



Charles Gruppe. *Minnehaha Creek in Winter*. Oil on canvas, 36" × 40". The Hainsworth Collection. Photo credit: R. H. Love Galleries, Chicago, Illinois.

Several strategies have been developed to open the blood-brain tumor barrier and increase anticancer drug delivery to brain tumors.

Modulation of Brain Tumor Capillaries for Enhanced Drug Delivery Selectively to Brain Tumor

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Background: *The blood-brain tumor barrier (BTB) significantly impedes delivery of most hydrophilic molecules to brain tumors. Several promising strategies, however, have been developed to overcome this problem.*

Methods: *We discuss several drug delivery methods to brain tumor, including intracerebroventricular, convection-enhanced delivery, BBB/BTB disruption, and BTB permeability modulation, which was developed in our laboratory.*

Results: *Using immunolocalization, immunoblotting, and potentiometric studies, we found that brain tumor capillary endothelial cells overexpress certain unique protein markers that are absent or barely detectable in normal capillary endothelial cells. We biochemically modulated these markers to sustain and enhance drug delivery, including molecules of varying sizes, selectively to tumors in rat syngeneic and xenograft brain tumor models. We also demonstrated that the cellular mechanism for vasomodulator-mediated BTB permeability increase is due to accelerated formation of pinocytotic vesicles that transport therapeutic molecules across the BTB.*

Conclusions: *Other methods to deliver drugs across the BTB are effective but have severe drawbacks. Our strategy targets BTB-specific proteins to increase antineoplastic drug delivery selectively to brain tumors with few or no side effects, thus increasing the possibility of improving brain tumor treatment.*

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Introduction

The cerebral microvessels and capillaries that form the blood-brain barrier (BBB) protect the brain, but they also pose an obstacle to the delivery to the brain of small and large therapeutic molecules. In fact, Pardridge¹ reported that the BBB blocks delivery of more than 98% of central nervous system (CNS) drugs. As large water-soluble molecules (eg, therapeutic humanized monoclonal antibodies) are developed for the treatment of neurological diseases, the challenge to deliver them across the BBB has assumed critical importance. This article focuses on the biochemical modulation of the blood-brain tumor barrier (BTB), which surrounds brain tumors and has key characteristics that differentiate it from the BBB, as a strategy to enhance anticancer drug delivery to brain tumor. Our research has also shed light on the properties of the abnormal BBB, which results from damage caused by cerebral ischemia and other neurological disorders, yet is still somewhat different from the BTB.

Every year in the United States, approximately 25,000 new primary brain tumor and more than 200,000 secondary (metastatic) brain tumor cases are reported. For the most part, even after surgery to remove a malignant brain tumor, brain cancer recurs, severely shortening life expectancy. Conventional treatment using radiation and intravenous (IV) chemotherapy are often unsuccessful primarily because the anticancer drugs fail to cross the BTB in sufficient quantities.² Therefore, understanding the BTB and the biochemical regulation of the BBB in its normal and abnormal states will be increasingly important as efforts continue to deliver therapeutic compounds to CNS targets. In particular, successful treatment of brain tumors involves efficient anticancer drug delivery to brain tumors across the BTB. Although the BTB is leaky in the tumor center, the established microvessels feeding the proliferating edge of the tumor and the brain adjacent to the tumor are nearly as impermeable as the BBB.² Therefore, the BTB still poses a major hurdle to anticancer drug delivery to tumors. Over the past 10 years, several promising strategies have been developed to open the BTB to increase anticancer drug delivery to brain tumors. In this article, we review the advantages and disadvantages of the major drug delivery methods.

Invasive Drug Delivery

Invasive drug delivery strategies circumvent the BTB but require either a craniotomy or insertion of catheters into the carotid artery. This strategy includes either intracerebroventricular (ICV) infusion of drugs, which is similar to a slow IV infusion,³ or intracerebral implantation of controlled-release anticancer drugs encased in biodegradable polymers.⁴ While effective in delivering anticancer drugs to tumor, the drugs in this delivery strat-

egy are not targeted specifically at brain tumor cells, so potentially noxious anticancer agents are also delivered to normal, healthy brain cells that can result in undesirable side effects.

In relation to the obstacle of developing a method to deliver drugs across the BTB, the drugs infused via the ICV strategy may not necessarily cross the BBB or the BTB. Such a strategy may deliver drugs to cerebrospinal fluid (CSF) via a circumventricular brain region (CVR) that surrounds the ventricular system but is not protected by the BBB or the BTB. Furthermore, the BBB and the blood-CSF barrier are anatomically and functionally distinct. Therefore, entry of a drug into CSF via CVR does not necessarily mean that the drug has crossed the BBB but is only a measure of blood-CSF barrier permeability.¹ A recent study concluded that temozolomide (Temodar) crossed the intact BBB by showing the presence of temozolomide in CSF after systemic administration.⁵ However, their observation may not mean that the drug crossed the BTB or BBB because CVR lacks a BBB or BTB, and temozolomide or its metabolite methyl-triazenyl imidazole carboxamide (MTIC) level was not quantified in brain tumor following a systemic administration. In contrast, we found that [¹⁴C]-labeled temozolomide hardly crossed the BTB and, in fact, only a small amount was taken up by the tumor in a rat brain glioma model (unpublished data). Therefore, drug entry into CSF is not an index of BTB permeability unless drug levels in the tumor are quantified by quantitative autoradiography⁶ or by detection and identification of a drug or its metabolites in brain tumor tissue by a quantitative assay such as the high-pressure liquid chromatography-mass spectrum-mass spectrum method.⁷ For example, a circulating drug such as azidothymidine (AZT) gains access to CSF from the blood following a systemic administration. The AZT molecules are rapidly exported back to blood by the CSF, possibly due to an active efflux mechanism,⁸ but they do not cross the BBB or BTB and would not reach a brain tumor. This complicates the ability of a drug to effectively reach tumor across the BBB or BTB. The goal of crossing the BTB instead of going around it via the CVR is crucial in developing a delivery method for most brain tumors. The CVR is surrounded by more permeable capillaries than BBB capillaries, which may offer an opportunity to deliver drugs to some brain tumors. However, it is a limited opportunity since the surface area of the CVR is small compared with the surface area of the BBB and BTB. Therefore, while the CVR is an effective portal for anticancer drug delivery to tumors in close proximity to the CVR, it is not an effective route to most brain tumors.

Intracerebral Infusion

Intracerebral implants have a limited, narrow effect that is effective against small brain tumors. However, they are

clinically ineffective against larger (greater than 500 μm) and highly diffused tumors such as gliomas because they do not allow anticancer agents to diffuse. This is a particularly critical factor in glioblastoma multiforme because it is essential that anticancer drugs be able to reach small pockets of tumor cells well away from the tumor core. For instance, the diffusion of 1,3-bis (2-cholorethyl) 1-nitrosourea (BCNU), a commonly used anticancer biodegradable implant, is limited to a tumor radius of 500 μm from the implanted site.⁴

Convection-Enhanced Delivery

Convection-enhanced delivery (CED) is a novel direct method to deliver anticancer drugs to brain tumor core and adjacent brain tissue surrounding the tumor core. Normally, CED uses positive pressure infusion to generate a pressure gradient to obtain uniform distribution of anticancer drugs. A recent clinical trial, however, showed that CED is an effective drug delivery strategy to treat human nondiffused brain tumors with intratumoral injection of anticancer agents.⁹ However, while effective, CED is highly invasive and is also best used against small solid tumors since it, like intracerebral infusion, has poor penetration of drug molecules into diffused tumor tissue. The application of this drug delivery strategy is reported in detail elsewhere.^{9,10}

BBB/BTB Disruption

This strategy causes global changes in brain microvasculature permeability that allow therapeutic molecules to reach the brain. Enhanced chemotherapeutic drug delivery using osmotic BBB disruption following intracarotid

infusion of mannitol has been demonstrated in rats¹¹ and humans.¹² In certain cases where the tumors are highly malignant, the delivery of anticancer drug across the entire brain vasculature may be warranted. This strategy, however, results in greater disruption of the normal BBB relative to the BTB.¹ It also causes several undesired side effects in humans, including physiological stress, transient increase in intracranial pressure, and unwanted delivery of anticancer agents to normal brain tissue.¹³ Clinicians may decide on the therapeutic strategies to treat brain tumors based on individual assessment of BBB/BTB intactness by using noninvasive BBB markers.¹⁴⁻¹⁶

Biochemical Modulation of the BTB

Much of our work for the past 10 years has focused on the role of certain vasoactive molecules on BBB permeability. We have observed that selected vasoactive molecules such as leukotriene (LTC_4), bradykinin (BK), and certain potassium channel agonists selectively increase permeability in brain tumor capillaries but not in normal brain capillaries.¹⁷⁻²⁸ These observations are being translated into strategies to increase drug delivery selectively to brain tumor tissue in patients in clinical studies.^{19,29-31} Hence, the biochemical modulation strategy involves selective increase in BTB permeability to anticancer drugs such as carboplatin, without affecting the normal BBB using vasoactive agents (Fig 1) and thus without serious side effects in preclinical rat brain tumor models. Many questions remain, however, regarding the mechanisms by which these vasoactive compounds increase abnormal brain capillary permeability including the BTB permeability. After extensive research, we are beginning to understand, for example, why normal brain capillaries appear to resist the permeability effects of these molecules and why their permeability effects are selective to abnormal brain capillaries.

Leukotriene

LTC_4 is a biologically active molecule formed from the unsaturated fatty acid, arachidonic acid (AA), via the 5-lipoxygenase pathway. The conversion of AA to LTC_4 via the epoxide intermediate LTA_4 is Ca^{2+} dependent. Further, the levels of LTC_4 in the brain are elevated in brain tumors.¹⁷ We demonstrated that intracarotid infusion of LTC_4 selectively increases capillary permeability in brain tumors but not in normal brain.^{18,20} We found that normal brain capillaries are rich in the BBB enzyme γ -glutamyl transpeptidase (γ -GTP), which acts as an "enzyme barrier," rapidly degrading LTC_4 and preventing it from acting on capillary permeability. We also found that abnormal brain capillaries and tumor capillaries lack γ -GTP and its enzymatic barrier effect,¹⁸ thereby allowing LTC_4 to increase the permeability of abnormal brain capillaries.

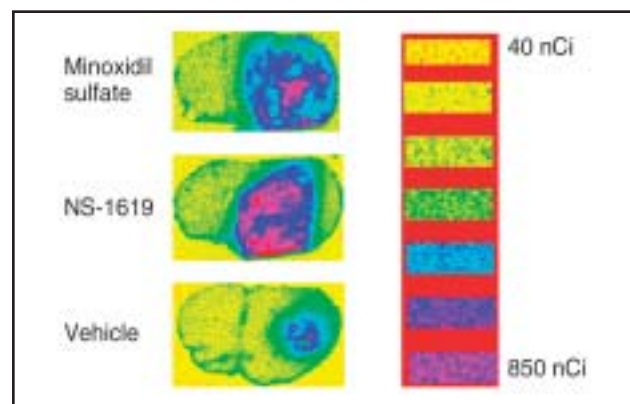


Fig 1. — Potassium channel agonists (NS-1619 and MS) increase the permeability of the blood-brain tumor barrier (BTB) and enhance the delivery of $[^{14}\text{C}]$ -carboplatin (305 daltons) to brain tumor. Color-enhanced autoradiographs of coronal brain sections show little $[^{14}\text{C}]$ -carboplatin uptake into rat brain tumor (RG2) in rats treated with phosphate-buffered saline (PBS) but significant uptake of $[^{14}\text{C}]$ -carboplatin in NS-1619- and MS-treated rats. The scale at left shows pseudocolor intensities of tissue-calibrated $[^{14}\text{C}]$ standards from 40 to 850 nCi/g specific activities for comparison.

We observed that other vasoactive molecules similar to LTC₄ also selectively increase permeability in tumor¹⁷ and abnormal brain capillaries²⁵ but do not increase normal brain capillary permeability except at high doses. While the selective effect of LTC₄ on permeability could be explained by γ -GTP-enriched normal brain capillaries, the lack of γ -GTP alone does not explain the selective effects on abnormal capillaries of other vasoactive compounds such as histamines,²² BK and BK analogs,^{19,23,24} and potassium channel agonists.^{24,26}

Bradykinin

BK, a non-peptide (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), is formed from kinogen.³² Intracarotid infusion of BK selectively increases permeability 2- to 12-fold in brain tumor capillaries, which allows transport across the BTB of molecules ranging in mass from 100 to 70,000 daltons.^{21,23} BK does not increase permeability in the normal BBB except at extremely high doses.²¹ BK-induced BTB opening, however, is transient, lasting 15 to 20 minutes, and tumor capillaries become refractory to the effect of BK for up to 60 minutes after administration.^{21,26} BK mediates its effect via BK type 2 (B2) receptors, and this observation led to clinical trials using the BK analog RMP-7 (Cereport) to increase delivery of chemotherapeutic agents to brain tumors. However, RMP-7 elicited a varying BTB permeability increase in patients with glioblastoma multiforme.¹⁹ We showed that this variable response appeared to be due to differential expression of B2 receptors in gliomas (Fig 3).²⁴ Modulation of B2 receptors to enhance drug delivery has four major limitations: (1) the effect of BK is transient, lasting only 20 minutes,^{21,26} due to B2 receptor internalization,³³ (2) at high doses, BK permeabilizes the normal BBB,³² (3) B2 receptor expression levels vary,²⁴ and (4) IV administration causes an unde-

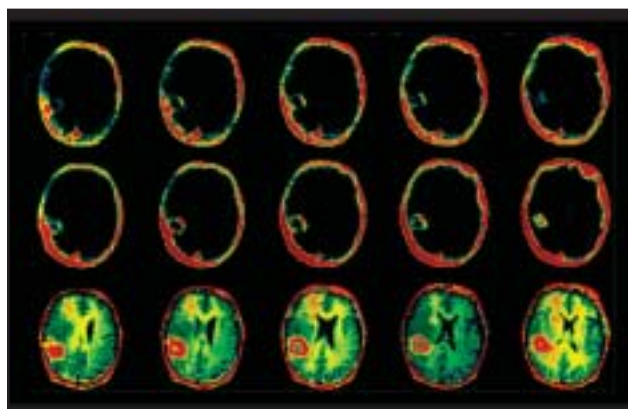


Fig 2. — Images obtained from patients with malignant gliomas. Top row: baseline axial positron emission tomographic (PET) images. Center row: axial PET images obtained after infusion of RMP-7. Bottom row: axial T1-weighted magnetic resonance (MR) images showing co-registration of baseline MR and RMP-7 PET images. From Black KL, Cloughesy T, Huang SC, et al. Intracarotid infusion of RMP-7, a bradykinin analog, and transport of gallium-68 ethylenediamine tetraacetic acid into human gliomas. *J Neurosurg.* 1997;86:603-609. Reprinted with permission.

sired hypotensive effect. Therefore, BK does not work when IV administered,² rendering this strategy unsuitable to achieve consistent drug delivery selectively to brain tumors in a clinical environment.

Mechanisms of BK-Induced BTB Permeability Increase

BK-B2 receptor interaction increases intracellular Ca²⁺ either by mobilization of Ca²⁺ from internal sites or through Ca²⁺ influx,³² both of which result in nitric oxide synthase (NOS) activation and NO production. Pretreatment of animals with L-NAME (an inhibitor of NOS) prior to BK infusion negated the permeability effect of BK.³⁴ Administration of L-arginine, a substrate for NOS, however, restored the effect of BK. These findings suggest that the effect of BK on permeability is mediated through NOS and NO. We showed that infusion of certain short-acting NO donors also selectively opens tumor capillaries but not normal capillaries,²⁶ suggesting that the lack of B2 receptors in normal brain microvessels may not be the only factor that prevents BK from opening the normal BBB. In human gliomas,¹⁹ however, intracarotid infusion of BK results in a wide range of increases in delivery of gallium-68 ethylenediamine tetraacetic acid (EDTA) in human gliomas from 2% to 58% (Fig 2). We showed that this variability could result from differences in B2 receptor density (Fig 3), differences in the ability of tumor microvessels to degrade cyclic guanosine monophosphate (cGMP), and/or other unexplored factors.²⁴ We also showed that BK's BTB permeability-enhancing effect is mediated by potassium channels (Fig 4) and, in particular, calcium-activated potassium (K_{Ca}) channels (Fig 5).²⁶

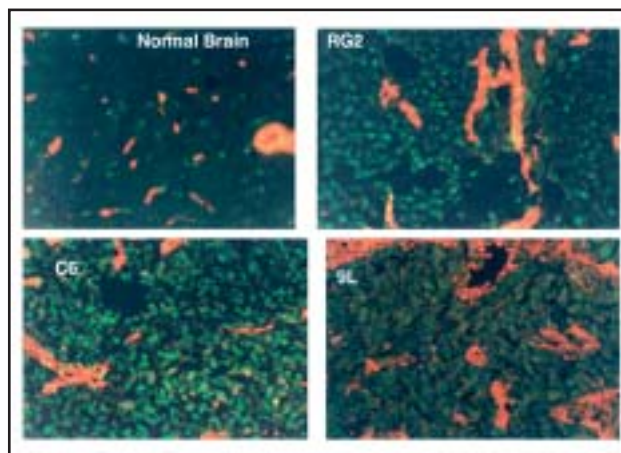


Fig 3. — Immuno-co-localization of B2 receptors (FITC-green) and endothelial cell marker, von Willebrand factor (TRITC-red). Normal rat brain and rat brain tumor capillary endothelium lack B2 receptor expression, although tumor cells (RG2, C6, and 9L) overexpress B2 receptors compared with normal brain cells. We also demonstrated a differential expression of B2 receptors that correlates with the degree of BTB permeability response to bradykinin infusion. From Liu Y, Hashizume K, Chen Z, et al. Correlation between bradykinin-induced blood-tumor barrier permeability and B2 receptor expression in experimental brain tumors. *Neurol Res.* 2001;23:379-387.

Nitric Oxide, cGMP, and cGMP-Dependent Protein Kinase G

NO is a major biological signaling molecule that modulates a number of physiological functions including cerebral microvascular tone in humans.³⁵ NO activates the heme-containing enzyme, soluble guanylate cyclase (sGC),³⁵ resulting in cGMP formation. We demonstrated that inhibiting sGC with LY83583 blocks BK-induced BTB permeability increase.²¹ Conversely, blocking the phosphodiesterase (PDE)-mediated breakdown of cGMP with Zaprinast further enhanced the effect of BK on tumor capillaries. A correlation was shown between the levels of cGMP in several experimental brain tumor cell lines and their response to BK infusion in rat brain tumor models. NO and cGMP effects are reported to be dependent on protein kinase G (PKG) in the CNS. Evidence for this comes from studies where dibutylryl (db)-cGMP increased vesicles in endothelial cells by activating PKG,³⁶ while Rp-8pCPT-cGMP, a selective inhibitor of PKG, blocked db-cGMP-induced changes.³⁶ We showed that PKG also plays a role in BTB permeability regulation in rat glioma model (unpublished data, 2004). It

was previously shown that db-cGMP, a cGMP analog resistant to PDE hydrolysis, increased vesicle formation in brain endothelial cells. Interestingly, after db-cGMP administration, brain endothelial cell tight junctions (TJs) remained closed.³⁶ The opening of TJs should result in an increase in brain capillary permeability, although the transport of molecules via TJs is dependent on the molecular size of the tracer. In contrast, LTC₄²⁰ and BK-induced BTB permeability increase is due to increased formation of transport vesicles,²⁶ which transport molecules across the endothelium in a size-independent process (Fig 6). Further, LTC₄²⁰ and BK²⁶ did not alter endothelial TJs, which accounts for their ability to allow the transport of molecules across the BTB independent of their molecular size.

In an effort to explore other potential targets to increase BTB permeability, we recently reported that two major potassium channels, K_{Ca} and ATP-sensitive potassium (K_{ATP}) channels,^{26,28} are overexpressed in brain tumor capillaries and tumor cells (Fig 4). We also showed that these potassium channels play a major role in BTB permeability regulation.²⁶⁻²⁸ Our findings support the observation that endothelium-dependent regulation of cerebral

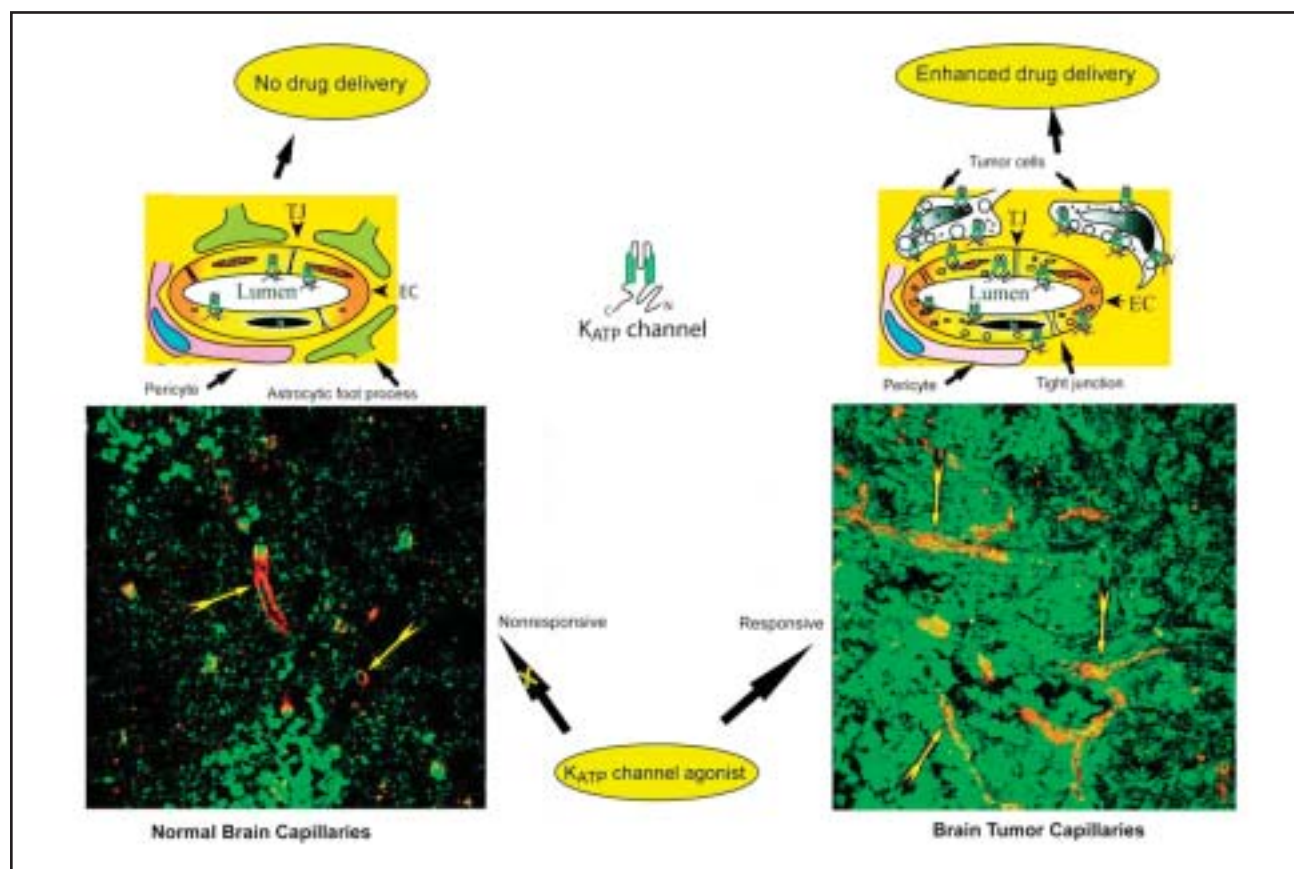


Fig 4. — Schematic representation of the differences between the BBB and BTB with regard to their morphology and biochemical response to K_{Ca} and K_{ATP} channel agonists. BTB capillaries and surrounding tumor cells overexpress K_{Ca} and K_{ATP} channels. Therefore, they may readily respond to activation by K_{Ca} and K_{ATP} channel agonists resulting in enhanced formation of transport vesicles, which appear to transport molecules to tumor tissue across the BTB. In contrast, K_{Ca} and K_{ATP} channels are barely detected in normal brain microvessel endothelial cells and may not readily respond to K_{Ca} and K_{ATP} channel agonists.^{26,28} Our strategy involves biochemical modulation of K_{Ca} and K_{ATP} channels for selective and enhanced drug delivery to tumors with little or no drug delivery to normal brain tissue. From Ningaraj NS, Rao MK, Black KL. Adenosine 5'-triphosphate-sensitive potassium channel-mediated blood-brain tumor barrier permeability increase in a rat brain tumor model. *Cancer Res.* 2003;63:8899-8911. Reprinted with permission of American Association of Cancer Research.

blood vessel functions is impaired in several disease states, including brain tumors.²⁶⁻²⁸ Unlike normal brain capillaries, brain tumor capillaries may respond readily to potassium channel modulators because of cancer-induced altered signaling mechanisms.

Role of Potassium Channels in BTB Permeability Modulation

Potassium channels are the most diverse class of ion channels and have critical roles in cell function. During the past 10 years, rapid identification of new genes encoding potassium channels has created both a challenge in discerning their physiological roles and an opportunity for exploring their use in drug target discovery. Increasing evidence suggests that vascular endothelial cells from

cerebral blood vessels express ion channels and that these ion channels play an important role in modulating endothelial cell functions.²⁶⁻²⁸

K_{Ca} Channels — Several physiological features of the large conductance *K_{Ca}* channels, also referred to as maxi K and BK channels, are well documented. *K_{Ca}* channels are unique because their activity is triggered by depolarization and enhanced by an increase in cytosolic calcium (in the μM range), providing a link between the metabolic and electrical state of cells. Endothelium-dependent regulation of cerebral blood vessel functions is impaired in brain tumors,^{26,27} which may affect tumor capillary permeability in response to vasomodulators. These critical observations prompted us to determine whether these channels have an important role in BTB permeability regulation. Using immunoblot and immunolocalization studies, we showed

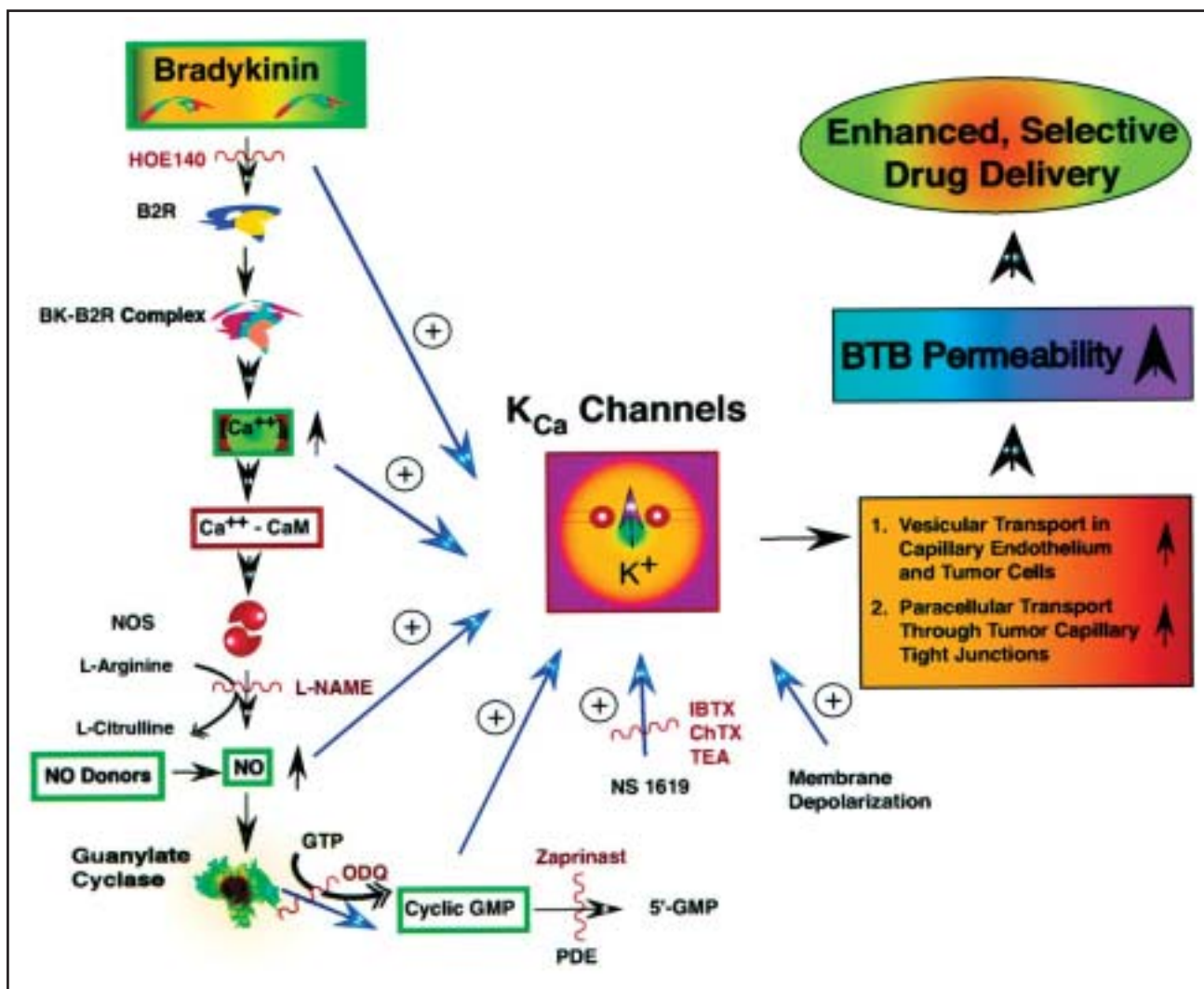


Fig 5. — The biochemical and cellular pathways for BTB permeability regulation by specific vasomodulators for enhanced drug delivery to brain tumors.²⁶ Overall, these studies will further delineate the role of *K_{Ca}*²⁶ and *K_{ATP}*²⁸ channel activation as a mechanism for selective delivery of anticancer agents across the BTB and could potentially result in improved control of disease in patients with metastatic brain tumors. The activators used include bradykinin, guanylate cyclase activator YC-1, nitric oxide (NO) donors, *K_{Ca}* channel agonist NS-1619, and *K_{ATP}* channel agonist minoxidil sulfate. The inhibitors used include NO synthase inhibitor L-NAME, guanylate cyclase inhibitor OGDG, phosphodiesterase inhibitor zaprinast, *K_{Ca}* channel inhibitor iberiotoxin (IBTX), and *K_{ATP}* channel antagonist glibenclamide. From Ningaraj NS, Rao M, Hashizume K, et al. Regulation of blood-brain tumor barrier permeability by calcium-activated potassium channels. *J Pharmacol Exp Ther.* 2002; 301:838-851.

that K_{Ca} channels are overexpressed in rat brain tumor capillary endothelium and tumor cells, and we demonstrated the functional presence of K_{Ca} channels in isolated rat brain tumor capillary endothelial and tumor cells.²⁶

K_{ATP} Channels — K_{ATP} channels couple intracellular metabolic changes to the electrical activity of the plasma membrane to regulate cerebral vascular tone and the relaxation of cerebral vessels in response to diverse stimuli, including vasomodulators, in both normal³⁷ and disease states.³⁸ K_{ATP} channels, heteromultimers expressed in cerebral vessels, are composed of pore-forming (inward-rectifying K_{ir} 6.1 or 6.2) and sulfonylurea receptor subunits.³⁸ K_{ATP} channels are widely distributed in a variety of tissues and cell types, including rat aorta and brain microvascular endothelial cells. Activation of these channels produces hyperpolarization, relaxation, and dilatation of cerebral arteries in humans. Sulfonylurea analogs, minoxidil sulfate, pinacidil, cromakalim, and diazoxide stimulate K_{ATP} channels. We showed that K_{ATP} channels take part in the regulation of BTB permeability.²⁸

K_{Ca} and K_{ATP} Channels in Brain Microvessels — Certain vasoactive molecules such as BK32 and vascular

endothelial growth factor³⁹ disrupt the BBB. In experimental brain tumors, low concentrations of BK selectively increased BTB permeability¹⁹⁻²⁴ due to overexpression of B2 receptors on tumor cells but not in tumor microvessels.²⁴ Also, the level of B2R expression on tumors directly correlated with the ability of BK to increase BTB permeability.²⁴ In contrast, K_{Ca} channels are overexpressed on both glioma cells and tumor microvessels and, therefore, a K_{Ca} channel agonist (NS-1619) selectively increased BTB permeability. We also showed the co-localization of K_{Ca} channels in normal and tumor brain microcapillary endothelium (using endothelial cell-specific von Willebrand factor antibody) and tumor cells.²⁶ These observations are crucial in that overexpressed K_{Ca} ²⁶ and K_{ATP} ²⁸ channels in the tumor, capillary endothelium, or tumor cells could be targets for opening the BTB with K_{Ca} and K_{ATP} channel agonists. Furthermore, infusion of K_{Ca} and K_{ATP} channel agonists in tumor-bearing Wistar rats significantly enhanced BTB permeability to allow delivery of [¹⁴C]-carboplatin to the tumor area with no permeability increase in normal brain tissue (Fig 1), and co-infusion of carboplatin with K_{Ca} ²⁷ and K_{ATP} ²⁸ channel agonists significantly enhanced survival. Further, NS-1619²⁷ and MS²⁸ enhanced HER-2 (*erbB-2*) monoclonal antibody delivery to brain tumor in a rat model with a human xenograft.

Conclusions

The BBB capillaries hamper delivery of therapeutic agents from circulation to the brain. Similarly, brain tumor capillaries that form the BTB also prevent delivery of most hydrophilic molecules and antitumor agents to brain tumor.¹ During the past decade, a considerable research effort has been made and various strategies employed to increase BTB permeability in order to enhance delivery of anticancer drugs selectively to brain tumors. Intracarotid infusion of hyperosmotic agents mannitol and arabinose opens the BBB in animals¹¹ and humans¹² and has produced some clinical success, but can also increase delivery of potentially toxic drugs to normal nonneoplastic brain tissue.¹ Another drug delivery strategy was developed to allow drug delivery to the brain using vectors, which carry modified proteins, peptides, or monoclonal antibodies that undergo receptor-mediated transcytosis.¹ This strategy is complex and can allow delivery of therapeutic agents to normal brain tissue.

In an effort to improve on these methods, we employed certain vasomodulators, such as potassium channel agonists for targeted and enhanced delivery of chemotherapeutics selectively to brain tumor in rodents (Fig 1) and BK or its analog, RMP-7 in humans (Fig 2). Our biochemical approach increases BTB permeability, enhances delivery of therapeutic drugs to brain tumors selectively with little or no drug delivery to normal brain, and can deliver small- to large-sized substances, including

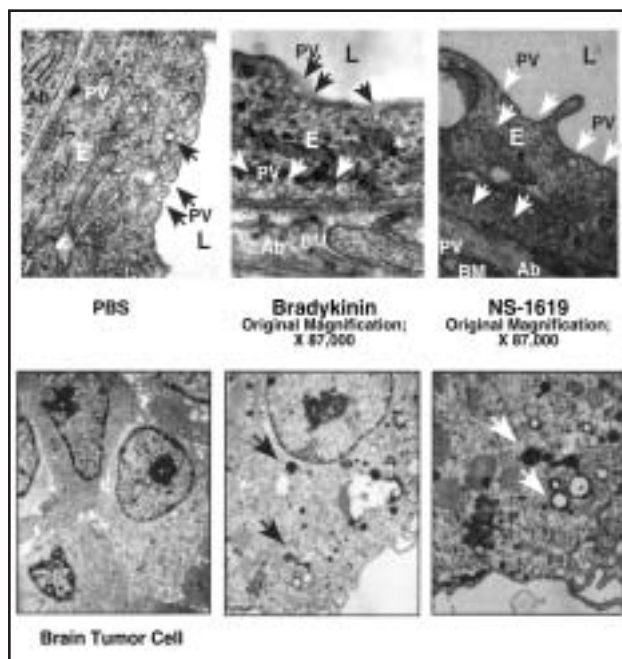


Fig 6. — Induction of vesicular transport in tumor capillary endothelium and tumor cells in vivo. Top row: In PBS-infused rat, a brain tumor microvessel endothelial cell shows very few vesicles (arrows). Bradykinin (BK) infusion caused an increased formation of vesicles (arrows) by luminal membrane invaginations. These vesicles, with average diameter of 75 to 80 nm, dock and fuse with the basal membrane. NS-1619 induces rapid formation of pinocytotic vesicles (arrows) in tumor capillary endothelial cytoplasm. Bottom row: No horseradish peroxidase (HRP)-laden vesicles are seen in a tumor cell of a vehicle-treated rat. A number of electron-dense, HRP-laden vesicles (arrows) are seen in tumor cells following intracarotid infusion of BK or NS-1619, and intravenous injection of HRP in rats with RG2 tumors (Ab = abluminal, BM = basal membrane, E = endothelial cytoplasm, L = luminal, PV = pinocytotic vesicles [arrows]). Adapted from Ningraj NS, Rao M, Hashizume K, et al. Regulation of blood-brain tumor barrier permeability by calcium-activated potassium channels. *J Pharmacol Exp Ther.* 2002;301:838-851.

contrast-enhancing agents, antitumor compounds, and therapeutic proteins and viral vectors.²⁶⁻²⁸ We demonstrated that brain tumor capillaries are responsive to intravascular infusion of low doses of tested vasomodulators, such as BK, causing BTB permeability increase via a mechanism involving BK type 2 (B2) receptors,²³ NO,³⁴ cGMP,²¹ and K_{Ca} channels.^{26,27} We have employed selected vasomodulators such as BK, NO donors, soluble GC activator and a K_{Ca} channel agonist (NS-1619) to increase BTB permeability, resulting in significantly enhanced tracer delivery specifically to brain tumor.

Furthermore, we demonstrated that activation of K_{Ca} channels by BK and NS-1619^{26,27} and K_{ATP} channels by minoxidil sulfate²⁸ induces accelerated formation of transport vesicles in both brain tumor capillary endothelium and tumor cells. These results provide evidence that vesicular transport is largely responsible for enhanced delivery of drugs across brain tumor capillaries to tumor tissue. Most importantly, our study demonstrated that rat brain tumor microvessels form an even greater number of vesicles in response to vasomodulator treatment, resulting in increased BTB permeability compared with untreated rats.

Our studies demonstrate that activation of potassium channels by channel-specific agonists and by agents that produce NO and cGMP in situ can sustain enhanced drug delivery selectively to brain tumors. This drug delivery system is independent of B2 receptors and circumvents the disadvantages of BK, including variable and refractory BTB permeability responses. The biochemical modulation strategy may improve the delivery of antineoplastic agents, including humanized monoclonal antibodies and therapeutic viral vectors selectively to brain tumors and neuropharmaceutics to diseased brain regions, while leaving normal brain unaffected and thus potentially increasing the survival rate for patients with debilitating neurological diseases and tumors.

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