglandin (PGE) generation from arachidonic acid, has been shown to regulate angiogenesis induced by colon cancer cells in a coculture model of colon carcinoma and endothelial cells, where the selective COX-2 inhibitor NS-398 inhibited the production of angiogenic factors by colon carcinoma cells. In a study by Jones et al. the most effective antiangiogenic compound was again NS-398. NS-398 also inhibited angiogenesis and in vivo growth of tumors derived by the PC-3 human prostate cancer cell line.

The antiangiogenic effect of the selective COX-2 inhibitor celecoxib has been demonstrated in a rat model of angiogenesis. COX-2 overexpression seems to occur first in stromal cells, and results in angiogenic growth factor release that is capable of inducing endothelial cell proliferation in a paracrine fashion. COX also provides a superoxide radical-generating pathway and could contribute in part to the establishment of a more oxidized intracellular environment, which in turn can stimulate proliferation of tumor cells. Inhibition of angiogenesis by NSAIDs apparently follows more than one pathway, prostaglandin dependent and independent. All types of NSAIDs inhibit the mitogen-activated protein (MAP) kinase Erk2. Early angiogenic stimuli also use the MAP kinase pathway, which in turn can lead to activation of nitric oxide synthase (NOS). Although the inhibitory effects of NO on tumorigenesis have been associated with an antiangiogenic effect, the importance of the different isoforms of NOS for tumor vascularization is not yet clear. Many angiogenic molecules also stimulate NOS activity; endothelial NOS have been shown to play an essential role in VEGF-induced angiogenesis.

Nuclear localization of MAP kinase is essential for its function, and NSAIDs interfere with ERK-2 nuclear translocation. However, the antiangiogenic effect of NSAIDs may not to be limited to inhibition of COX-2 and PGE synthesis. Future detailed analysis of the antiangiogenic effects of NSAIDs could be interesting for chemoprevention of those cancers in which COX-2 overexpression is observed.

NATURAL AND SYNTHETIC STEROID RECEPTOR SUPERFAMILY LIGANDS

Retinoid

Like steroids and vitamin D, retinoids exert most of their effects by regulating gene expression through specific receptors belonging to the steroid/thyroid hormone superfamily. Chemopreventive protocols using these drugs target tissues that express the corresponding receptors. Carotenoids, retinol, and retinoids are fundamental regulators of cell growth, differentiation, and development. Retinoids bind and activate their nuclear receptors, the RARs and RXRs. They enhance apoptosis and prevent the conversion of in situ cancer to locally invasive malignancy by suppressing the invasive process. Retinoids have been reported to be active in treating specific premalignant lesions and reducing the incidence of second primary tumors in patients with prior head and neck, lung, or liver cancer. However, a carotenoid (ß-carotene) was shown to significantly increase the mortality for primary lung tumors in smokers and asbestos-exposed individuals. This underscores the need for relevant experimental models to identify pathways signaling chemopreventive effects.

All-*trans*-retinoic acid (RA) has shown antiangiogenic effects in several systems. In one study, it caused the endothelial cells of large and small vessels to become refractory to stimulation of migration by tumor-conditioned media or purified angiogenic factors (α-fibroblast growth factor, vascular endothelial growth factor, TGFß-1, and IL-8) without affecting cell proliferation. Rats given all-*trans*-RA were unable to mount an angiogenic response to tumors implanted in their corneas. These results indicated that all-*trans*-RA can directly affect both tumor and endothelial cells and thereby suppress the formation of new blood vessels in vivo.

13-*cis*-RA was found to synergize with interferons in the inhibition of angiogenesis in experimental models of Kaposi's sarcoma tumors and breast and vulval carcinomas. 9-*cis*-RA, which binds and activates RARs and RXRs, showed antiangiogenic activity synergistic with interferon α in a experimental mouse model of tumor-induced angiogenesis.

Retinoid receptors can regulate gene expression by interacting directly with cognate response element in target gene promoters, but they can also cross-talk with other signaling pathways. All-*trans*-RA interferes with AP-1 signaling, a key regulatory pathway in angiogenesis. Transcriptional interference is not restricted to AP-1 but can involve other transcription factors such as NF-κB and CCAT/enhancer binding protein beta. Examples of genes that have been shown to be suppressed by all-*trans*-RA through antagonism of AP-1 include VEGF, stromelysin, collagenases, and TGF-ß. Retinoids also exert transcriptional repression on interstitial collagenase. Although retinoids are inhibitors of in vitro angiogenesis, they have distinct effects on the plasminogen-dependent proteolytic system: all-*trans*-RA and 9-*cis*-RA increase u-PA activity in human microvascular endothelial cells, and t-PA synthesis is induced in the absence of altered PAI-1 synthesis in HUVEC cells exposed to *all-trans* RA. In a defined cell-free system, plasminogen activators (uPA and t-PA) have been shown to generate angiostatin, a naturally occurring inhibitor of angiogenesis, from plasminogen. TIMPs are also angiogenesis inhibitors: retinoids positively

modulate endothelial cell production of TIMP-1 and TIMP-2. COX-2-derived PGEs contribute to tumor growth by inducing newly formed blood vessels; retinoids suppress the basal expression and EGF- or TPA-mediated induction of COX-2 in human oral squamous carcinoma cells. The recent discovery of STAT1 as a novel retinoid-regulated gene supports further evidence for the interference of retinoids with signaling pathways involved in angiogenesis.

The future in this area will depend on the development of new agents whose mechanism of action is well understood and that show increased preventive or therapeutic efficacy, and less toxicity, than the parent natural compounds. Rexinoids such as SR11246, LG100268, and LGD1069 (Targretin or bexarotene) are RXR nuclear receptor selective ligands. Different from RARs, RXR can heterodimerize with partners of the steroid receptor superfamily, including vitamin D receptor (VDR) and peroxisome proliferator-activated receptor (PPAR). In preclinical studies of breast cancer chemoprevention, the RXR selective ligand LGD1069 showed evidence of activity on tamoxifen-resistant cancer, and a less toxic profile compared to RAR agonists in the *N*-methylnitrosourea (NMNU) -induced rat mammary carcinogenesis model, where it strongly inhibited tumor burden and tumor incidence. The antitumoral effects of LGD1069 have been related to inhibition of tumor angiogenesis. LGD1069 (bexarotene) is under evaluation in a phase II trial for the treatment of the highly vascular AIDS-associated Kaposi sarcoma. RXR synergistic or modulatory activity on other hormone receptors can influence the activity and toxicity of therapeutic ligands relevant in cancer prevention and cure.

The synthetic retinoid 4-HPR, or fenretinide, has been shown to prevent breast, prostate, and ovarian cancer in preclinical models and has been evaluated in clinical trials of cancer prevention. Pienta et al using three different angiogenesis inhibition assays, demonstrated that 4-HPR inhibits angiogenesis as well as endothelial cell motility and tubule formation, thus providing a putative mechanism responsible for the proven chemopreventive effect of 4-HPR on prostate cancer development.

Cell–matrix interactions have been investigated in 4-HPR-treated BALB/c 3T3 cells whose invasive potential is lowered by 4-HPR. Preliminary experiments obtained in our laboratory in an in vivo angiogenesis assay show that 4-HPR significantly inhibits angiogenic growth factor-stimulated neovascularization. Based on these data, we hypothesized that the antitumor effect of 4-HPR could be due at least in part to its inhibitory effect on endothelial cell growth and tubular morphogenesis, thus preventing neoangiogenesis at an early stage of tumor development. 4-HPR acts through a mechanism involving ceramide biosynthesis in neuroblastoma cells and, more generally, by mitochondrial damage in several tumor cell types. C2 ceramide has been demonstrated to be involved in detachment-induced endothelial cell apoptosis derived by αVß3 and αVß5 RGD binding integrins blockade, with consequent c-*jun* N-terminal kinase activation. 4-HPR action on endothelial cells has not been investigated at the molecular level, but 4-HPR-dependent long-term production of ROS in endothelial cells might also inhibit endothelial cell proliferation and neoangiogenesis. 4-HPR decreases cellular release of insulin-like growth factors (IGFI and II) and enhances insulin-like growth factor binding protein synthesis and secretion, thus reducing the circulating levels of the biologically active molecule, a potent proangiogenic growth factor. The networking pathways of IGFs and VEGF signaling, as shown by recent findings on IGF-I-regulated VEGF mRNA expression in NIH3T3 and endometrial adenocarcinoma cells, indirectly support a role for 4-HPR as an antiangiogenic agent.

PPARγ ligands:

Another member of the steroid hormone receptor superfamily, PPARγ, is activated by eicosanoids, including the natural ligand 15-deoxy-delta12, 14-prostaglandin J2 (15D-PGJ2), a prostanoid derived from the cyclooxygenase product PGD2, and by antidiabetic agents such as thiazoldinediones. PPARγ is a key transcription factor involved in adipogenesis and monocyte differentiation. The role of PPARs in cancer chemoprevention has recently been reviewed .PPARγ, activated by 15D-PGJ2 or by new antidiabetic agents (BRL49653 and ciglitazone) showed a potent antiangiogenic activity by inhibiting differentiation of HUVEC cells into tube-like structures in a tridimensional collagen matrix. VEGF receptors and uPA mRNA were decreased in the same cells whereas PAI levels were elevated. 15D-PGJ2 has been shown to induce endothelial cell apoptosis via a PPAR-dependent pathway involving nuclear translocation of PPAR as well as caspase activation. PPARγ ligands also inhibited choroidal neovascularization in response to VEGF. In addition, 15D-PGJ2 and cyclopentenone prostaglandins have been shown to inhibit the NF-κB-dependent transcription of target genes, including COX-2, by directly blocking IkappaB kinase in a PPARγ-independent manner. Again, blockade of NF-κB signaling inhibits angiogenesis of ovarian cancer by suppressing the expression of VEGF and IL-8. As the NF-κB signaling pathway appears to be a key regulatory pathway in inflammation and angiogenesis, this novel mechanism could enhance the antiangiogenic activity of COX-2 inhibitors.

Ligands for PPARγ have been shown to have marked synergism with rexinoids in the treatment of experimental diabetes. It seems reasonable to speculate that a similar synergism between the two classes of agents could be also found in chemoprevention and angioprevention of cancer.

Steroids:

Angiogenic activity has been reported for ligands of the nuclear hormone receptor superfamily such as androgens and estrogens. Inhibition of the proangiogenic effects of estrogens could underlie the chemopreventive action of tamoxifen and selective estrogen receptor antagonists on mammary carcinogenesis. Recently, the molecular mechanism underlying angiogenic growth factors regulation by estrogens in the female reproductive tract has been elucidated by the finding that a functional estrogen responsive element is present in the promoter region of VEGF, and that estrogens regulate VEGF mRNA and protein expression in human breast cancer cells. Tamoxifen and raloxifene both bind to the estrogen receptors ER-α and ER-ß and can act as estrogen antagonists or agonists, depending on the target tissue, thus promoting the beneficial effects of estrogens on bone or suppressing cancer-promoting effects in the breast. Tissue-specific antiangiogenic/angiogenic effects of estrogen antagonists could also explain the increased incidence of endometrial cancer in tamoxifen-treated women. Further development of steroid receptor modulators devoid of angiogenic effects would be crucial to avoid the insurgence of secondary neoplasms in treated patients. 2-Methoxyestradiol (2-ME), an endogenous estrogen metabolite that disrupts microtubule formation, induces apoptosis in endothelial cells and inhibits angiogenesis by a mechanism involving stress-activated protein kinase signaling and modulation of Fas expression. In an animal model, 2-ME inhibited estrogen-induced pituitary tumor growth and angiogenesis, probably by down-regulating VEGF expression.

Hormonally regulated tissues such as the prostate are angiogenesis-dependent, and androgens have been studied extensively as regulators of prostatic angiogenesis. The ongoing large-scale Prostate Cancer Prevention Trial begun in 1997 is using finasteride as a chemopreventive agent. Finasteride, a testosterone analog, competitively inhibits the enzyme 5α reductase that converts testosterone into the more potent dihydrotestosterone. The use of finasteride in prostate cancer chemoprevention is based on the rationale that androgens promote prostate tumorigenesis. Several authors have reported studies of the effect of androgens on VEGF release. Recent work from the group of Folkman reports that androgens modulate the angiogenic activity of hormone responsive human prostate cancer by regulating VEGF mRNA and protein expression. Androgen withdrawal also inhibits hypoxic induction of VEGF mRNA. In the case of prostate cancer, antioxidants such as vitamin E and selenium protect from tumor insurgence, suggesting that a combination of hormonal therapy and antioxidants could synergistically contribute to the antitumoral and antiangiogenic effect of the chemopreventive treatment.

Vitamin D

Vitamin D (calciferol) and its analogs (deltanoids) are well-recognized regulators of cell proliferation and differentiation besides their classic function in mineral homeostasis. The active form of vitamin D, 1α,25-dihydroxy vitamin D_3, binds to the nuclear receptor VDR belonging to the steroid/thyroid hormone receptor superfamily, which can heterodimerize with the rexinoid RXR receptor.

The antitumoral effect of vitamin D has been studied in prostate and breast cancer cells, where it induces cell death. Vitamin D_3 receptors have been identified on bovine aortic endothelial cells and human capillaries, where a 4.5-fold up-regulation of VDR is detectable in activated proliferating cells. Vitamin D_3 showed antiangiogenic properties in several cellular contexts, interfering with the action of angiogenic molecules. 1α,25-Dihydroxy vitamin D_3 has been shown to inhibit VEGF-induced endothelial cell sprouting and elongation in vitro due to induction of apoptosis and to reduce in vivo vascularization of tumors derived by MCF-7 breast carcinoma cells overexpressing VEGF. Angiogenesis was also inhibited by vitamin D_3 in a transgenic murine model of retinoblastoma, with the tumors from treated animals showing significantly lower vessel counts than controls. 1α,25-Dihydroxy vitamin D_3 has been shown to inhibit Kaposi's sarcoma (KS) cell growth in vitro by reducing the production of the angiogenic cytokines IL-6 and IL-8, which are autocrine growth factors for the highly vascular KS. This effect of vitamin D_3 is particularly relevant in that tumor cells in KS, which is a strong inflammatory disease, display the phenotypic and morphological characteristics of activated endothelial cells. A DNA sequence mediating this repression was localized in the 5' flanking region of the IL-6 gene. In the same study, a synthetic VDR agonist, calcipotriol, showed antitumor effects in KS patients. The potential clinical use and possible toxic side effects of less calcemic vitamin D analogs are under evaluation to prevent prostate, colon, and breast cancer. [33 to 46]

Diet

Some common components of human diets also act as mild angiogenesis inhibitors and have therefore been proposed for angioprevention, the prevention of metastasis through the inhibition of angiogenesis. In particular, the following foodstuffs contain significant inhibitors and have been suggested as part of a healthy diet for this and other benefits:

- Soy products such as tofu and tempeh, (which contain the inhibitor "genistein")
- *Agaricus blazei* mushrooms (angiogenesis inhibitors found in the mushroom include
- sodium pyroglutamate and ergosterol)
- Black raspberry extract (Rubus occidentalis)
- Reishi mushrooms (via inhibition of VEGF and TGF-beta)

- *Trametes versicolor* mushrooms
- *Maitake* mushrooms (via inhibition of VEGF)
- *Phellinus linteus* mushrooms
- Green tea (catechins)
- Liquorice (glycyrrhizic acid)
- Red Wine (resveratrol)
- Bananas produce a substance known as TNF which combats cancer cells.
- Antiangiogenic phytochemicals and medicinal herbs.
- Royal Jelly[46,47]

CURRENT RESEARCH

- High-Dose Atorvastatin Improves Hypercholesterolemic Coronary Endothelial Dysfunction Without Improving the Angiogenic Response
- Intramyocardial delivery provides a superior mode of delivery for therapeutic angiogenesis
- Dr Rana Reports on the powerful placebo effect in endstage coronary artery disease
- Dr Voisine Reports on the deleterious effect of elevated cholesterol on angiogenesis and endothelial function
- Dr Shie describes a Novel Transcriptional Stimulator of VEGF: RTEF-1
- Angiogenesis Research Center and Interventional cardiology Section receives a BIRD foundation grant to study a novel mechanism to induce angiogenesis: MicroTissue transplant for sustained and efficient angiogenesis
- Drs. Wu and Li report the anti-apoptotic and myocardial protective effects of PR39
- Dr Ruel discusses the effect of elevated cholesterol on angiogenesis
- Dr Wu publishes on COX-2 cardioprotective effect
- ARC publishes on spatial heterogeneity in VEGF-iduced vasodilation
- Dr. Tanveer Khan discuss gene therapy for angiogenesis in Gene Therapy (Nature Publishing Group)
- Study at BIDMC discussed on Boston's WCVB
- Study at BIDMC discussed on New England Cable News
- Study at BIDMC discussed on BBC
- Cell Therapy for cardiovascular disease: *fact or fiction*. Editorial in Lancet
- The Angiogenesis Research Center reports on Phase II randomized, placebo controlled study of FGF-2
- The Angiogenesis Research Center reports on safer subxyhoid access strategies for pericardial drainage and Pericardial access
- Angiogenesis Research Center characterize PR39 as a novel peptide regulator of angiogenesis [37-39]

Fig. 1. Strategies for inhibition of tumor growth by anti-angiogenic therapeutic drugs

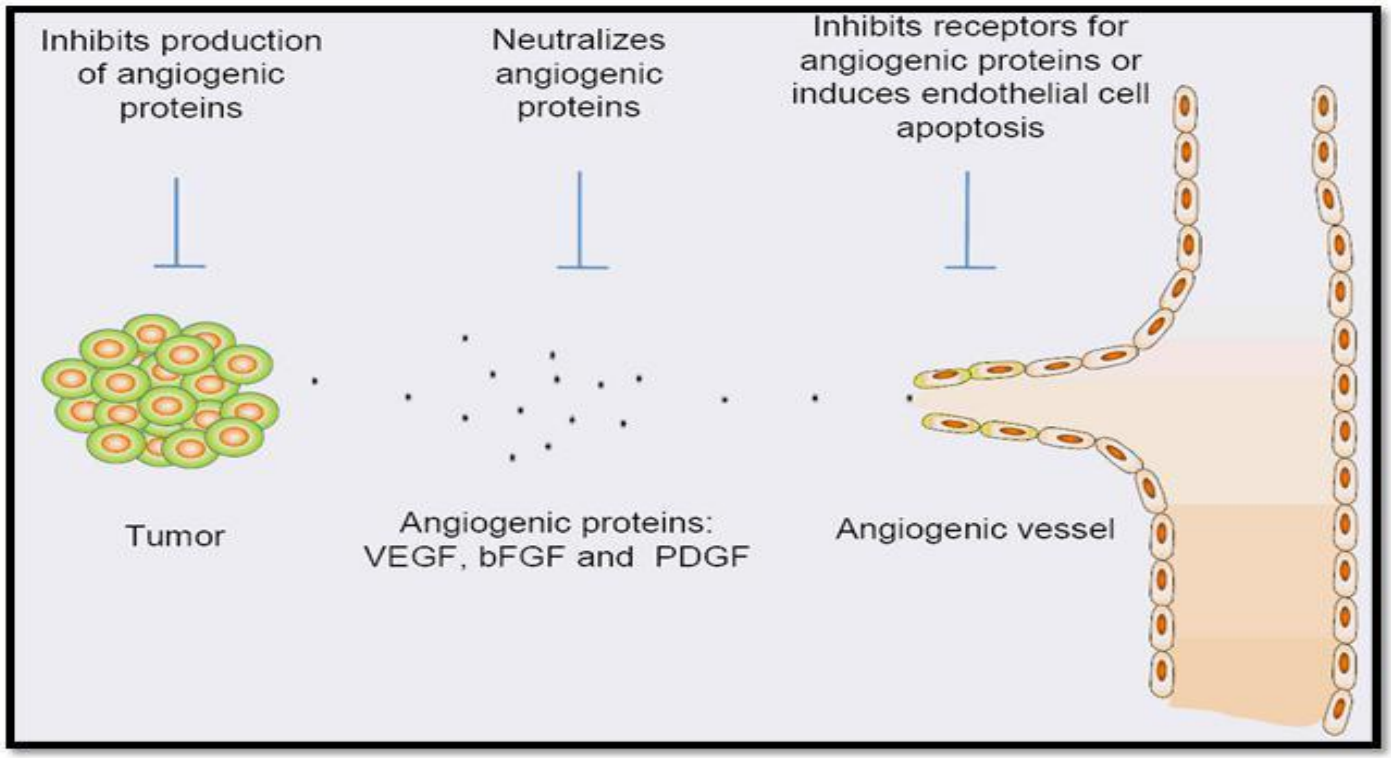


Fig No. 2. A key binding domain of VEGFR1 and a key binding domain of VEGFR2 (left) are fused for tight binding affinity for both VEGF-A isomers and PlGF (center). Two dual-domain arms are used for one VEGF Trap-Eye molecule to mimic the natural receptor pairing necessary for growth factor signaling (right).

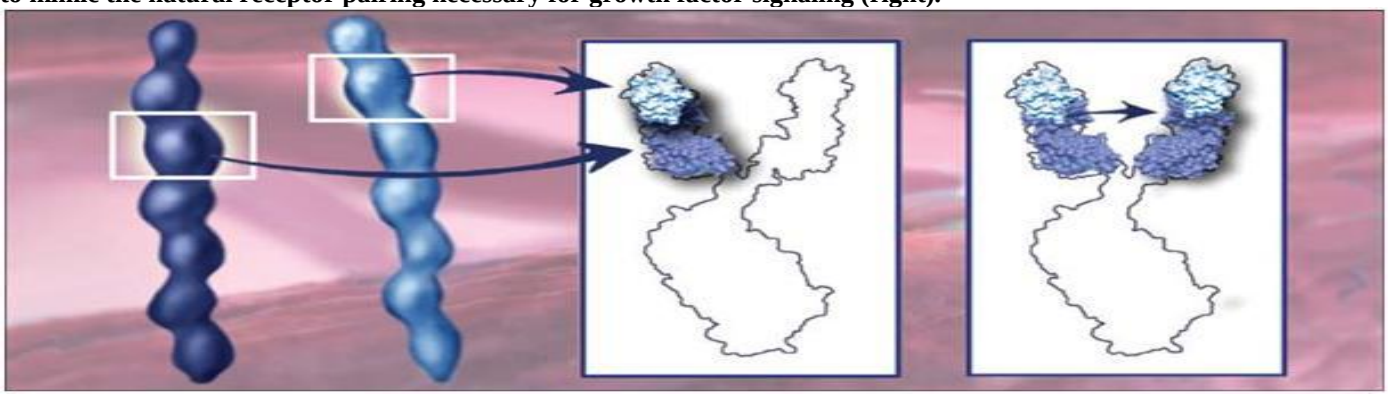
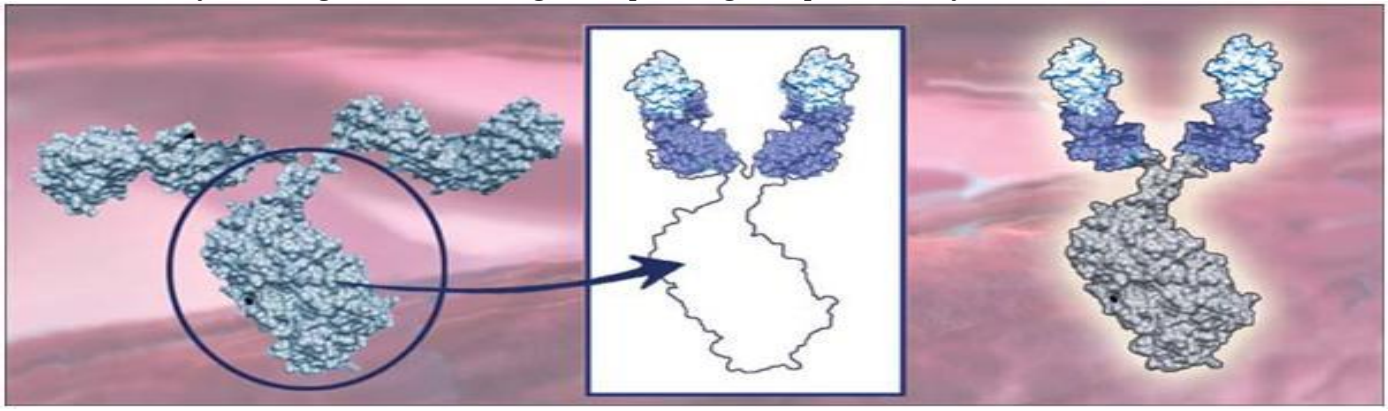
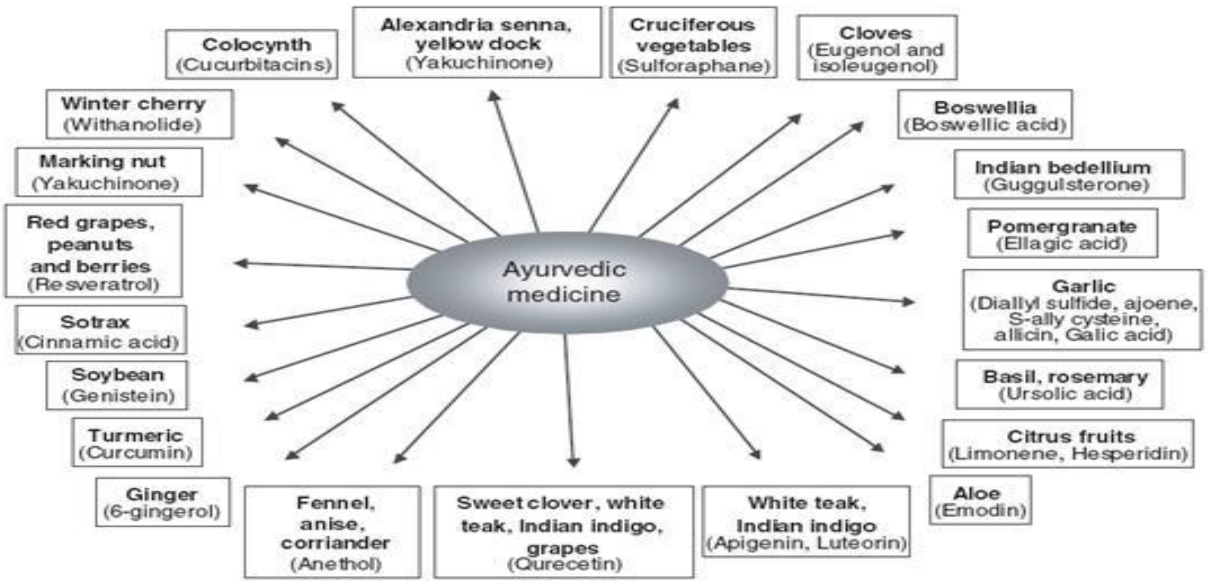


Fig No. 3. The Fc portion of IgG1 (left) is fused to the two dual-domain arms (center) resulting in the engineered molecule of VEGF Trap-Eye (right). This exemplifies how a molecule can be designed to possess specific properties of different naturally-occurring molecules with a goal of optimizing therapeutic activity.



NATURAL THERAPY



CONCLUSION

Anti-angiogenic drugs are the recent outcome of the fruits of a prolonged period of basic research, mostly undertaken over the last 20 years. Conversely, inhibitors of angiogenesis are now being used to treat a variety of 'angiogenic diseases' characterized by unwanted or over-exuberant angiogenesis such as diabetic retinopathy and age-related macular degeneration, and perhaps even endometriosis and atherosclerotic plaques. Successes in treating such diseases with drugs which modulate angiogenesis is bound to increase the efforts in the area of cancer, and vice versa. Exciting clinical results in the area of 'therapeutic angiogenesis' are being obtained using bFGF, VEGF or the genes which encode these proteins, to stimulate vessel growth in ischemic heart disease and peripheral vascular disease. Further research work is needed for development new more potent agents used against the high rate of angiogenesis in disorders.

REFERENCES

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*., 285, 1971, 1182–1186.
2. Alicia Mundy. "Medicare Eye Study Finds Untapped Savings". 2010.
3. Tufail A, Patel PJ, Egan C, Hykin P, Da Cruz L, Gregor Z, Dowler J, Majid MA. et al. Bevacizumab for neovascular age related macular degeneration (ABCTrial): multicentre randomised double masked study. *BMJ*, 340, 2010, c2459–c2459.
4. Haberfeld H, ed. *Austria-Codex* (2009/2010 ed.). Vienna: Österreichischer Apothekerverlag, 2009.
5. Gehrs KM, Anderson DH, Johnson LV, Hageman GS. Age-related macular degeneration—emerging pathogenetic and therapeutic concepts. *Ann Med.*, 38, 2006, 450-471.
6. Schmier JK, Jones ML, Halpern MT. The burden of age-related macular degeneration. *Pharmacoeconomics*, 24, 2006, 319-334.
7. Kleinman ME, Ambati J. Molecular Mechanisms of Neovascularization in AMD. *Retinal Physician*, 2009.
8. Carmeliet P. Oncology, 69 (3), 2005, 4-10.
9. Garcia M et al. Global Cancer Facts & Figures. Atlanta, GA: American Cancer Society, 2007.
10. WHO Cancer Factsheet N°297 – updated July 2008. Last accessed 24 August 2009.
11. Anonymous 1. http://www.drugs.com/cg/non-pharmacological-pain-management-therapies-for-adults.html