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Review paper

Inhibitors of angiogenesis in a clinical perspective

Emile E Voest
Department of Internal Medicine and Medical Oncology, Laboratory of Angiogenesis Research, University Hospital Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands. Tel: (+31) 30 2506265; Fax: (+31) 30 2523741.

Inhibition of angiogenesis or preventing the outgrowth of new blood vessels towards a tumor is one of the most promising areas of anticancer drug development. Several angiogenesis inhibitors are now on the verge of being tested in clinical trials. This review discusses possible applications of inhibitors of angiogenesis and whether the current methodologies for testing novel anticancer drugs are appropriate to evaluate the efficacy of inhibitors of angiogenesis.

Key words: Angiogenesis, applications, cancer, clinical testing.

Introduction

In the last decade enormous progress has been made in understanding the development of cancer. It is now recognized that tumor development is a multistep process which may take years, if ever, to become clinically relevant.[1] One of the most promising approaches to influence the tumor–host interaction is the inhibition of angiogenesis.[2,3] Angiogenesis is an absolute requirement for tumors to grow beyond the maximal size (1–2 mm) which can be reached when the tumor solely depends on diffusion for an adequate supply of oxygen and nutrients. In other words, every tumor, regardless of the tissue it originates from or of the genetic changes it has undergone, needs angiogenesis to grow. The concept that tumor growth could be controlled by depriving the tumor of vascularization was originally proposed by Folkman in 1971.[4] In the past quarter century the induction of angiogenesis by tumors was found to be a carefully controlled process by positive and negative regulators.[5–7] The contribution of endothelial cells to the growth of tumors is underlined by several recent observations. Firstly, the proliferation rate of avascular dormant metastases was found to be similar to that of a rapidly growing primary tumor.[8] The reason that the metastases remained dormant was a high apoptotic index of tumor cells which balanced proliferation. The ingrowth of capillaries into the avascular dormant tumor tilted the balance between proliferation and apoptosis towards proliferation with as the net end result a rapidly growing tumor. Secondly, tumor vascularization creates an access to the circulation and may lead to metastases.[9–11] Because most cancer patients are killed by metastases of the primary tumor, depriving the tumor of blood vessels may effectively block the hematogenous spreading of tumor cells. Thirdly, endothelial cells may have a paracrine effect on tumor cells. Endothelial cells have been shown to stimulate growth in the absence of blood flow.[12,13] Inhibition of angiogenesis prevents these events from occurring. The variety of stimulatory factors produced by different tumors may present a problem to effectively suppress endothelial migration or proliferation. Antiangiogenic drugs which cause complete anergy of endothelium will probably be the most effective compounds.

An important question in antiangiogenic therapy is whether it can induce regression of tumors. Because of the cytostatic rather than cytotoxic character of antiangiogenic therapy, mature vessels are not affected. It is therefore unlikely that inhibition of angiogenesis will lead to complete regression of the tumor because the mature tumor vessels are not affected. A significant problem is how to test these drugs in clinical studies. Normally, new drugs are tested in patients who are heavily pretreated with conventional chemotherapy and have large residual tumor masses. Based on the efficacy and toxicity profile in phase I/II clinical studies, new drugs are either discarded or further tested in phase III studies. This conventional line of testing poses a significant problem to angiogenesis inhibitors because it is unlikely that this type of drug will have an

effect in those patients. Cancer patients entered in phase II trials often have bulky disease. A large tumor burden may produce large quantities of angiogenic factors which locally may overwhelm the angiogenesis inhibitor under investigation. To evaluate different types of angiogenesis inhibitors, it may be useful to divide them into two categories: class 1 (specific and semi-specific inhibitors) and class 2 (non-specific inhibitors). Class 1 drugs are defined as compounds which inhibit proliferation and/or migration of endothelial cells without a substantial effect on non-endothelial cells. Examples of class 1 inhibitors are described in this review in detail. Class 2 drugs are defined as compounds with a substantial effect both on endothelial and tumor cells. The concept of angiogenesis inhibition has encouraged testing of large numbers of conventional anticancer drugs for a potential additional effect on the tumor vasculature. Several of these drugs were found to have an additional inhibitory effect on angiogenesis. From a conventional treatment point of view (eradication of all tumor cells) this has a definite advantage: the vascular supply of the tumor is attacked in addition to the cytoxic effects on the tumor compartment. However, because these drugs affect a variety of different cell types they can be expected to cause more side effects then drugs specifically affecting endothelial cells. This may limit the use of class 2 drugs in long-term treatment regimens. Examples of class 2 drugs are taxol and interleukin-12.[14,15]

It is important to know the mechanism of action of the different angiogenesis inhibitors to be able to interpret the outcome of phase I/II studies with these drugs. Class 2 drugs are more likely to induce a remission of established tumors then class 1 drugs. However, the absence of tumor remissions induced by class 1 drugs in conventional phase I/II studies does not exclude significant antitumor activity of these drugs when administered to patients in an adjuvant setting.

Clinical applications of antiangiogenic therapy

How should inhibitors of angiogenesis be used in cancer treatment? Effective suppression of angiogenesis may keep tumors dormant and prevent clinically relevant tumor development or recurrence. However, because tumor cells themselves are not affected by inhibition of angiogenesis, this implies a continuous and life-long treatment with drugs that suppresses neovascularization. Inhibitors of angiogenesis may be used as an adjuvant to conventional treatment modalities. Patients who are surgically cured but at risk for tumor recurrence (e.g. patients with node-positive breast cancer or Dukes C colorectal cancer) may benefit from an antiangiogenic treatment regimen. Likewise, patients who are in complete remission after chemotherapy but are at risk for tumor recurrence (e.g. patients treated for small cell lung cancer) may be given maintenance treatment to suppress angiogenesis (Table 1). Clinical trials are underway to test angiogenesis inhibitors as adjuvant treatment to prolong tumor dormancy. A novel application could be the use of angiogenesis inhibitors as chemopreventive drugs. The term chemoprevention is not appropriate in this case because it is unlikely that inhibitors of angiogenesis prevent the transformation of cells into cancer cells. However, they may prevent tumors developing in subjects at risk for cancer from becoming clinically relevant. For example, patients with head and neck cancer are at risk for developing a second primary tumor in the lungs or head and neck area. Treatment with angiogenesis inhibitors may suppress the growth of this second primary tumor.

A recently reported novel application of angiogenesis inhibitors is the combination of these drugs with conventional anticancer treatment modalities. It is now recognized that besides inhibiting neovascularization, inhibitors of angiogenesis may also enhance the efficacy of other treatment modalities such as chemotherapy and immunotherapy.[15,16] The mechanism through which this synergism occurs remains to be elucidated and warrants further study.

In the next decade additional ways to modulate the endothelial compartment of tumors will undoubtedly make a contribution to the treatment of cancer patients. An interesting approach is the restoration of tumor endothelial cell adhesion molecule expression, which is downregulated on tumor

Table 1. Applications of antiangiogenic therapy

Short-term treatment
(Class 1 and 2 angiogenesis inhibitors)
Treatment of the primary tumor
(a) Combination with:
Chemotherapy
Radiotherapy
Immunotherapy
(b) Multi-drug antiangiogenic therapy
Long-term treatment (class 1 angiogenesis inhibitors)
Adjuvant treatment
Maintenance treatment after tumor remission
Chemoprevention

endothelium, by blocking the effects of angiogenic molecules. This may enhance the effects of the immunotherapy directed against the tumor.[17] Other examples of novel approaches directed against the tumor vasculature are antiangiogenic gene therapy[18] and the combination of antiangiogenic drugs with hypoxic activated drugs.[19]

The remainder of this overview discusses some of the most promising class 1 antiangiogenic drugs which are currently tested in clinical trials or will be tested in the near future.

Vascular endothelial growth factor (VEGF) antagonists

VEGF is an endothelial cell specific mitogen which activates specific receptors which are only present on endothelial cells.[11] VEGF is considered the most important angiogenic factor. A dominant-negative VEGF receptor (flk-1) was found to inhibit tumor growth in animal models.[18,20] A similar inhibitory effect on tumor growth was seen when antibodies against VEGF were used.[21,22] A possible limitation of these drugs is the dependence on vascular endothelial growth factor as the single endothelial growth factor. As mentioned earlier, a large number of endothelial cell mitogens have been reported to be produced by tumor cells. Blocking of the effects of VEGF may be insufficient to prevent all neovascularization because the tumor may utilize other angiogenic factors to induce angiogenesis. This potential problem could be overcome by combination therapy with other inhibitors of angiogenesis. VEGF antagonists are on the verge of being tested in phase I studies.

Angiostatin

Angiostatin is a fragment of plasminogen which was purified from the urine of mice bearing Lewis lung carcinoma.[23] The presence of the primary tumor induced the production of angiostatin which was found to suppress angiogenesis in distant lung metastases. Angiostatin inhibited the proliferation of endothelial cells *in vitro*, but had no effect on the proliferation of a panel of tumor cell lines. Long-term daily treatment of mice with purified angiostatin could suppress growth and even cause regression of a large panel of tumors without causing apparent side effects. A distinct advantage of angiostatin over other inhibitors is the relatively slow clearance from the circulation.[23] This will, in the future, enable treating cancer patients with daily injections to create a continuous systemic antiangiogenic environment. The experience with angiostatin has provided proof of principle that inhibition of angiogenesis may prolong tumor dormancy. The mechanism of action of angiostatin is presently unclear. Angiostatin is not yet available for clinical studies.

Platelet factor 4

Platelet factor 4 was originally purified from the α-granules of platelets.[24] Functional studies on the protein revealed that platelet factor 4 has an effect on endothelial cell migration and proliferation *in vitro* and inhibited angiogenesis *in vivo*.[16] The mechanism through which platelet factor 4 exerts its effect may be through blocking the mitogenic response of endothelial cells to basic fibroblast growth factor. Subsequent studies showed that tumor growth was inhibited in mice treated with platelet factor 4.[25] Platelet factor 4 is currently being tested in phase I clinical trials. The short half-life of platelet factor 4 may present a significant problem.[24] Suppression of angiogenesis should preferably be continuous and periods without inhibition may result in the attraction of blood vessels by the tumors.

$\alpha_v\beta_3$-antagonists

Proliferating endothelial cells express a specific integrin on their cell mebrane: $\alpha_v\beta_3$-integrin.[26] Antibodies directed against this integrin were found to be antiangiogenic in several *in vitro* and *in vivo* models of angiogenesis.[27–29] Antibodies against related integrins had no antiangiogenic effect. A unique property of $\alpha_v\beta_3$-antagonists is their ability to induce apoptosis in proliferating endothelial cells.[28] This leads to disrupted blood vessels and results in tumor regression. $\alpha_v\beta_3$-antagonists are not yet available for clinical testing.

TNP-470

TNP-470, formerly called AGM-1470, is a synthetic analog of fumagillin. Fumagillin is a molecule derived from *Aspergillus fumigatus* which, by serendipity, was found to have potent effects on endothelial cell proliferation.[30] In animal models TNP-470 suppresses angiogenesis and tumor growth.[31,32] This drug is now tested in phase I and II clinical trials in

the US. Recently it was found that TNP-470 stimulates B cell proliferation.[33] The clinical relevance of this observation has to be further studied.

Metalloproteinase inhibitors

In order to migrate, cells have to degrade the extracellular matrix. Both endothelial cells and tumor cells are equiped with several matrix metalloproteinases. Because migration is a crucial step in the development of new vessels and metastasis, drugs were designed to inhibit metalloproteinases.[34] Treatment of tumor-bearing mice with the metalloproteinase inhibitor batimastat resulted in effective inhibition of angiogenesis and tumor growth.[35,36] This suppression could be achieved at very low concentrations of these drugs. Batimastat and marimastat, an analog which can be administered orally, are now tested in phase I and II clinical trials. Oral administration of angiogenesis inhibitors is a definite advantage if maintenance treatment is indicated. Although animal studies are promising, the long-term effect of continuous suppression of remodeling of the extracellular matrix has to be awaited.

Conclusion

Modulation of angiogenesis may be considered as one of the most promising new anticancer strategies. Several drugs with potent antiangiogenic activity in animal models are being or on the verge of being tested in clinical trials. The toxicity profile of each antiangiogenic drug will be established in phase I trials. The side effects will largely determine whether these drugs may be given as maintenance treatment to prolong tumor dormancy in patients at risk for tumor recurrence by inhibiting angiogenesis or in combination with conventional anticancer modalities to enhance treatment efficacy in patients with established tumors.

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