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# Local anti-angiogenic brain tumor therapies

Eric P. Sipos[1,2,3] and Henry Brem[3,4,5]
[1]*Division of Neurosurgery, Walter Reed Army Medical Center, Washington D.C., USA;* [2]*Department of Surgery, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA;* [3]*Department of Neurosurgery;* [4]*Department of Oncology, and* [5]*Department of Ophthalmology, The Johns Hopkins Medical Institutions, Baltimore, Maryland, USA*

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**Summary**

The critical role of angiogenesis in the growth of solid tumors, including neoplasms of the central nervous system, has provided the impetus for research leading to the discovery of inhibitors of tumor neovascularization. The therapeutic potential of systemically administered antiangiogenic drugs for brain tumors, however, is limited by a variety of anatomic and physiologic barriers to drug delivery. Implantable controlled-release polymers for local drug administration directly into the tumor parenchyma have therefore been developed to achieve therapeutic concentrations of these drugs within the brain while minimizing systemic toxicity. With use of these polymers, successful antiangiogenic therapy for treatment of experimental intracranial malignancies has been achieved. This has been demonstrated with a variety of otherwise unrelated drugs – including the angiostatic steroids, tetracycline derivatives, and amiloride – which modulate collagenase activity, and thus, basement membrane and interstitial matrix metabolism. Controlled-release polymers provide a clinically practicable method of achieving sustained antiangiogenic therapy which can be readily used in combination with other treatment modalities such as cytoreductive surgery, radiation, and cytotoxic chemotherapy.

## Introduction

The growth of solid tumors in three dimensions is critically dependent upon the presence of a blood supply. During the initial, avascular phase of growth, tumors exist as small aggregates of neoplastic cells sustained by simple diffusion for delivery of nutrients and removal of cellular waste products [1]. Avascular tumors grow at a slow, linear pace until they reach the limits of diffusion and an equilibrium is achieved between the rate of cellular proliferation at the periphery and cell death at the center [2]. Within the anterior chamber of the rabbit eye, the upper limit of avascular tumor growth is 1–2 mm in diameter [3]. Similarly, the growth of tumor spheroids *in vitro* plateaus once aggregates of fewer than $10^6$ cells reach diameters less than 4 mm [2]. These avascular neoplasms are dormant from a growth perspective and have little metastatic potential; however, they may remain viable for prolonged periods.

Tumors produce diffusable angiogenic factors which activate endothelial cells in nearby established capillaries or venules and induce endothelial proliferation, migration, and new vessel formation through a series of sequential but partially overlapping steps [4–9]. Once vascularized, previously dormant tumors begin to grow rapidly and their volumes increase exponentially [1,2,10]. Mitotic activity within such tumors occurs predominantly wisthin cylindrical clusters around new microvessels [11]. For malignant neoplasms, it is during this phase of tumor growth that metastatic colonies are most commonly detected [4].

The potential therapeutic value of inhibition of tumor angiogenesis, first recognized nearly three decades ago [12], is also supported by a variety of indirect clinical and experimental evidence in neuro-oncology. The angiogenic capacity of brain tumor cells correlates with tumor malignancy [13] and a histologic grading system developed to quantify tumor angiogenesis predicts survival of patients with astrocytomas [14]. On the basis of

this system, the glioblastoma multiforme is among the most 'endothelial-rich' tumors studied [15]. Similarly, microvascular density correlates with tumor recurrence and patient mortality in children with brain tumors [16]. Other less infiltrative intracranial neoplasms such as meningiomas and hemangiopericytomas are also dependent upon angiogenesis for growth [17]. Cerebrospinal fluid (CSF) from brain tumor patients is up to 10 times as potent in inducing capillary endothelial cell migration than CSF from patients without tumors [18]. Furthermore, in a study of 26 pediatric patients with intracranial tumors, basic fibroblast growth factor, a potent angiogenic factor, was detected in the CSF of 62% of the children studied and this CSF was mitogenic for cultured endothelial cells [16]. Observations such as these provide the rationale for development of anti-angiogenic therapies for treatment of patients with brain tumors.

## Barriers to systemic drug delivery for intracranial neoplasms

The normal blood–brain barrier is disrupted in newly-formed tumor vessels. Altered expression of biochemical barrier proteins such as molecular transporters has been shown to correlate with the histologic grade of central nervous system tumors [19]. The permeability of newly induced capillary endothelial cells is also increased as a direct result of their location in the peritumoral environment of malignant glial neoplasms [20]. The contrast enhancement and cerebral edema seen clinically on computerized tomography or magnetic resonance imaging scans of patients with malignant gliomas provides a radiographic correlate of the disruption in the normal blood–brain barrier. Despite the permeability of the tumor microvasculature, however, a variety of structural and physiologic anomalies provide barriers to effective systemic drug delivery for treatment of brain tumor patients.

The microvasculature of malignant brain tumors such as the glioblastoma multiforme differs structurally and physiologically from normal cerebral capillaries in several important ways. Histologically, a normal brain capillary has one or two endothelial cells per lumen when viewed in cross-section. In malignant tumors, however, there may be dramatic endothelial hyperplasia with up to 10 endothelial cells per lumen [15]. These vessels are often dilated, saccular, and tortuous. The microvascular architecture often varies from one intratumoral location to another and abnormal branching patterns are common. Tumors may contain giant capillaries, arteriovenous shunts, or anastomotic channels between venules [21–23].

Because of the elevated interstitial pressures resulting from increased vascular permeability and the decreased intravascular pressures resulting from the anatomic peculiarities of the tumor microvasculature, many of the small tumor vessels are partially or totally collapsed [24]. These factors probably contribute to the diminished blood flow measured in brain tumor vessels compared to that in adjacent normal pial vessels [25] and may explain the areas of necrosis seen histologically and radiographically in glioblastoma multiforme. Furthermore, these observations predict that systemic administration of drugs for patients with malignant brain tumors would yield inhomogeneous and problematic penetration within the tumor and the surrounding brain.

High systemic drug levels are often required to achieve therapeutic concentrations within the central nervous system. Conventional strategies for improving systemic drug delivery to brain tumors have typically relied upon increased doses, frequency, or duration of drug administration. This approach is generally limited by systemic toxicity. Several alternative methods have therefore been developed in attempts to improve drug delivery to brain tumors [26]. These methods have included chemical modification of pharmacological agents to make them more lipophilic, and biochemical or osmotic modification of the blood–brain barrier. Alternative routes of drug delivery include intraarterial, intrathecal, intraventricular, and interstitial drug administration [27].

## Controlled-release polymers for interstitial drug delivery

The most direct method of bypassing the many barriers to drug delivery is to deliver drugs directly into the tumor parenchyma, thus maximizing drug concentrations within the tumor while minimizing systemic toxicity. Theoretically, with local delivery, interstitial pressure gradients and drug concentration gradients would favor redistribution from the center to the periphery of the tumor by a combination of diffusion and convection [28]. Additionally, local drug delivery is appealing for the treatment of malignant gliomas because these tumors usually recur locally and systemic metastases are rare [29]. Examples of local drug delivery strategies include implantable

catheters and implantable controlled-release polymer systems [26].

One of the first applications of controlled-release polymers for *in vivo* drug delivery was to provide a method for testing small quantities of putative inhibitors of angiogenesis in simple experimental models such as the rabbit cornea assay and the chick chorioallantoic membrane assay [30,31]. These models continue to serve as valuable tools in the search for new anti-angiogenic compounds; however, the role of drug delivery polymers has since been translated to therapeutic setting, providing a new method for local delivery of antiangiogenic agents directly within the substance of malignant brain tumors [32,33].

The biodegradable polyanhydride polymer, polybis(p-carboxyphenoxy)propane–sebacic acid (pCPP : SA) copolymer, undergoes slow but complete hydrolytic degradation in the presence of water, thus releasing biologically active drugs incorporated within the polymer matrix gradually over time [27]. Biocompatibility studies of p(CPP : SA) have demonstrated that the parent compound and its degradation products are noncytotoxic, nonmutagenic, and nonteratogenic [34]. Since clinical testing (including prospective, double-blind, placebo-controlled multicenter trials in North America and Europe) demonstrated its safety and efficacy [35–37], this drug delivery polymer has become widely used clinically for the local delivery of carmustine in patients with malignant gliomas. It is currently being studied in the laboratory for local delivery of a variety of antiangiogenic agents [38].

## Local anti-angiogenic therapy

The therapeutic potential of angiogenesis inhibition for treatment of tumors and other diseases has provided a strong impetus for research and has led to the discovery of many new antiangiogenic compounds which have been summarized elsewhere [38]. The antiangiogenic agents reviewed below have been effective when delivered locally using controlled-release polymers.

### *Cartilage-derived inhibitor*

Cartilage is a normally avascular tissue generally resistant to tumor invasion [39]. The vascularized embryonic precursor of cartilage loses its blood vessels in the early neonatal period [40,41]. Observations such as these led Brem and Folkman to hypothesize and demonstrate that cartilage produces a potent diffusible inhibitor of tumor angiogenesis [42]. Once isolated and purified, the cartilage extract was incorporated into the nonbiodegradable ethylene–vinyl acetate copolymer and was tested in the rabbit cornea angiogenesis assay [30]. This was the first reported use of a polymer for local delivery of an antiangiogenic compound. Subsequently, the purified cartilage-derived inhibitor was found to be a protein with potent anticollagenase properties and a high sequence homology to a collagenase inhibitor isolated from cultured human skin fibroblasts [43,44]. More recently, a potent and specific cartilage-derived angiogenesis inhibitor was isolated, cloned, purified, sequenced, and identified as troponin I, a subunit of the troponin complex [45].

### *Heparin and the angiostatic steroids*

The observation that cortisone administered in combination with heparin is antiangiogenic, causes tumor regression, and inhibits tumor metastases [46] led to the discovery of a new class of compounds referred to as the ‘angiostatic steroids’. The potency of the antiangiogenic effects of these steroids in the presence of heparin is independent of their relative mineralocorticoid or glucocorticoid activities [47]. Tetrahydrocortisol, for example, is the most potent of the known angiostatic steroids and is completely lacking in glucocorticoid or mineralocorticoid activity [48]. The mechanism of action for the angiostatic steroids appears related to modulation of collagen metabolism [49,50], induction of basement membrane dissolution [51], and promotion of plasminogen activator inhibitor synthesis which in turn reduces synthesis of plasminogen activator, an enzyme involved in remodeling the extracellular matrix and the capillary basement membrane [52,53].

The antitumor effects of angiostatic steroids in the presence of heparin have been confirmed in a series of *in vivo* studies. In a nude mouse model, orally administered heparin and hydrocortisone inhibited angiogensis and growth of human neurofibrosarcoma xenografts [54]. When incorporated into an implantable controlled-release polymer for local delivery, the combination of heparin and cortisone inhibited angiogenesis in the rabbit cornea assay and inhibited tumor growth in the rat 9L-gliosarcoma model [55]. This study established that local delivery of antiangiogenic agents using implantable biodegradable

polymers was technically feasible and effective. It also demonstrated that these polymers could be used to deliver a combination of drugs simultaneously.

*Tetracycline derivatives*

As the mechanisms of action for many of the seemingly unrelated inhibitors of angiogenesis become better understood, a recurrent theme involves collagenase inhibition and alteration of basement membrane and extracellular matrix metabolism, critical components of the series of events leading to tumor angiogenesis, cancer invasion, and metastasis. This observation stimulated interest in other commercially available drugs known to have similar effects and led to study of the antitumor effects of the tetracycline derivatives.

The tetracyclines are inhibitors of mitochondrial protein synthesis which have been used for many years as broad-spectrum antibiotics, and more recently have been found to inhibit tumor growth [56]. Although the antineoplastic activity of these drugs were initially attributed to inhibition of mitochondrial protein synthesis within tumor cells, the tetracyclines also inhibit extracellular matrix metalloproteinases [57–59]. Thus, the antitumor effect of the tetracyclines may result in part or entirely from modulation of collagen synthesis and consequently inhibition of angiogenesis.

Of the tetracycline derivatives in commercial use as antibiotics, minocycline is the most lipid-soluble and therefore has the best tissue-penetration [60,61]. It is also a potent inhibitor of collagenase [62] and a potent inhibitor of tumor angiogenesis in the rabbit cornea [63]. Minocycline inhibits the growth of early-passage endothelial cells at concentrations that have minimal effects on the growth of pericytes, smooth-muscle cells, and C6 glioma cells. Deoxyribonucleic acid and protein synthesis in endothelial cells are also selectively inhibited by minocyline. These effects on endothelial cell growth *in vitro* correlate with the anticollagenase potency of minocycline and other tetracycline derivatives rather than their antimicrobial activity [64]. Others have demonstrated that minocycline has minimal cytotoxic effects against tumor cells *in vitro* [65]. These studies lend further support to the hypothesis that the antineoplastic effects of minocycline are mediated by inhibition of angiogenesis.

When delivered systemically, minocycline prolongs survival of mice with subcutaneously implanted Lewis lung carcinoma [65–67]. In the intracranial 9L-gliosarcoma model, however, systemic administration of minocycline was ineffective. In this tumor model, local delivery of minocycline using controlled release polymers significantly improved survival [68].

Col-3 (6-dimethyl-6-doxy-4-dedimethylaminotetracycline, Metastat), is another non-antimicrobial tetracycline derivative with antineoplastic and antiangiogenic properties. In addition to its potent anticollagenase activity, col-3 is as effective as minocyline in inhibition of brain endothelial cell proliferation [69]. Col-3 is one of several newly developed synthetic matrix metalloproteinase inhibitors currently in clinical trials [70] and is now being studied in a New Approaches to Brain Tumor Therapy (NABTT) clinical consortium trial for patients with malignant gliomas [71]. Although oral or parenteral administration of col-3 may prove effective for extracranial malignancies, the barriers to effective drug delivery summarized above may warrant trials of local delivery for this drug.

*Amiloride*

Amiloride is another drug which was available commercially for many years before its antiangiogenic properties were recognized. Originally developed as a potassium-sparing diuretic, amiloride was subsequently found to inhibit urokinase-type plasminogen activator (u-PA) [72], a serine protease which has an important role in the proteolytic degradation of the extracellular matrix. In central nervous system gliomas, immunohistochemical staining for u-PA correlates with tumor grade [73,74] and shorter patient survival [74,75] and the genes for both u-PA and its receptor are upregulated [76]. Perhaps through its effects on u-PA, amiloride inhibits primary tumor growth [77] and metastasis [78] and has antiangiogenic effects [79,80]. When administered systemically, amiloride inhibits the growth of rat 9L-gliomas implanted subcutaneously but has no effect on the survival of rats with intracranial 9L-gliomas [81], as one might expect given the poor central nervous system penetration of this drug [82,83]. When treated with drug delivery polymers containing amiloride, however, the median survival of rats with intracranial 9L-gliomas was increased 50% [81].

## Future applications for antiangiogenic brain tumor therapy

As a treatment strategy for intracranial or extracranial neoplasms, successful antiangiogenic therapy will

provide tumoristatic rather than tumoricidal effects. Once the antiangiogenic effect is eliminated, tumor growth suppression might no longer be expected. Thus, long-term therapy could be required for any antiangiogenic drug. Recent experimental evidence, however, provides cause for greater optimism [84]. These experiments demonstrated no development of drug resistance to an inhibitor of angiogenesis in three tumor types tested. Furthermore, repeated cycles of treatment resulted in prolonged tumor dormancy after cessation of antiangiogenic therapy [84].

The recent discovery that thalidomide is an orally effective antiangiogenic agent [85] provides an obvious potential solution for clinically achievable long-term therapy of extracranial malignancies and this drug is now being tested clinically. Similarly, clinical trials of col-3 administered as a single daily oral dose are underway as noted above. Development of an effective oral agent for antiangiogenic therapy of intracranial malignancies remains a challenge. It is, however, technically feasible to alter the duration of local drug delivery by changing the composition of implantable controlled-release polymers [86,87]. Thus, long-term local antiangiogenic brain tumor therapy should be possible for these and other promising new antiangiogenic drugs such as Squalamine [88], Angiostatin [89], and Endostatin [90], which are currently in clinical trials for extracranial malignancies. The efficacy of these new drugs against malignant brain tumors has yet to be determined clinically; however, by providing a therapeutic alternative to systemic drug administration, local drug delivery polymers may allow us to use drugs that otherwise would be effective only against extracranial malignancies.

In order to maximize the therapeutic benefits of antiangiogenic therapy, it will be necessary to use these drugs in combination with cytoreductive treatment modalities such as radiation, chemotherapy, and surgical resection. Minocycline, for example, synergistically potentiates the survival benefits achieved with surgical tumor resection or systemic carmustine (BCNU) in the rat intracranial 9L-glioma model [68]. Similarly, minocycline potentiates the therapeutic benefits of radiation and a variety of cytotoxic drugs in the murine Lewis lung carcinoma model [65,66]. Greater than additive effects have also been achieved when different antiangiogenic drugs have been used in combination [91–93]. As we learn more about the inhibitors of angiogenesis, additional synergistic drug combinations may become obvious on the basis of complementary mechanisms of action.

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*Address for offprints:* Henry Brem, Department of Neurosurgery, The Johns Hopkins School of Medicine, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA; Tel.: 410-614-0477; Fax: 410-614-0478; E-mail: hbrem@jhmi.edu