Recent Research in Science and Technology 2011, 3(7): 08-15
ISSN: 2076-5061
www.scholarjournals.org

www.recent-science.com

RRST-Pharmacology

A Review on "Anti-angiogenic Therapy – Past, Present and Future"

Kinja K*, Rohit S, Mandloi A, Sharma I, Savita S

Department of Pharmacology, NIMS Institute of Pharmacy, NIMS University, Shobha Nagar, Jaipur-303121

Article Info

Article History

Received : 17/02/2011
Revised : 24/03/2011
Accepted : 24/03/2011

*Corresponding Author

Tel : 9529155588

Email:
kkinja_pharma@yahoo.com

Abstract

Angiogenesis, or the formation of new blood vessels out of pre-existing capillaries, is a sequence of events that is fundamental to many physiologic and pathologic processes such as cancer, ischemic diseases, and chronic inflammation. With the identification of several proangiogenic molecules such as the vascular endothelial cell growth factor, the fibroblast growth factors (like in FGFs), and the angiopoietins, and the recent description of specific inhibitors of angiogenesis such as platelet factor-4, angiostatin, endostatin, and vasostatin, it is recognized that therapeutic interference with vasculature formation offers a tool for clinical applications in various pathologies. Whereas inhibition of angiogenesis can prevent diseases with excessive vessel growth such as cancer, diabetes retinopathy, and arthritis, stimulation of angiogenesis would be beneficial in the treatment of diseases such as coronary artery disease and critical limb ischemia in diabetes. In this review we highlight the current knowledge on angiogenesis regulation and report on the recent findings in angiogenesis research and mainly highlight the anti-angiogenic drugs/therapy.

Key Words: Angiogenesis, anti-angiogenic drugs, tumour angiogenesis, vascular remodelli

Introduction

The formation of new blood vessels out of pre-existing capillaries, or angiogenesis, is a sequence of events that is of key importance in a broad array of physiologic and pathologic processes. Normal tissue growth, such as in embryonic development, wound healing, and the menstrual cycle, is characterized by dependence on new vessel formation for the supply of oxygen and nutrients as well as removal of waste products. Also, a large number of different and nonrelated diseases are associated with formation of new vasculature. Among these pathologies are diseases, such as tissue damage after reperfusion of ischemic tissue or cardiac failure, where angiogenesis is low and should be enhanced to improve disease conditions [1,2]. In several diseases, excessive angiogenesis is part of the pathology. These diseases include cancer (both solid and hematologic tumors), cardiovascular diseases (atherosclerosis), chronic inflammation (rheumatoid arthritis, Crohn's disease), diabetes (diabetic retinopathy), psoriasis, endometriosis, and adiposity. These diseases may benefit from therapeutic inhibition of angiogenesis. This article mainly gives information about these anti-angiogenic drugs [3]. (Figure-1)

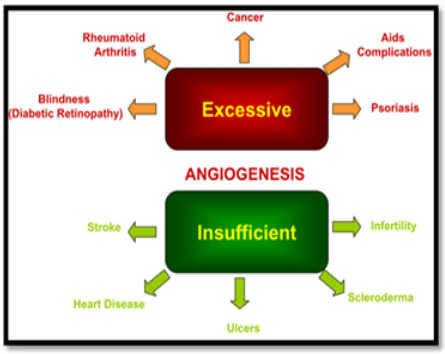


Fig-1- Diseases associated with angiogenesis

History

The initial recognition of angiogenesis being a therapeutically interesting process began in the area of oncology in the early 1970s, when Dr. Folkman and Denekamp put forward the idea that tumors are highly vascularized and thereby vulnerable at the level of their blood supply. In those early years, it was already hypothesized that the process of angiogenesis might be a target for therapy. It was only after the discovery of the first compounds with specific angiostatic effects in the early 1990s that the research field of angiogenesis rapidly expanded and provided an increasing body of evidence that inhibition of angiogenesis could attenuate tumour growth. More recently, novel angiogenesis inhibitors have shown great potential in the treatment of cancer in preclinical studies. Several of those compounds are

currently being tested in clinical trials. With increasing insight into the role of angiogenesis in other diseases as well, modulation of vascular outgrowth is now also regarded as a therapeutic target in these diseases. Anti-angiogenesis therapy is considered, worldwide, a promising approach, supposedly leading to the desperately needed breakthrough in cancer therapy and other proangiogenic diseases [4,5].

Function of Endothelial Cells in Normal Physiology

The blood vessels in the body have long been considered to merely function as a transport compartment of the blood. Nowadays, it is appreciated that the vasculature is one of the main organs in the body, extending more than 900 m2 and playing a major role in maintaining the body's integrity in various ways. Blood vessels consist of endothelial cells that are in direct contact with the blood, and subendothelially located pericytes, smooth muscle cells, fibroblasts, basement membrane (BM),1 and extracellular matrix (ECM). Depending on the location in the body, the organ microenvironment, the cellular constituents, BM, and ECM of the vasculature differ in phenotype, composition, and function [6].

The endothelial cells form a monolayer in every single blood vessel in the circulation and are actively involved in several regulatory processes in the body. Besides being metabolically active and selectively permeable for small solutes and peptides/proteins, they regulate blood coagulation. When their integrity is maintained, endothelial cells exert anticoagulative properties via the synthesis of thrombomodulin, tissue factor (TF) pathway inhibitor and tissue-type plasminogen activator (t-PA). On activation or damage, endothelial cells quickly release proteins like multimeric von Willebrand factor (vWF), which promotes platelet adhesion and aggregation, and plasminogen activator inhibitor-1, a member of the serpin family. In addition, TF expression by endothelium leads to initiation of the extrinsic blood coagulation pathway. Another important feature of endothelial cells is their ability to direct cells of the immune system to specific sites in the body. Constitutively expressed or cytokine-inducible cellular adhesion molecules [e.g., E-selectin and intercellular adhesion molecule-1 (ICAM-1)] and soluble factors such as chemoattractants, cytokines, and chemokines act in concert to recruit the immune cells to lymphoid organs or inflammatory sites [7].

Molecular Control of Angiogenesis: In vasculogenesis during embryonic development, new endothelial cells differentiate from stem cells. In contrast, in angiogenesis new blood vessels mainly emerge from pre-existing ones [8]. In adult life, physiologic stimuli during wound healing and the reproductive cycle in women lead to angiogenesis, whereas vasculogenesis is absent. Pathologic conditions such as tumor growth, rheumatoid arthritis, and diabetic retinopathy are characterized by abundant angiogenesis. The active vascular remodelling phase in tumors, e.g., is reflected by the fact that tumor endothelial cells proliferate 20 to 2000 times faster than normal tissue endothelium in the adult [9]. (Figure-2)

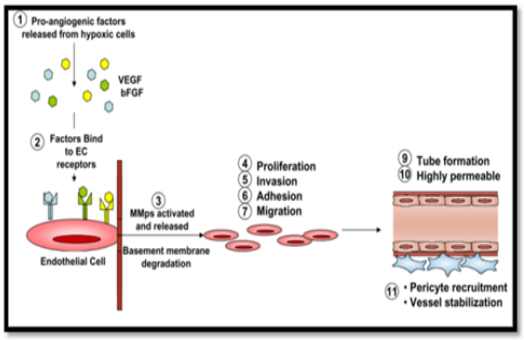


Fig-2- Mechanism of Angiogenesis

1. Initiation of the Angiogenic Response
2. Endothelial Cell Migration and Proliferation
3. Maturation of the Neovasculature
4. Other Mechanisms Implicated in Angiogenesis Control

Hypoxia is an important environmental factor that leads to neovascularization. In the case of tumor growth, however, cancer-causing genetic changes, possibly in conjunction with environmental influences, are able to induce angiogenesis as well [10]. Many oncogenes, among which c-myb, sis, and src, were shown to stimulate the expression of a wide variety of molecules that induce angiogenesis. Furthermore, mutant ras oncogenes strongly up-regulated the proangiogenic factors TGF-, TGF-, and VEGF. Activated oncogenes can also indirectly contribute to the angiogenic phenotype by affecting the production and activation of BM and ECM-degrading enzymes [11]. Tumor suppressor genes have now also been identified to play a role in angiogenic activities of cells. Inactivation of p53, for example, down-regulated the antiangiogenic ECM component thrombospondin [12]. In nonsmall cell lung cancer, loss of p53 was associated with a high-vascular maturation index. Besides the involvement of tumor cell-associated changes in p53, this tumor suppressor gene also plays a role in endothelial cell-mediated control of angiogenesis. Adenovirus-mediated overexpression of endothelial p53 inhibited human umbilical vein endothelial cells (HUVEC) proliferation and capillary network formation in vitro [13]. In endothelial cells existing in atherosclerotic and normal human aorta, variations in p53 expression levels could be detected. Moreover, the multinucleated variant endothelial cells expressed a mutant p53 type, which may be indicative for loss of endothelial cell growth control [14]. As with tumor cells, it is most likely that in vivo a combination of mutations in various tumor suppressor genes and oncogenes leads to a proliferative proangiogenic character of the endothelial cells. (Figure-3)

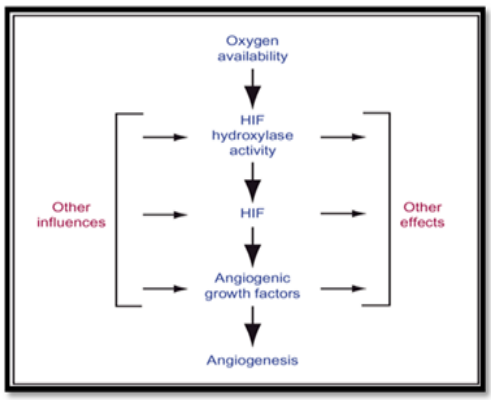


Fig-3- Effect of Hypoxia on Angiogenesis

Past and Present- What follows are 10 of the most significant reasons (with no particular significance attached to their order) for the explosive growth in angiogenesis research development of anti-angiogenic drugs.

1. The discovery of basic fibroblast growth factor (bFGF) as the first pro-angiogenic molecule: - Despite the logic of Folkman's ideas sceptics awaited the precise identification of the hypothetical TAF molecule. This did not occur until the mid-1980s, when Shing et al. [15] reported that basic fibroblast growth factor (bFGF, also known as FGF-2) isolated from a chondrosarcoma could function as a tumor-derived capillary growth factor and stimulate angiogenesis in various models [16]. However, a puzzling feature about bFGF is that it lacks a signal sequence for secretion and generally remains intracellular, at least in cell culture. This cast some doubt on its authenticity as a major inducer of tumor angiogenesis. Nevertheless, the discovery provoked new interest in the field, and there is no doubt that bFGF is recognized as a potent inducer of angiogenesis, including in the clinic for non-cancerous diseases such as hind limb ischemia and ischemic heart disease [17,18]. (Figure-4)

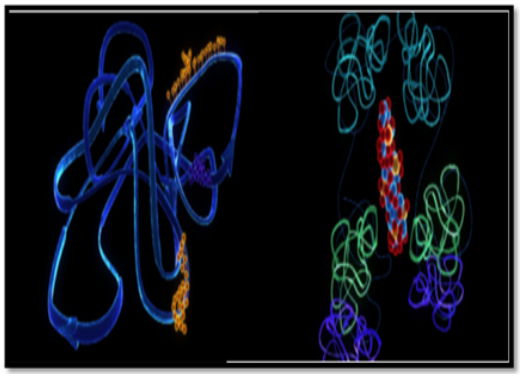


Fig-4- Three dimensional structures of Fibroblast Factor-1 (FGF-1) and FGF-1 receptor (FGFR)

2. The discovery of vascular endothelial growth factor (VEGF), and VEGF receptor tyrosine kinases on activated endothelial cells: - VEGF was initially termed vascular permeability factor (VPF), and still retains this term [19]. Its first function (vascular permeability) was discovered by Dvorak and colleagues [20] and was first molecularly defined by Ferrara et al [21]. There are a number of families (VEGF, VEGF-B, VEGF-C, VEGF-D) of which VEGF (or VEGF-A as it is sometimes called) is the most important; there are several VEGF isoforms and a number (e.g.VEGF121 and VEGF165) are readily secreted. It also functions as a potent pro-survival (anti-apoptotic) factor for endothelial cells in newly formed vessels [22,23] and indeed this may be one of its most significant functions. VEGF is expressed by the vast majority of cancers, often at elevated levels, and blocking its activity, e.g. by specific neutralizing antibodies to VEGF or to VEGF receptors expressed by 'activated' endothelial cells, can inhibit experimental tissue growth *In-vivo*, but not *In-vitro*. This is because of the highly elevated and restricted expression of two receptor tyrosine kinases, called flk-1/KDR (also known as VEGF-receptor-2) and flt-1 (also known as VEGF receptor-1), by the endothelial cells of newly formed blood vessels, which bind VEGF with high affinity. Another significant feature of VEGF is that levels of this growth factor in tumor cells can be significantly enhanced by hypoxia. This can occur through both transcriptional and post-transcriptional mechanisms, e.g. mRNA stabilization [24]. (Figure-5)

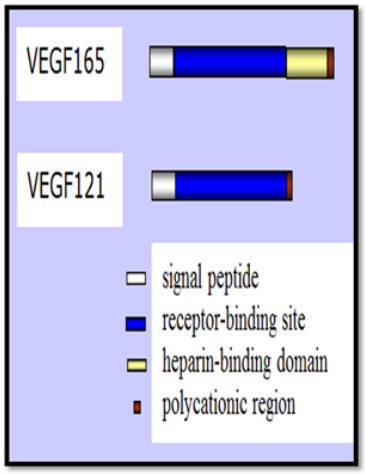


Fig-5- Structure of VEGF Receptors

3. The discovery of the angiopoietins and their tyrosine kinase receptors: - A second family of ligands and receptors specific for vascular endothelial cells have been uncovered more recently [25]. In this case, it was the receptors that were discovered first, i.e. tie-2/tek and tie-1 [26]. They remained as orphan receptors until 1996, when the ligands for one of them (tie-2), called angiopoietin-1 and angiopoietin-2 (ang-1 and ang-2), were discovered by Yancopolous and colleagues [27]. Of considerable interest is the fact that although both ang-1 and ang-2 behaves in a contrary manner. Indeed, ang-2 can cause regression of newly formed vessels by endothelial cell apoptosis, unless VEGF is present, in which case the two collaborate to promote angiogenesis. The ligand for tie1 remains unknown. Similar to VEGF and its receptors, the use of transgenic and gene knockout mice has been a powerful methodology to

uncover the functional significance of the angiopoietin–angiopoietin receptor systems in both embryonic vasculogenesis and angiogenesis [28].

4. The discovery of endogenous inhibitors of angiogenesis: - A large growing and structurally diverse family of endogenous protein adhesion molecules such as E-selectin, endoglin, inhibitors of angiogenesis has been discovered, e.g. thrombo-spondin-1, interferonα/β, the16kDa fragment of prolactin, endostatin, vascular endothelial cell growth inhibitor (VEGI), angiostatin, vasostatin, Meth-1 and Meth-2 and cleavage products of platelet factor 4 (63), or anti-thrombin III (64), among many others. Some of these are internal fragments of various proteins which normally lack any anti-angiogenic activity e.g. angiostatin is one or more fragment(s) of plasminogen and endostatin is a fragment of type XVIII collagen. Many of the precursor proteins are components of the extracellular matrix (ECM)/basement membranes (e.g. type XVIII collagen and thrombospondin) or members of the clotting/fibrinolytic pathways (e.g. plasminogen and anti-thrombin III). It is now thought that the angiogenic switch is triggered as a result of a shift in the balance of stimulators to inhibitors [29-31].(Table-1)

Table-1: - Some endogenous inhibitors of angiogenesis

Name	Description
Thrombospondin-1 and internal fragments of thrombospondin-1	Thrombospondin is a 180 kDa, large, modular extracellular matrix protein
Angiostatin	A 38 kDa fragment of plasminogen involving either kringle domains 1–3, or smaller kringle 5 fragments
Endostatin	A 20 kDa zinc-binding fragment of type XVIII collagen
Vasostatin	An N-terminal fragment (amino acids 1–80) of calreticulin
Vascular endothelial growth factor inhibitor (VEGI)	A 174 amino acid protein with 20–30% homology to tumor necrosis factor superfamily
Fragment of platelet factor 4 (PP4)	An N-terminal fragment of PP4
Derivative of prolactin	16 kDa fragment of the hormone
Restin	NC10 domain of human collagen XV
Proliferin-related protein (PRP)	A protein related to the pro-angiogenic proliferin molecule
SPARC cleavage product	Fragments of secreted protein, acid and rich in cysteine
Osteopontin cleavage product	Thrombin-generated fragment containing an RGD sequence
Interferonα/β	Well known anti-viral proteins
Meth 1 and Meth 1	Proteins containing metalloprotease and thrombospondin domains, and disintegrin domains in NH2 termini
Angiopoietin-2	Antagonist of angiopoietin-1 which binds to tie-2 receptors
Anti-thrombin III fragment	A fragment missing C-terminal loop of anti-thrombin III (a member of the serpin family)

5. The discovery of additional molecular markers in newly formed blood vessels, especially integrins and cell adhesion molecules: - The discovery of VEGF receptors and their upregulation in newly formed blood vessels highlights the fact that indeed, there can be major phenotypic differences between mature, quiescent vessels, and their newly formed counterparts. Such differences are essential to avoiding unwanted toxicity to normal vessels when using anti-angiogenic drugs, and thus achieving a sufficient therapeutic index. A number of such differences are now known, and include a very significant such as αvβ3 or αvβ5. This was first reported by Cheresh and colleagues who exploited such differences using specific neutralizing antibodies or small molecule peptide antagonists to block angiogenesis, which occurs, at least in part, by induction of endothelial cell apoptosis. Other 'markers' that are upregulated in activated endothelial cells include adhesion molecules such as E-selectin, endoglin, glycoproteins such as 'prostate-specific' membrane antigen, the ED-B domain of fibronectin and various proteases [32]. Integrins are transmembrane proteins composed of an and subunit in over 20 different heterodimeric combinations. They bind to ECM proteins or cell surface ligands through short peptide sequences. Combinations of different integrins on cell surfaces allow cells to recognize and respond to a variety of different ECM proteins. They are able to transduce signals from within the cells to the outside as well as from the outside into the cell [33].

6. The development of quantitative assays for angiogenesis: - It is of course difficult to evaluate the function of angiogenic growth regulators in the absence of reliable assays. This was a particularly serious problem in the early days of angiogenesis research since it had not been possible to successfully grow vascular endothelial cells in long term culture until Folkman's group established the appropriate conditions in 1980, and used them to elucidate the functional/sequential steps in angiogenesis. Since then a number of semi-quantitative or quantitative assays have appeared that involve sprouting angiogenesis in tumor (cell) free systems, such as the corneal micropocket assay, the subcutaneous 'Matrigel plug' assay and assays involving growth of cut sections (slices) of blood vessels in three dimensional gels of an ECM-associated material such as collagen. The development of these systems has been a boost to the discovery of new stimulators and inhibitors of angiogenesis, especially the latter [34,34].

7. Recognition of the prognostic significance of angiogenesis: - Another major finding which attracted interest in the field was reported by Weidner et al. (85) who found that the greater the degree of angiogenesis detected in a primary tissue, the worse the prognosis. This established a direct relationship between metastasis and

angiogenesis. Tissue vascularity is measured by staining tissue sections with antibodies specific (or highly specific) for antigens expressed by vascular endothelial cells such as factor VIII (von Willibrand factor), CD-31 or CD-34 and then counting (under high power) the number of highlighted vessels in so-called vascular 'hotspots' i.e. localized areas where there are unusually high numbers of vessels, as detected first under lower power magnification. Aside from the prognostic implications of this work, it also served to highlight the extent to which tissue masses can become 'contaminated' by blood vessels. Detection of blood vessels in tissue sections has recently been modified by Benjamin et al., so that it is now possible to discriminate between newly formed immature vessels and those that are more established and mature. It is based on the use of antibodies toα-smooth muscle action (α-SMA), which appears to stain the latter type of vessel as a result of mature vessels attracting a 'coat' of periendothelial support cells, i.e. pericytes and smooth muscle (α-SMA-antigen-positive) cells. A change in the ratio of immature/mature vessels because of the relative vulnerability of the immature vessels to this, and most other, forms of anti-angiogenic therapy [36,37].

8. Lack of acquired resistance to direct-acting anti-angiogenic drugs: - It was first proposed in 1991 by Kerbel that anti-angiogenic therapy might bypass a major problem encountered in virtually all strategies used to treat cancer, especially chemotherapy and hormonal ablation/antagonist therapies, namely acquired drug resistance. The underlying rationale was that the cellular target of anti-angiogenic drugs is a normal, and hence genetically stable, host cell, i.e. the vascular endothelial cells which line newly formed blood vessels in tissues. Finally, with respect to the issue of drug resistance, anti-angiogenic therapies may also circumvent what may be a major mechanism of intrinsic drug resistance, namely insufficient drug penetration into the interior of a tissue mass due to high interstitial pressure gradients within tissues [38,39].

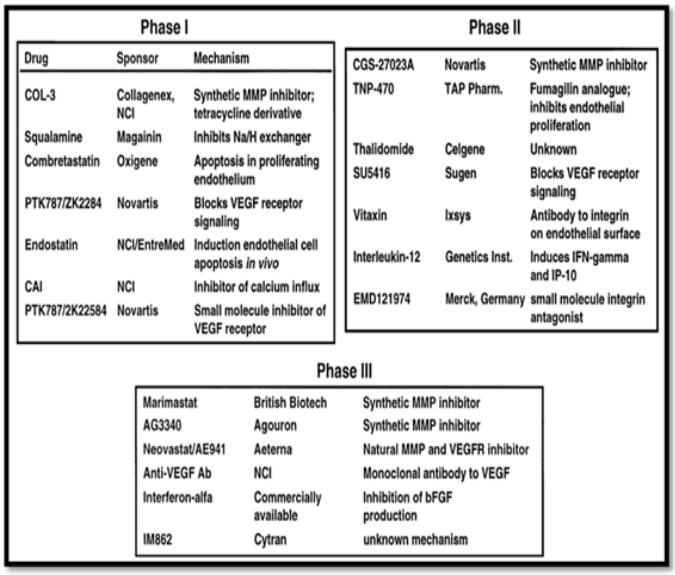
9. The discovery of the impact of angiogenesis on 'liquid' hematologic malignancies: - Another remarkable recent development, and one that is clearly counter-intuitive as well, is the recent realization that so-called 'liquid' hematologic malignancies are angiogenesis dependent. The discovery was based on findings such as elevated levels of pro-angiogenic growth factors, i.e. bFGF and VEGF, in the serum and urine of patients with acute lymphatic leukaemia and multiple myeloma. Similarly, a sharp increase in bone marrow angiogenesis, as measured by means of vessel density in vascular hot spots, has also been detected in such patients. This work has already resulted in the initiation of early phase I clinical trials to test putative anti-angiogenic drugs. If this holds up and is found to be a consequence of an anti-angiogenic effect it would provide major impetus in treating these excess angiogenic disorders [40,41].

10. The discovery of the 'accidental' anti-angiogenic effects of various conventional or new anti-cancer drugs and treatment strategies: - A number of discoveries led to the provocative conclusion that the use of anti-angiogenic drugs and therapies—in the clinic—is, in reality, probably not a new development. Denekamp and colleagues were the first to have the insight to point out these 'accidental' anti-vascular effects using various rodent tumor models and a diverse spectrum of anti-cancer agents ranging from cytokines/biological response modifiers and ionizing radiation to chemotherapeutic drugs and photodynamic therapy. More recently, much interest has been aroused by findings which relate to how withdrawal of androgens may cause regression of hormone-sensitive cancers, such as prostate cancer, at least in part through an anti-angiogenic mechanism. Finally, with respect to the effects of traditional/conventional therapeutics, mention should be made of the findings of Teicher and colleagues, where it has been shown preclinically that the combination of an anti-angiogenic drug (or drugs) (such as TNP-470) with a conventional cytotoxic agent, such as cisplatinum, taxol or cyclophosphamide, can significantly improve the anti-tumor efficacy of the cytotoxic drug [42].

The future: opportunities to be seized, problems to overcome

While this is an exciting time, there are a number of problems which could hamper progress in the field and overall enthusiasm for the concept of anti-angiogenic therapy. Tackling these problems presents some challenging opportunities; success in doing so would help foster a new era of cancer therapeutics in the clinic. Some of the problems and opportunities are discussed below.

1. The difficulties associated with clinical evaluation of anti-angiogenic drug efficacy: - The nature of most anti-angiogenic drugs makes it difficult to evaluate their potential efficacy in early phase clinical trials since, with few known exceptions [43]; they do not cause overt or rapid tumor regression, as can conventional cytotoxic agents. Indeed, a number of anti-angiogenic clinical trials currently in progress have been designed to compare the effects of a particular cytotoxic agent alone compared with this same agent in combination with an angiogenesis inhibitor. Another possible resolution to this problem could come with the increased use of improved 'anti-vascular targeting' strategies which can cause acute tumor regressions [44]. The use of various non-invasive medical imaging strategies (e.g. MRI, Doppler ultrasound) to monitor changes in tumor blood flow, vascular structure and permeability may be the most fruitful approach and, indeed, there are considerable research efforts (and some successes) underway in this area. (Figure-6)



Phase I

Drug	Sponsor	Mechanism
COL-3	Collagenex, NCI	Synthetic MMP inhibitor; tetracycline derivative
Squalamine	Magainin	Inhibits Na/H exchanger
Combretastatin	Oxigene	Apoptosis in proliferating endothelium
PTK787/ZK2284	Novartis	Blocks VEGF receptor signaling
Endostatin	NCI/EntreMed	Induction endothelial cell apoptosis *in vivo*
CAI	NCI	Inhibitor of calcium influx
PTK787/2K22584	Novartis	Small molecule inhibitor of VEGF receptor

Phase II

CGS-27023A	Novartis	Synthetic MMP inhibitor
TNP-470	TAP Pharm.	Fumagilin analogue; inhibits endothelial proliferation
Thalidomide	Celgene	Unknown
SU5416	Sugen	Blocks VEGF receptor signaling
Vitaxin	Ixsys	Antibody to integrin on endothelial surface
Interleukin-12	Genetics Inst.	Induces IFN-gamma and IP-10
EMD121974	Merck, Germany	small molecule integrin antagonist

Phase III

Marimastat	British Biotech	Synthetic MMP inhibitor
AG3340	Agouron	Synthetic MMP inhibitor
Neovastat/AE941	Aeterna	Natural MMP and VEGFR inhibitor
Anti-VEGF Ab	NCI	Monoclonal antibody to VEGF
Interferon-alfa	Commercially available	Inhibition of bFGF production
IM862	Cytran	unknown mechanism

Fig-6- Angiogenesis inhibitors in clinical trials

2. The prospect of delayed toxicity associated with long-term anti-angiogenic therapy: - Another obvious problem is that toxic effects associated with chronic anti-angiogenic therapy may not show up in short term early phase clinical trials or animal models, but rather, only after very protracted courses of therapy using these new drugs. The development of spastic diplegia in some infants or children who had been treated previously and successfully over 1 year with anti-angiogenic (interferonα2β) therapy for their life threatening hemangiomas is an example of such delayed toxic side effects. This undoubtedly will increase the need for very highly specific or completely specific targets expressed by blood vessels. The growing inter-relationship between the clotting and fibrinolytic pathways, and angiogenesis raises the possibility of bleeding/coagulation disorders in patients who receive certain anti-angiogenic drugs, as well as causing or exacerbating existing cardiovascular defects in older patients. Growth in neonates may also be compromised by angiogenesis inhibitor therapies. This possibility could turn out to be an important factor in selecting the optimal angiogenesis inhibitors for clinical development and their use in patients [45].

3. The need for better animal therapy models: - More emphasis should be placed upon evaluating anti-angiogenic therapies on metastases growing in such sites as the lungs, liver, brain and bone, especially since it cannot be assumed that an angiogenesis inhibitor that is effective in the particular organ site, e.g. the lungs, will be equally effective in other sites. Hence some anti-angiogenic drugs, depending on their target, may show large differences in anti-angiogenic efficacy as a function of organ site. In this regard, new and improved metastasis models are being developed, such as the use of firefly luciferase on green fluorescent protein-tagged cell lines which greatly enhance detection of microscopic and macroscopic metastases in many different organ sites [46]. There may be a dynamic equilibrium established between regression of 'established' vessels and the formation of new ones, a process which may favour a relatively constant and very high ratio of immature to mature vessels in spontaneous human tumors. These studies await confirmation but, if correct, could bode well for the clinical use of anti-angiogenic drugs which target newly formed immature vessels[47].

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] Carmeliet P, Ng Y S, Nuyens D, Theilmeier G, Brusselmans K, Cornelissen I, Ehler E, Kakkar V V, Stalmans I, Mattot V, Perriard J C, Dewerchin M, Flameng W, Nagy A, Lupu F, Moons L, Collen D, D'Amore P A and Shima D T (1999) Impaired myocardial angiogenesis and ischemic cardiomyopathy in mice lacking the vascular endothelial growth factor isoforms VEGF164 and VEGF188. Nat Med 5: 495-502.

[2] Ferrara N and Alitalo K (1999) Clinical Applications of angiogenic growth factors and their inhibitors. Nat Med 5: 1359-1364.

[3] Hanahan D and Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86: 353-364.

[4] Ingber D, Fujita T, Kishimoto S, Sudo K, Kanamaru T, Brem H and Folkman J (1990) Synthetic analogues of

fumagillin that inhibit angiogenesis and suppress tumour growth. Nature 348: 555-557.
[5] O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH and Folkman J (1994) Angiostatin: A novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 79: 315-328.
[6] Rajotte D, Arap W, Hagedorn M, Koivunen E, Pasqualini R and Ruoslahti E (1998) Molecular heterogeneity of the vascular endothelium revealed by in vivo phage display. J Clin Invest 102: 430-437.
[7] Carlos TM and Harlan JM (1994) Leukocyte-endothelial adhesion molecules. Blood 84: 2068-2101.
[8] Risau W (1997) Mechanisms of angiogenesis. Nature 386: 671-674.
[9] Denekamp J (1984). Vascular endothelium as the vulnerable element in tumours. Acta Radiol Oncol 23: 217-225.
[10] Rak J, Filmus J, Finkenzeller G, Grugel S, Marme D and Kerbel RS (1995) Oncogenes as inducers of tumor angiogenesis. Cancer Metastasis Rev 14: 263-277.
[11] Bouck N, Stellmach V and Hsu SC (1996). How tumors become angiogenic. Adv Cancer Res 69: 135-174.
[12] Dameron KM, Volpert O V, Tainsky MA and Bouck N (1994) The P53 tumor suppressor gene inhibits angiogenesis by stimulating the production of thrombospondin. Cold Spring Harbor Symp Quant Biol 59: 483-489.
[13] Riccioni T, Cirielli C, Wang X, Passaniti A and Capogrossi MC (1998) Adenovirus-mediated wild-type P53 overexpression inhibits endothelial cell differentiation in vitro and angiogenesis in vivo. Gene Ther 5: 747-754.
[14] Satoh T, Sasatomi E, Yamasaki F, Ishida H, Wu L and Tokunaga O (1998) Multinucleated variant endothelial cells (MVECs) of human aorta: Expression of tumor suppressor gene P53 and relationship to atherosclerosis and aging. Endothelium 6: 123-132.
[15] Shing,Y., Folkman,J., Sullivan,R., Butterfield,C., Murray,J. and Klagsbrun,M. (1984) Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. Science, 223: 1296–1299.
[16] Folkman J, and Klagsbrun M.(1987). Angiogenic factors. Science, 235, 442-447.
[17] Sellke,F.W., Laham,R.J., Edelman,E.R., Pearlman,J.D. and Simons,M. (1998) Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. Ann. Thorac. Surg., 65: 1540-1544.
[18] Schumacher,B., von Specht,B.U., Haberstroh,J. and Pecher,P. (1998) The stimulation of neo-angiogenesis in the ischemic heart by the human growth factor FGF. J. Cardiovasc. Surg., 39: 445–453.
[19] Veikkola T. and Alitalo K. (1999) VEGFs receptors and angiogenesis. Semin. Cancer Biol., 9: 211-220.
[20] Dvorak, H.F., Brown,L.F., Detmar,M. and Dvorak,A.M. (1995) Review: Vascular permeability factor/vascular endothelial growth factor, microvascular hyper-permeability and angiogenesis. Am. J. Pathol., 146: 1029–1039.
[21] Ferrara,N. and Davis-Smyth,T. (1997) The biology of vascular endothelial growth factor. Endocr. Rev., 18: 4–25.
[22] Benjamin,L.E. and Keshet,E. (1997) Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. Proc. Natl Acad. Sci. USA, 94: 8761–8766.
[23] Alon,T., Hemo,I., Itin,A., Pe'er,J., Stone,J. and Keshet,E. (1995) Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nature Med., 1: 1024–1028.
[24] Ikeda,E., Achen,M.G., Breier,G. and Risau,W. (1995) Hypoxia-induced transcriptional activation and increased mRNA stability of vascular endothelial growth factor in C6 glioma cells. J. Biol. Chem., 270: 19761–19766.
[25] Davis,S. and Yancopoulos,G.D. (1999) The angiopoietins: Yin and Yang in angiogenesis. Curr. Top. Microbiol. Immunol., 237: 173–185.
[26] Dumont, D.J., Yamaguchi, T.P., Conlon, R.A., Rossant, J. and Breitman, M.L. (1992) tek, a novel tyrosine kinase gene located on mouse chromosome 4, is expressed in endothelial cells and their presumptive precursors. Oncogene, 7, 1471–1480.
[27] Davis,S., Aldrich,T.H., Jones,P.F., Acheson,A., Compton, D.L., Vivek, J., Ryan, T., Bruno, J., Radjiewski, C., Maisonpierre,P.C. and Yancopoulos,G.D. (1996) Isolation of Angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell, 87: 1161–1169.
[28] Hanahan,D. (1997) Signaling vascular morphogenesis and maintenance. Science, 277: 48-50.
[29] Zhai,Y.,Ni,J.,Jiang,G.W.,Lu,J.,Xing,L.,Lincoln,C.,Carter,K. C.,Janat,F., Kozak,D., Xu,S., Rojas,L., Aggarwal,B.B., Ruben,S., Li,L.Y., Gentz,R. and Yu,G.L. (1999) VEGI, a novel cytokine of the tumor necrosis factor family, is an angiogenesis inhibitor that suppresses the growth of colon carcinomas in vivo. FASEB J., 13: 181–189.
[30] Sage,E.H. (1999) Pieces of eight: bioactive fragments of extracellular proteins as regulators of angiogenesis. Trends Biol. Sci., 7: 182.
[31] Gupta,S.K., Hassel,T. and Singh,J.P. (1995) A potent inhibitor of endothelial cell proliferation is generated by proteolytic cleavage of the chemokine platelet factor 4. Proc. Natl Acad. Sci. USA, 92: 7799–7803.
[32] Brooks,P.C., Montgomery,A.M.P., Rosenfeld,M., Reisfeld, R.A., Hu, T., Klier,G. and Cheresh,D.A. (1994) Integrin αvβ3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell, 79: 1157–1164.
[33] Stromblad,S. and Cheresh,D.A. (1996) Cell adhesion and angiogenesis. Trends Cell Biol., 6: 462.
[34] Passaniti,A., Taylor,R.M., Pili,R., Guo,Y., Long,P.V., Haney, J.A., Pauly,R.R., Grant,D.S. and Martin,G.R. (1992) Methods in laboratory investigation: a simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin and fibroblast growth factor. Lab. Invest., 67: 519.

[35] Parish, C.R., Freeman,C., Brown,K.J., Francis,D.J. and Cowden, W.B (1999) Identification of sulphated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. Cancer Res., 59: 3433–3441.

[36] Weidner,N., Semple,J.P., Welch,W.R. and Folkman,J. (1991) Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. N. Engl. J. Med., 324: 1–8.

[37] Rak,J.W., Hegmann,E.J., Lu,C. and Kerbel,R.S. (1994) Progressive loss of sensitivity to endothelium-derived growth inhibitors expressed by human melanoma cells during disease progression. J. Cell. Physiol., 159: 245–255.

[38] Kerbel,R.S. (1991) Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anti-cancer therapeutic agents. BioEssays, 13: 31–36.

[39] Boehm,T., Folkman,J., Browder,T. and O'Reilly,M.S. (1997) Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature, 390: 404–407.

[40] Perez-Atayde,A.R., Sallan,S.E., Tedrow,U., Connors,S., Allred, E. and Folkman,J. (1997) Spectrum of tumor angiogenesis in the bone marrow children with acute lymphoblastic leukemia. Am. J. Pathol., 150: 815-820.

[41] Vacca,A., Ribatti,D., Ruco,L., Giacchetta,F., Nico,B., Quondamatteo,F., Ria, R., Iurlaro,M. and Dammacco,F. (1999) Angiogenesis extent and macrophage density increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. Br. J. Cancer, 79: 965–970.

[42] Gerber,H.P., McMurtrey,A., Kowalski,J., Yan,M., Keyt, B.A., Dixit, V. and Ferrara,N. (1998) Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. J. Biol. Chem., 273: 30336–30343.

[43] Offodile,R.,Walton,T.,Lee,M.,Stiles,A.andNguyen,M.1999. Regression of metastatic breast cancer in a patient treated with the anti-angiogenic drug TNP-470.Tumori,85: 51–53.

[44] Gradishar,W.J. (1999) Endpoints for determination of efficacy of anti angiogenic agents in clinical trials. In Teicher, B.A. (ed.) Antiangiogenic Agents in Cancer Therapy, Human Press Inc. Totowa, NJ: pp. 341-353.

[45] Auerbach,R., Alby,L., Morrissey,L.W., Tu,M. and Joseph, J. (1985) Expression of organ-specific antigens on capillary endothelial cells. Microvas.Res., 29: 401–411.

[46] Bergers,G., Javaherian,K., Lo,K.M., Folkman,J. and Hanahan, D. (1999) Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. Science, 284: 808–812.

[47] Kong,H.L. and Crystal,R.G. (1998) Gene therapy strategies for tumor antiangiogenesis. J. Natl Cancer Inst., 90:273–286.