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Vascular targeted therapies in oncology

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Abstract

Neovascularization is intimately involved in tumor survival, progression, and spread, factors known to contribute significantly to treatment failures. Thus, strategies targeting the tumor blood vessel support network may offer not only unique therapeutic opportunities in their own right, but also novel means of enhancing the efficacies of conventional anticancer treatments. This article reviews one such therapeutic approach directed at the tumor blood vessel support network. Vascular disrupting therapies seek the destruction of the established neovasculature of actively growing tumors. The goal of these therapies is to cause a rapid and catastrophic shutdown in the vascular function of the tumor in order to arrest the blood flow and produce tumor cell death as a result of oxygen and nutrient deprivation and the build up of waste products.

Keywords

Tumor vasculature; Vascular disrupting agents; Small-molecule vascular disrupting agents;
Conventional anticancer therapies; Combined modality; Treatments

Introduction

Conventional anticancer therapies aim to maximize tumor destruction while maintaining acceptable levels of normal tissue side-effects. However, most such therapies have poor selectivity and a low therapeutic ratio. Thus, a major pharmacological goal remains the development of highly selective non-toxic therapies for the treatment of cancer. Efforts aimed at improving treatment outcomes have traditionally been directed at developing therapeutic strategies that seek to enhance neoplastic cell-killing by cytotoxic therapies or, to a lesser degree, to protect normal tissues from the collateral damage associated with the application of such agents. However, during the past few decades, another treatment approach has emerged that is currently receiving considerable attention. Rather than targeting the neoplastic cell population directly, this treatment strategy involves the impairment of the nutritional support of the tumor by targeting the tumor blood vessel network. Such vascular targeting approaches are based on the recognition that a continuously expanding vasculature is an essential requirement for tumor initiation, progression, and metastasis.

As is generally well accepted, most tumors remain dormant and fail to develop beyond a few millimeters in size in the absence of angiogenic growth (Ausprunk and Folkman 1977; Folkman 1986). The process involved, viz., neovascularization, is relatively uncommon in most normal

tissues but is an essential feature of solid tumors (Folkman 2002). Indeed, continued tumor growth is widely considered to be dependent on nutrient supply from a network of microvessels that may originate from angiogenesis, vasculogenesis, vessel intussusception, vascular mimicry, or any combination thereof (Hendrix et al. 2003; Streubel et al. 2004). However, neovascularization invariably lags behind the aggressively expanding tumor mass (Tannock 1970) resulting in a tumor vasculature that is morphologically and functionally abnormal and that differs greatly from the vascular network found in most normal adult tissues (Konerding et al. 1995; Konerding et al. 2002). Tumor vasculature is primitive in nature, highly abnormal, and chaotic. Given its pivotal role in tumor survival, progression, and spread, all of which are known to contribute significantly to treatment failures, agents capable of targeting tumor blood vessels have been actively pursued (Arap et al. 1998; Ruoslahti 2002; Ellis et al. 2001; Kerbel 2000; Siemann and Horsman 2008).

Vascular targeting agents

The field of vascular directed therapies has expanded rapidly, and a large number of investigational drugs have begun to undergo clinical evaluation. Not only are these agents distinct from conventional anticancer treatments, but the application of vascular targeting strategies as adjuvants to standard therapeutic modalities may offer unique opportunities to develop even more effective cancer therapies.

Vascular targeting therapies fall into two general categories based on whether they interfere with new blood vessel development or damage the established tumor vasculature (Thorpe 2004; Ellis et al. 2001; Bloemendal et al. 1999; Siemann et al. 2004). Angiogenesis inhibitors (AIs) seek to inhibit the tumor-initiated angiogenic process by interrupting essential aspects of angiogenesis, most notably signaling between the tumor and endothelial and stromal cells, and of endothelial cell function in order to prevent new blood vessel formation. Antiangiogenic therapies are the subject of another review of this Special Issue on endothelial cell biology and pathology and will therefore not be discussed further in the present article. An alternative approach involves the application of therapeutics seeking the preferential destruction of the established tumor vessel network. These vascular disrupting agents (VDAs) cause direct damage to the previously established tumor endothelium resulting in a rapid and selective vascular shutdown and secondary tumor cell death caused by ischemia (Chaplin and Dougherty 1999; Siemann et al. 2004; Thorpe 2004; Chaplin et al. 2006; Siemann and Horsman 2008). They comprise two main classes: ligand-based therapies, which deliver toxins, procoagulant, or pro-apoptotic effectors to disease-associated vessels, and small-molecule VDAs, which do not specifically localize to such vessels but exploit the known differences between them to induce selective vascular dysfunction.

The ligand-based therapies include (1) biological response modifiers or cytokines such as tumor necrosis factor (TNF) and interleukins, (2) certain established chemotherapeutic drugs such as vinka alkaloids and arsenic trioxide, and (3) a variety of strategies that use either antibodies, peptides, or growth factors that can selectively bind to tumor vessels (Chaplin and Dougherty 1999; Siemann et al. 2004; Thorpe 2004; Siemann 2004; Chaplin et al. 2006). Gene therapy approaches utilizing endothelial cell-specific promoter elements and vectors with restricted cellular tropisms have been examined, and encouraging results have been reported (Chaplin and Dougherty 1999). A variety of approaches based on linking antibodies or peptides that recognize tumor-associated vasculature to toxins, pro-coagulant, and pro-apoptotic effector molecules that can induce endothelial cell damage have also been explored (Thorpe 2004; Siemann et al. 2004). Preclinical investigations employing such ligand-directed VDA targeting have shown not only the localization of the therapeutic moiety to tumor vessels but also the selective destruction of tumor vasculature (Thorpe 2004).

Small-molecule VDAs include the flavone acetic acid (FAA) and its derivative DMXAA. The main effect of these agents on endothelial cells is believed to involve the induction of cytokines, especially TNF- α , leading to the induction of extensive hemorrhagic necrosis in tumors as a result of vascular collapse (Baguley and Ching 2002). Tubulin-depolymerizing drugs, including CA4P, ZD6126, AVE8062, NPI2358, MN-029, and OXi4503, comprise a second group of small molecules shown to have vascular disrupting properties (Siemann et al. 2004; Chaplin et al. 2006). These agents selectively disrupt the cytoskeleton of proliferating endothelial cells (Galbraith et al. 2001a) and vascular endothelial-cadherin signaling (Vincent et al. 2005) resulting in endothelial cell-shape changes and detachment ultimately leading to vascular collapse and the induction of widespread tumor necrosis. Since they preferentially target dividing endothelial cells, this accounts for their tumor specificity. Both the flavonoids and the tubulin-depolymerizing small-molecule drugs have been shown to have potent anti-vascular and anti-tumor efficacy in a wide variety of preclinical models, and the lead agents are undergoing clinical evaluation (Chaplin et al. 2006).

VDAs: single agent antitumor effects

The in situ consequences of VDA exposure on tumor pathophysiology have been extensively studied and described (Siemann 2004; Tozer et al. 2005; Horsman and Siemann 2006; Salmon and Siemann 2006). Within minutes of treatment, tumor perfusion begins to be compromised. The suppression of functional blood vessels is dose-dependent and typically lasts for 24–48 h (Fig. 1, Fig. 2). The result is wide-spread tumor necrosis (Fig. 3) and loss of viable tumor cells. A striking characteristic of tumors that have undergone VDA therapy is that despite extensive necrosis, a viable rim of neoplastic cells survives at the periphery (Fig. 3; Grosios et al. 1999; Davis et al. 2002; Siemann et al. 2004; Tozer et al. 2005). These residual areas of tumor tissue, believed to survive VDA treatment because their nutritional support is derived from vasculature in the adjacent normal tissue (Siemann 2004), can act as a source of tumor regrowth. One consequence of the viable rim is that, typically, only repeated multiple-dose treatments with such agents impact tumor growth (Li et al. 2002; Blakey et al. 2002; Davis et al. 2002; Siemann and Shi 2004). Thus, in general, VDA treatment alone is highly unlikely to eradicate the tumor mass. Nevertheless, the destruction of large tumor areas, particularly in the central and typically most radiation- and chemotherapy-treatment-resistant regions of the tumors, is clearly highly beneficial and desirable. Consequently, such agents will probably find their greatest utility when applied in a combined modality setting.

Combining VDAs with conventional anticancer therapies

One approach to eliminating the tumor cells surviving VDA treatment is to combine it with standard anticancer therapies such as radiotherapy or chemotherapy. Cells comprising this viable rim of tumor tissue are likely to be in a state of high proliferation and excellent nutrition. These factors, coupled with their accessibility to systemically administered agents, make the surviving tumor cells susceptible to killing by radiation and anticancer drugs. Conversely, VDAs cause the destruction of large areas of the interior of tumors and show excellent activity against large bulky disease (Landuyt et al. 2001; Siemann and Shi 2003) that is typically resistant to conventional anticancer therapies.

The concept of combining VDAs with radiotherapy and chemotherapy has been extensively tested in preclinical settings by using a variety of tumor models and small-molecule targeting agents. We and others have extensively reviewed this topic (see Horsman and Siemann 2006), and results from numerous preclinical investigations in a large variety of model systems can be summarized as follows. In general, marked enhancements in antitumor activities were observed when VDAs and radiation or chemotherapy were combined. Typically, these enhanced responses were obtained by administering the VDA after treatment with conventional

anticancer agents (Fig. 4). The rationale for such a sequence was usually based on a desire to avoid any possible pharmacologic interference by the VDA on the action of the anticancer drug or the possibility of inducing radiation detrimental effects of tumor hypoxia. Indeed, several preclinical investigations that specifically studied the issue of timing and sequencing of agents concluded that VDA-chemotherapy/radiotherapy combinations may be less effective when the VDA is administered shortly prior to the conventional anticancer treatment. Importantly, in preclinical investigations of combinations of VDAs with anticancer drugs or radiotherapy, improved antitumor efficacy has been achieved in the absence of the concomitant enhancement of chemotherapeutic agent-specific side-effects (Siemann et al. 2002; Siemann and Rojiani 2002) or of the increased radiation response of normal tissues (Wilson et al. 1998; Murata et al. 2001a, b; Horsman et al. 2002) suggesting that the combination of VDAs and conventional anticancer treatments yields a therapeutic benefit.

Combining vascular targeting therapies

Vascular directed therapies have shown impressive antitumor effects in preclinical tumor models, and recent clinical observations are encouraging. Nevertheless, the achievement of tumor cures in patients with either AI or VDA monotherapy is likely to be extremely difficult. In the case of the former, the complexity of pathways available for neovascularization implies that disrupting only a single aspect of angiogenesis probably will not suffice (Ellis et al. 2000). VDAs, on the other hand, by the very nature of their highly selective mechanism of damaging tumor blood vessels, will not be able to eliminate those pockets of tumor cells whose nutritional supply is derived from blood vessels in the surrounding normal tissues (Siemann et al. 2004).

A logical extension of vascular targeting is the application of these therapies in concert in a manner not unlike the clinical norm of utilizing multi-agent chemotherapy, i.e., the development of a vascular targeted therapy that comprises several agents directed against different aspects of the tumor blood vessel network. Although several strategies are possible, the combined application of AIs and VDAs is likely to lead to complementary antitumor effects. AIs and VDAs differ not only in their mode of action, but also in their likely therapeutic application (Siemann et al. 2005). Briefly, the objective of antiangiogenic therapies is to interfere with new vessel formation thereby preventing tumor growth and limiting metastatic potential. Consequently, antiangiogenic therapies are typically administered chronically over months and years. VDAs compromise established tumor vasculature and have the potential to destroy tumor masses and to prevent progression. Such agents are designed to be used in an intermittent fashion rather than by means of long-term exposures. Since both the initiation of new vessel formation and the integrity of the existing blood vessel network are critical to the growth and survival of a tumor, such a double assault on the tumor vasculature would appear to be a highly logical approach.

This possibility has been examined experimentally in studies combining a selective inhibitor of VEGFR2 (vascular endothelial growth factor receptor 2)-associated tyrosine kinase with a microtubulin-disrupting VDA (Siemann and Shi 2004). The results showed not only that such a combination therapy could significantly enhance the tumor response beyond that achieved with either vascular targeting therapy alone, but also that, from a therapeutic perspective, the targeting of the tumor vasculature with AIs and VDAs was complementary and not redundant (Fig. 5). A likely explanation for these observations is that, whereas the VDA significantly reduced the viable tumor mass, the AI impaired subsequent tumor regrowth by interfering with the re-establishment of the tumor blood vessel network. Combinations of other agents seeking to exploit the approach of dual targeting of the tumor vasculature are currently under active investigation.

Clinical investigation with VDAs

A number of VDAs have progressed into clinical trial evaluations. These include DMXAA and several VDAs that target the inhibition of tubulin assembly (for a detailed reviewed, see Siemann and Chaplin 2007). Several key observations supporting VDA proof-of-concept have been achieved in man. Importantly, dynamic contrast-enhanced magnetic resonance imaging and positron emission tomography have been utilized to demonstrate that tumor blood flow reductions can readily be detected in tumors of patients at VDA doses well below the maximum tolerated dose. Although VDAs are generally well tolerated with different side-effect profiles to current oncologic therapies, cardiovascular effects such as transient hypertension and tachycardia were seen in most studies. Consequently, in light of this early clinical experience, the monitoring and controlling of hypertension and the exclusion of patients with a recent history of cardiovascular disease will probably be key elements in the phase II/III protocols with these agents, much as it has been with the antiangiogenic agent Avastin (bevacizumab). Lead VDAs have now advanced into Phase II studies in combination with conventional treatment modalities. Trials dealing with a variety of tumor sites including prostate, ovarian, and non-small cell lung cancer are under active study.

Future directions of VDA investigations

Early observations in patients treated with VDAs have been encouraging. Preclinical studies had suggested that markers of tumor vascular function could serve as important indicators of biologic activity for this class of agents. In patients, blood flow reductions within tumors as measured by magnetic resonance imaging or positron emission tomography have been observed with all VDAs tested (Dowlati et al. 2002; Galbraith et al. 2001b), and some hints of clinical activity have been noted (Dowlati et al. 2002). However, such blood flow measurements are impractical to use routinely on large patient populations, and there remains a need to develop other biomarkers of VDA activity. In the case of DMXAA, plasma levels of the serotonin metabolite 5-hydroxy indoleacetic acid have been used to monitor response to treatment, both in experimental animals and in the clinic (McKeage et al. 2006; Kestell et al. 2001). Recent studies have indicated that treatment of tumor-bearing animals with the tubulin-binding VDA OXi4503 leads to a rapid mobilization of circulating endothelial cell progenitors (Shaked et al. 2006). Although the exact mechanism leading to the mobilization of these cells is as yet unknown, it might be a response to induced vascular damage and could contribute to the revascularization process that occurs in the absence of additional treatments.

Concluding remarks

The appeal of vascular directed anticancer therapy lies not only in the recognition of the critical function of the vasculature in the growth and spread of a tumor, but also in the realization that by targeting a component distinct from that targeted by cytotoxic agents there exists great potential for complementary therapeutic activity. Rapid developments in recent years have now resulted in the identification of a variety of potential targets and a large number of investigational drugs.

In the case of VDAs, interest in tubulin-depolymerizing drugs, the most widely studied group of compounds, stems from evidence obtained many years ago that colchicine induces hemorrhagic necrosis in tumors. With several compounds in patient trials and others due to enter clinical evaluation, research efforts have focused on establishing preclinical data supporting the clinical advancement of these agents. In particular, the combination of VDAs with radiotherapy and anticancer drugs and the possibility of advancing the use of VDAs with emerging therapeutic strategies, including antiangiogenics, are being actively pursued, a concept now also beginning to be explored in the clinic. Whereas the advancement of VDAs into the treatment of patients is clearly encouraging, these trials have also raised additional

questions. We believe that preclinical studies can aid the resolution of these issues. Given their possible future clinical impact, the continued pursuit of agents exhibiting the ability to affect the growth and survival of tumors by targeting their endothelium is clearly warranted.

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Fig. 1.

Effect of VDA treatment on patent tumor blood vessels in KHT sarcomas. Tumor-bearing mice were treated with 100 mg/kg CA4P (a), 100 mg/kg ZD6126 (b), 100 mg/kg MN029 (c), or 25 mg/kg OXi4503 (d), and at various times thereafter, the numbers of functional blood vessels were determined by using the fluorescent dye Hoechst 33342. Results are the means $\pm$ SE of three to five tumors. From Siemann and Horsman 2008; with permission

Fig. 2. Tumor blood perfusion measured by using dynamic contrast-enhanced magnetic resonance imaging after treatment with a single dose of CA4P (100 mg/kg) or OXi4503 (25 mg/kg). Data points represent changes in tumor perfusion in individual mice as a function of time after treatment. Values given are the median group response as a function of time after treatment. From Salmon and Siemann 2006; with permission

Fig. 3. Histological assessment of KHT-sarcoma-bearing mice treated with ZD6126. **a** Hematoxylin and eosin staining of control KHT tumors showing little necrosis. **b** By 24 h after treatment with ZD6126 (150 mg/kg), extensive areas of necrosis are seen with viable tumor cells present only at the tumor periphery (*arrows*). **c, d** The viable rim (*arrows*) is more readily apparent at higher magnification. From Siemann 2002; with permission

Fig. 4. Survival of clonogens in KHT sarcomas 24 h after treatment with a range of doses of radiation given either alone (*open symbols*) or in combination with a 2.5-mg/kg dose of OXi4503 (*closed symbols*). In the combination treatment, OXi4503 was administered 1 h after radiotherapy. Data are the means $\pm$ SE of three to nine tumors. From Siemann and Horsman 2008; with permission

Fig. 5.

Response of KSY-1 xenografts in mice treated with ZD6474 (Zactima) and ZD6126 either alone or in combination. The antiangiogenic ZD6474 (25 mg/kg) was administered orally on a daily basis, starting on the day that the tumors reached a size of 200 mm³, for a period of 5 days. The VDA ZD6126 was given intraperitoneally on days (D) 1, 3 and 5 at a dose of 100 mg/kg. In the combination group, ZD6126 was given 1 h after ZD6474, on days when both agents were administered. The results show the growth of the median tumors of groups of eight mice. From Siemann and Shi 2004; with permission