Adenosine 5′-triphosphate-sensitive Potassium Channel-mediated Blood-Brain Tumor Barrier Permeability Increase in a Rat Brain Tumor Model

Nagendra S. Ningaraj, Mamatha K. Rao, and Keith L. Black
Maxine DuNitt, Neuromuscular Institute and Burns and Allen Research Institute, Cedars-Sinai Medical Center, Los Angeles, California

ABSTRACT

Brain tumor microvessels/capillaries limit drug delivery to tumors by forming a blood-brain tumor barrier (BTB). The BTB overexpresses ATP-sensitive potassium (K\textsubscript{ATP}) channels that are barely detectable in normal brain capillaries, and which were targeted for BTB permeability modulation. In a rat brain tumor model, we infused minoxidil sulfate (MS), a selective K\textsubscript{ATP} channel activator, to obtain sustained, enhanced, and selective drug delivery, including various sized molecules, across the BTB to brain tumors. Glibenclamide, a selective K\textsubscript{ATP} channel inhibitor, significantly attenuated the MS-induced BTB permeability increase. Immunocytochemistry and glibenclamide binding studies showed increased K\textsubscript{ATP} channel density on tumor cells and brain capillary endothelium, which was confirmed by K\textsubscript{ATP} channel potentiometric assay in tumor cells and brain endothelial cells cocultured with brain tumor cells. MS infusion in rats with brain tumors significantly increased transport vesicle density in tumor capillary endothelial and tumor cells. MS facilitated increased delivery of macromolecules, including Her-2 antibody, adenoviral-green fluorescent protein, and carboplatin, to brain tumors, with carboplatin significantly increasing survival in brain tumor-bearing rats. K\textsubscript{ATP} channel-mediated BTB permeability increase was also demonstrated in a human, brain tumor xenograft model. We conclude that K\textsubscript{ATP} channels are a potential target for biochemical modulation of BTB permeability to increase antineoplastic drug delivery selectively to brain tumors.

INTRODUCTION

Understanding the biochemical regulation of the blood-brain barrier (BBB) and blood-brain tumor barrier (BTB) is critical to developing methods to deliver therapeutic compounds to central nervous system targets. Despite evidence of the antineoplastic effect of drugs such as Trastuzumab, a humanized anti-Her-2 monoclonal antibody (MAb; developed by Genentech, Inc., San Francisco, CA), alone or with chemotherapeutic agents such as docetaxel (1, 2), their clinical use for neuro-oncology remains limited because of the high doses necessary to achieve in vivo therapeutically effective concentrations in brain tumors (2). Brain tumor capillaries constitute the BTB, which has different structural and functional characteristics to that of normal brain capillaries that form the BBB. Among many distinct differences (3), we showed that BTB capillaries are responsive to vasoactive agents (4–14). This knowledge allowed us to develop a biochemical approach to increase BTB permeability and to enhance delivery of hydrophilic therapeutic drugs or small- to large-sized molecules, including contrast-enhancing agents, antitumor compounds, therapeutic proteins, and viral vectors (4–14) in vivo to brain tumors selectively with little or no drug delivery to normal brain. This drug delivery strategy exploits the responsiveness of brain tumor capillaries to intravascular infusion of low doses of vasomodulators, such as bradykinin (BK), causing BTB permeability increase via a mechanism involving calcium-activated potassium (K\textsubscript{Ca}) channels (4, 5), BK type 2 receptors (6), nitric oxide (7), and cyclic GMP (11). Overall, our research has identified the molecular targets that selectively modulate BTB permeability and contributed to a better understanding of the biochemical changes that occur during permeability modulations. More recently, we observed that another major potassium channel subtype, the ATP-sensitive potassium (K\textsubscript{ATP}) channel, is involved in brain tumor microvessel permeability regulation and may serve as another target for anticancer drug delivery. We tested whether minoxidil sulfate (MS)-induced activation of K\textsubscript{ATP} channels increases BTB permeability and enhances carboplatin (CPN) delivery to tumor tissue, thereby increasing survival in rats with implanted brain tumor. We demonstrated that CPN could be delivered selectively to tumor tissue without increasing delivery to normal brain cells.

K\textsubscript{ATP} channels are heterodimers of sulfonyleurea receptors and inwardly rectifying potassium channel subunits (K\textsubscript{ir}6.x) with a (SUR-K\textsubscript{ir}6.x)4 stoichiometry. K\textsubscript{ATP} channels are widely distributed, including in the vasculature of the brain. They couple intracellular metabolic changes to the electrical activity of the plasma membrane regulating cerebral vascular tone and mediate the relaxation of cerebral vessels to diverse stimuli, including vasomodulators, in normal (15) and disease states (16). The role of K\textsubscript{ATP} channels in regulating the permeability of normal and brain tumor capillaries, however, has not been elucidated. Brain tumors thrive in a hypoxic environment; this fact could explain the up-regulation of K\textsubscript{ATP} channel expression detected in and around brain tumors but also shown in hypoxic and ischemic conditions (3, 16). Furthermore, endothelium-dependent regulation of cerebral blood vessel functions is impaired in brain tumors (17, 18), which might affect tumor capillary permeability in response to vasomodulators.

Brain tumors, particularly gliomas, frequently exhibit up-regulated epidermal growth factor receptor genes, which is associated with tumor aggressiveness, poor prognosis, and shortened patient survival (19, 20). One study found that 17–20% of primary brain tumors were Her-2 positive (20), and Her-2 has been linked to tumor progression. Mutant Her-2 MAb directed against Her-2 or Her-2 inhibitors have been shown to block kinase activation of epidermal growth factor receptor through the formation of nonfunctional heterodimeric receptor complexes (21), which prevent tumor growth. Trastuzumab was developed to treat Her-2-positive breast cancers and may, possibly, also be effective in treating gliomas. Although Trastuzumab alone or in combination with a chemotherapeutic drug (22) has the potential to treat breast and lung cancers metastasized to brain, as well as gliomas, their efficient delivery across the BTB to tumor is highly challenging. Our strategy involves selective modulation of BTB permeability via activation of K\textsubscript{ATP} channels in tumor capillaries to enhance delivery of Neu and Her-2 MAb in syngeneic and xenograft rat tumor models, respectively.

This study sought to elucidate the role of K\textsubscript{ATP} channels in BBB and BTB permeability modulation and the role of transendothelial vesicular transport in normal and tumor capillaries in rats harboring intracranial rat gliomas (RG2). We investigated whether MS causes BTB permeability increase in a human, brain tumor xenograft model. We also studied whether brain tumor cells induce overexpression of K\textsubscript{ATP} Channels in brain endothelial cells cocultured with tumor cells.
We further tested whether MS increases the transport of macromolecules, such as adenosinergic vectors carrying green fluorescent protein (GFP) genes and Her-2 MAb, across the BTB in a human tumor xenograft model.

MATERIALS AND METHODS

MS, glibenclamide (Sigma Chemical Co., St. Louis, MO), 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H benzimidazol-2-one (NS-1619), and ibotenic acid (RBI Chemical, Natik, MA), membrane potential assay kit (Molecular Devices, Sunnyvale, CA), and radioisotrons (NEN Co., Boston, MA) such as [14C]-α-aminoisobutyric acid ([14C]-AIB; 57.6 mCi/mmoll; M, 103,000), [14C]-CPN (25 mCi/mmoll; M, 371,000), [3H]-dextran (2 mCi/mmoll; M, 70,000), [3H]-glibenclamide (50 mCi/mmoll; M, 494,000), von Willebrand factor (vWF; DAKO, CA), Neu polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), gliarial fibrillary acidic protein (GFAP; Chemicon, Temecula, CA), Her-2 MAb (clone CB 11; Zymed, San Francisco, CA), and fluorescent-labeled secondary antibodies (Chemicon; and Molecular Probes, Eugene, OR) were used in the present study.

In Vivo BBB/BBT Permeability. All animal experiments were conducted in accordance with policies set by the Institutional Animal Care and Use Committee and NIH guidelines. A rat syngeneic tumor model was prepared using female Wistar rats, and a human tumor xenograft model in athymic nude rats weighing 180–200 g was prepared for the BBB/BBT permeability studies. Although brain tumor blood vessel development is rapid in rats compared with humans, this variable should not affect our findings or conclusions, because our study analyzes the response of blood vessels, regardless of how rapidly they developed, to vasomodulators. The optimum number of tumor cells and incubation period for in vivo tumor growth was determined in separate experiments. RG2 cells (1 × 103) in 5 μl of medium with 1% methylcellulose were injected into the basal ganglia of Wistar rats, whereas nude rats received injections of glioblastoma multiforme (GBM) primary cells (5 × 104). The coordinates were 5 mm lateral to the bregma and 4.5 mm deep to the basal ganglia. Seven days (for RG2 tumor) and 3–4 weeks (for GBM) after tumor implantation, the rats were prepared for permeability study, as described previously (4, 5). In regional permeability studies, 5 min after the start of the intracarotid (i.c.) infusion, 100 μCi/kg [14C]-AIB, [14C]-dextran, or [14C]-CPN in 1 ml of phosphate buffer saline (PBS) were injected as an i.v. bolus within a 15-sec period.

Kj Measurement. The unilateral transfer constant Kj (μl/g/min) is an initial rate for brain-to-blood transfer of a radioisotracer, as calculated described by Ohno et al. (23). The Kj was determined for radioisotrons [14C]-AIB, [14C]-CPN, and [14C]-dextran in the tumor core, tumor-adjacent brain tissue, and contralateral brain tissue using the quantitative autoradiographic (QAR) method as described previously (4, 5). An optimum dose (10 μg/kg/min, i.c.) of BK, which was established in previous studies (4, 5, 9–11), and optimal dose (30 μg/kg/min for 15 min, i.c.) of MS were used for Kj measurements in rats (n = 6/group). In a separate QAR study, various doses of glibenclamide (0–10 μg/kg/min) were used to establish an optimum dose, which maximally attenuated the MS-induced BTB permeability increase in RG2 tumor-bearing rats. Additional experiments were performed in rats with RG2 tumor by confining MS with glibenclamide (5 μg/kg/min) to investigate whether inhibition of KATP channels by glibenclamide attenuates MS-induced permeability increase. In addition, the effect of MS with optimum dose and time established in rats (n = 4/dose) harboring intracranial RG2 tumor on BTB permeability in nude rats harboring GBM was investigated.

Dose-Response Study. To establish the optimal dose range that would result in selective increases in BTB permeability without appreciably altering systemic blood pressure, various doses (0–60 μg/kg/min) of MS were administered. In a separate study, Evans blue dye was administered i.v. in rats (n = 4/group) with RG2 tumors to achieve a semiquantitative measure of BTB permeability increase.

Time Course. To determine whether vasomodulator-induced BTB permeability increase in RG2 tumor-bearing rats (n = 4/time period) was transient or could be sustained over a long period, a separate QAR study was performed. BK (10 μg/kg/min), MS (30 μg/kg/min), or a KATP channel activator (NS-1619) was infused (i.c.) separately for 15-, 30-, and 60-min periods, and Kj was determined as described above.

Synergistic Effect of KATP and KATP Channel Agonists on BTB Permeability. To investigate whether MS-induced BTB permeability increase was independent of KATP channels, MS was coinused with the KATP channel inhibitor glibenclamide (100 μg/kg/min) in RG2 tumor-bearing rats (n = 4/group). Furthermore, to investigate whether KATP and KATP channel agonists administered simultaneously would exert a synergistic effect on BTB permeability, NS-1619 and MS (30 μg/kg/min each) were coinused (i.c.) for 15 min, and Kj was measured by QAR.

Isolation of Brain Endothelial Cells. To investigate the expression and activity of KATP channels in rat brain endothelial cells (RBECs), we isolated endothelial cells from the brains of neonatal rats using the method described previously (4). The homogeneity (90–95%) of endothelial cells was verified by immunostaining with an endothelial cell marker, factor VIII/vWF. For potentiometric assays, RBECs were seeded alone and in coculture with RG2 tumor cells in a gelatin-coated, 96-well plate to obtain a monolayer.

Coculture Experiments. To investigate whether brain tumor cells can induce overexpression of KATP channels in RBECs and human brain microvessel endothelial cells (HBMECs), we cocultured RBECs with RG2 cells and HBMECs with GBM cells. We standardized suitable conditions for the growth of the coculture on glass coverslips in 6-well tissue culture plates. Initially, 104 cells of RBECs and HBMECs were cocoted with 103 cells of RG2 and GBM, respectively, and allowed to achieve 70% confluence. Reverse transcription (RT)-PCR and Western blot analyses in RBECs and HBMECs alone or in coculture with tumor cells were performed to study whether tumor cells induce overexpression of KATP channels at mRNA and protein levels in endothelial cells. In addition, immunocytochemical analysis of cocultures was performed with vWF and KATP channel antibodies to support RT-PCR and Western blot data.

Western Blot Analysis. To investigate the differential expression of KATP channels by the Western blot method, protein homogenates of normal brain and tumor tissues, tumor cells alone, or in cocultures were prepared by rapid homogenization in 10 volumes of lysis buffer (1% SDS, 1.0 mm sodium vanadate, 10 mM Tris (pH 7.4)). The extracts of normal brain and tumor tissues obtained from nude rats, which harbored intracerebral glioma for 3–4 weeks after tumor cell implantation, were used. Immunoblot analysis was performed on RG2 and GBM cells. Control protein lysates for the protein/receptor of interest were purchased from various vendors. Samples were fractionated on a 6–12% SDS-polyacrylamide gel, transferred to polyvinylidene difluoride membranes, and subjected to standard procedure. The membranes (Imobilon-P; Millipore, Bedford, MA) were probed with respective antibodies to support RT-PCR and Western blot data.

Immunocytochemistry (ICC). We colocalized KATP channels in rat and human brain and tumor sections with an anti-KATP channel antibody raised against a KATP6.2 subunit that was shown to be present in brain along with sulfonylurea receptor 2A/B (16, 25). ICC and confocal-laser-scanning microscope analysis were used to detect colocalization and differential expression of KATP channel subunits. Monoclonal anti-KATP6.2 antibody in normal and brain tumor microcapillary and in human and rat tumor tissues. In ICC studies of KATP channels, HBMECs and COS cells were positive and negative controls, respectively. All ICC experiments included either a control sample preincubated with a respective blocking peptide, whenever available, or control samples that did not contain primary antibodies. ICC analysis of KATP channels in athymic nude rats with intracerebral GBMs was performed as described recently (4, 5). To determine whether KATP channels are present on capillary endothelial and tumor cells, brain tumor sections were incubated with polyclonal KATP6.2 and monoclonal anti-vWF primary antibodies. The colocalization of KATP channels and vWF antibodies was accomplished with antirabbit IgG conjugated with a fluorescent
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FITC and antimouse IgG conjugated with rhodamine (tetracetylmethaneisocyanate). To demonstrate the expression of K_{ATP} channels in endothelial, RG2, and GBM tumor cells, monolayers of RG2 and GBM cells were fixed with 4% paraformaldehyde, processed simultaneously, and immunostained with anti-K_{ir}6.2 primary antibody and antirabbit IgG conjugated with FITC. To demonstrate that tumor cells in vitro and in vivo were of glial origin, RG2 and GBM cells were immunostained with antiarabbit polyclonal GFAP and antirabbit IgG conjugated with tetramethylrhodamine isothiocyanate. Immunostaining of rat brain sections after i.c. delivery of Neu (human Her-2 equivalent) in RG2-bearing rats was performed with antirabbit Neu and secondary antirabbit IgG conjugated with tetramethylrhodamine isothiocyanate. In contrast, immunostaining of rat brain sections after i.c. delivery of Her-2 MAb in GBM tumor-bearing rats was detected using a Zenon Immunostaining kit (Molecular Probes).

RT-PCR Analysis. RNA was isolated from endothelial (HBMEC, RBEcs), and tumor (RG2, GBM) cells and tissues samples and purified using TRIzol (Invitrogen Life Technologies, Inc., Carlsbad, CA), following the manufacturer’s instructions. First-strand synthesis of cDNA using random hexamers was performed in 2-μg aliquot of DNase I-treated total RNA using Superscript II. Subsequently, 4 μl of cDNA were amplified in a standard 50-μl PCR reaction (at appropriate conditions) containing 0.12 μmol/liter of each primer. An aliquot (20 μl) of the RT-PCR product was analyzed on a 1% agarose gel and visualized using ethidium bromide. The locations of the primers indicated are based on the subunit sequence information obtained from GenBank: K_{ir}6.2 subunit of K_{ATP} channel (human; accession no. D50582). The following primers were used for amplification of K_{ir}6.2 subunit of K_{ATP} channel (5'-GTACCGAGCCATGCTGCCCC-3' and 5'-GGGGGC-CCAGAGACCATTGCTCA-3'), and β-actin (5'-AATCTGGAACACCATCCTTAC-3' and 5'-CTCTCTCTTAATGGCAGCAG-3').

Confocal Microscopy. Immunoreactive visualization of K_{ATP} channels, Her-2, and vWF was performed as described previously (4, 5). In vivo, confocal images were captured using a Leica (Heidelberg, Germany) True Confocal Scanner Spectrophotometer laser scanning confocal microscope (inverted) equipped with argon (488 nm) and krypton (568 nm) lasers. Fluorescent signals for K_{ATP} and Neu/Her-2 expressed on cultured rat and human endothelial cells, brain tumor cells, and brain tumor-bearing rat brain sections were visualized using the 488-nm argon laser line, and fluorescent signals on vWF were visualized using the 568-nm krypton laser line. Fluorescence signals for flour-488 or -568 were displayed individually as green and red pseudocolor projections or merged as overlap projections to visualize possible colocalization of two different antigens within specific subcellular structures.

[3H]-Glibenclamide Binding Study. The densities of K_{ATP} channels in rat brain endothelial and tumor cells and in human normal brain and tumor tissue were determined by [3H]-Glibenclamide binding study. To quantify K_{ATP} channel density in vitro, RG2 cells alone or cocultured with GBM cells, RG2 tumor tissue, HBMEC alone or cocultured with GBM, and normal human brain and GBM tissues were incubated with glibenclamide (5 μM) and [3H]-glibenclamide (5 nM) for 1 h in sodium phosphate buffer (10 mM, pH 7.4). The assay was modified from Chullin-Rogers et al. (25). Samples were washed three times with sodium phosphate buffer, and cells/tissue were lysed with ice-cold sodium phosphate buffer, mixed with liquid scintillation fluid, and counted on a liquid scintillation counter. The actual binding of [3H]-glibenclamide was calculated by measuring the difference between nonspecific and specific bindings in three separate experiments conducted in duplicate.

Membrane Potential Assay. Functional activities of putative K_{ATP} channels in HBMEC and GBM cells were measured by detecting changes in voltage across the cell membrane, as described previously (4). We used the membrane potential assay kit (Molecular Devices), which provides a fast, simple, and parallel membrane- and cell-based brief, HBMECs and GBM cells obtained from cell passages 2–5 were suspended in 5 ml of growth medium. In two separate experiments, the cell density was adjusted to 1 × 10^6 cells/well and coated in sterile, clear-bottom, black 96-well plates (Corning Inc., Acton, MA), precoated with poly-l-lysine, and allowed to achieve a monolayer within 24 h. The monolayer cells were incubated with the membrane potential assay kit reagents for 30 min and read directly by FLEXstation. The anionic potentiometric dye that traverses between cells and the extracellular solution in a membrane potential-dependent manner serves as an indicator of vasomodulator-induced voltage changes across the cell membrane. To determine the optimum dose that elicits detectable voltage changes across HBMEC and GBM cell membranes, dose-response studies were performed with 0–50 μM K_{ATP} channel agonist MS and K_{ATP} channel antagonist glibenclamide (100 nM), using the Spectrofluorometer set to the following parameters: excitation/emission (530/565), and emission cutoff (550 nm) wavelengths. Additionally, the effects of the optimum dose (10 μM) of MS and glibenclamide (100 nM) on K_{ATP} channel activity in HBMECs and GBM cells were determined.

GFP-Adenoviral (GFP-Adv) and erbB-2 Antibody Delivery. GFP-Adv constructs were prepared as described by Smith et al. (26), with slight modifications. The GFP gene was first cloned into a shuttle vector, pAdTrack-CMV, and the resultant plasmid was linearized by digesting it with restriction endonuclease Pme I and subsequently cotransformed into Escherichia coli cells with an adenoviral backbone plasmid, pAdEasy-1. Recombinant plasmids were transfected into 293 cells. The presence of recombinant adenoviruses was verified by RT-PCR. GFP-Adv (1 × 10^9 pfu/ml) was infused i.c. with or without MS in rats with implanted GBM. Neur rabbit polyclonal and Her-2, which are specific for rat and human erbB-2 receptors, respectively, were used in this study. Both antibodies were dialyzed using M100,000 cutoff dialysis tubing (0.5-ml capacity, 10-μm pore size; Spectrum Laboratories) to remove BSA and other additives. MS (30 μg/kg/min) was infused (i.c.) for 15 min, followed by i.c. infusion of Neu or Her-2 MAb (1 mg/ml/kg) or GFP-Adv (1 × 10^9 pfu/ml) for 45 min. After 2 h, rats (n = 2) were perfused fixed with 4% paraformaldehyde transcardially, and the brain removed. Her-2 MAb bound to Her-2 receptors in vivo was detected by incubating rat brain cryosections as per the manufacturer’s (Molecular Probes) procedure with Alexa Flour-647-conjugated secondary antibody (Zion labeling kit) that specifically binds to the IgG fragment of Her-2 MAb. Neur antibody binding to erbB-2 receptors in RG2 brain tumor sections was detected using antirabbit IgG conjugated with tetracyethylrhodamine isothiocyanate. To investigate whether there was Adv-GFP delivery and expression of GFP in tumor cells, another group of rats (n = 2) were transcardially perfused fixed with 4% paraformaldehyde after 96 h, brains were removed, and cryosections were imaged for the presence of GFP by confocal microscopy. Furthermore, to demonstrate the expression of GFP on glial tumor cells, GFP was colocalized with GFAP.

Transmission Electron Microscopy. Seven days after RG2 tumor cell implantation, rats (n = 3/group) were infused i.c. with PBS, BK (10 μg/kg/min in 0.5% DMSO), NS-1619 (30 μg/kg/min in 0.5% ethanol), or MS (30 μg/kg/min in 0.5% DMSO) for 15 min. Rats were infused with 10 ml of cold PBS and perfuse fixed with 250 ml of 1% glutaraldehyde in PBS (pH 7.4) through the heart. Tumor-bearing brains were removed, and 1-μm tissue sections encompassing tumor mass, brain surrounding the tumor, and normal brain were cut, and samples were processed for transmission electron microscopy analysis (JEOL electron microscope operating at 80 kV), as described previously (4).

Quantitative Analysis. At least 10 profiles of capillaries from each group sectioned transversely and photographed at low magnification (∼7200) were evaluated for their general features, as described previously (4). Briefly, micrographs were placed on a digitizing screen, and structural features were measured using Scan Pro 4, a computer-assisted, image analysis system (Jandel Scientific, Corte Madera, CA). Abluminal and luminal circumferences, areas of endothelial cytoplasm excluding nuclei and vacuoles, and mean thickness of endothelial cytoplasm were measured and compared with those in the control group. The mean thickness of endothelium was calculated by subtraction of the luminal radius from the abluminal radius, which was obtained from the areas circumscribed by the abluminal and abluminal circumference, respectively. The proportion of total vesicular area to the cytoplasmic area was expressed as a percentage to derive another parameter to characterize vesicular transport.

Survival Study. Wistar rats with implanted intracranial tumors were used to study the effect of MS on increased CPM delivery and survival. RG2 cells (1 × 10^6) were implanted intracranially to form a tumor (2 mm in size) in rats within 1 week. After 1 week, rats were given saline, CPM (5 mg/kg), or CPM after 15 min of MS (30 μg/kg/min for 15 min) infusion through an exteriorized catheter once a day for 3 consecutive days. The rats were monitored carefully for mortality and clinical signs attributable to brain tumor growth for 90 days or until death, whichever came first. Brains of dead or moribund rats or those that survived beyond 90 days were removed, frozen, and cryosectioned for histological evaluation, to compare tumor volumes between treated and untreated.
treated groups. Kaplan-Meier analysis was performed to determine the statistical significance of this study.

**Statistics.** Results are expressed as mean ± SD, where applicable. For all *in vivo* permeability studies, we used *n* ≥ 4 rats/group, unless stated otherwise. Unpaired two-tailed Student’s *t* tests were used to compare the control and treated groups. The statistical analyses of *K*<sub>i</sub>, vesicle density, vesicular area, cleft index, and cleft area index comparison among different groups, with or without drug treatment, were performed using ANOVA, followed by either unpaired parametric analysis of Student’s *t* test or by nonparametric analysis of the Mann-Whitney *U* test. *P* < 0.05 was considered statistically significant. Statistical analysis of the vesicular density and proportion of cumulative vesicular area compared the effect of i.c. infusion of MS with that of PBS infusions.

**RESULTS**

**BBB Capillaries Differ from BTB Capillaries.** Fig. 1 shows the differences in the response of the BBB and BTB to *K*<sub>ATP</sub> channel agonists. BTB capillaries and surrounding tumor cells overexpress *K*<sub>ATP</sub> channels and, therefore, may readily respond to activation by *K*<sub>ATP</sub> channel agonists. In contrast, *K*<sub>ATP</sub> channels are hardly detected in normal brain microvessel endothelial cells, which, therefore, may not respond to *K*<sub>ATP</sub> channel agonists.

**K*<sub>ATP</sub> Channels Mediate MS-induced BTB Permeability Increase.** BTB permeability, *K*<sub>i</sub> (μl/g/min), was measured by QAR of cryosections obtained from RG2 or GBM tumor-bearing rat brains after the injection of a [14C]-labeled tracer, 5 min after i.c. MS infusion. To determine whether *K*<sub>ATP</sub> channels mediate MS-induced BTB permeability increase, rats with implanted intracerebral RG2 tumors received i.c. MS infusion alone or with glibenclamide. *K*<sub>i</sub> was determined for radiotracer [14C]-AIB in the tumor core, tumor-adjacent brain tissue, and contralateral brain tissue. Comparison of pseudocolor-enhanced autoradiographs of rat brain sections showed enhanced delivery of [14C]-AIB on i.c. MS infusion (Fig. 2A). Glibenclamide coinfusion blocked MS-induced [14C]-AIB uptake (Fig. 2A). After i.c. infusion of MS (30 μg/kg/min) for 15 min, *K*<sub>i</sub> significantly increased in the tumor center (32 ± 5 μl/g/min; *P* < 0.001) compared with PBS controls (10.5 ± 1.5 μl/g/min). MS-induced increase in BTB permeability was significantly inhibited (12.2 ± 3.0 μl/g/min; *P* < 0.01) by coinfusion of glibenclamide (5 μg/kg/min for 15 min; Fig. 2B). MS-induced *K*<sub>i</sub> increase was not blocked by iberio-
Intracarotid infusion of MS (30 μg/kg/min) also significantly increased BTB permeability (52 ± 8 μl/g/min; P < 0.001), which was attenuated (24 ± 6 μl/g/min; P < 0.01) when glibenclamide (5 μg/kg/min for 15 min) was coinfused with MS (Fig. 2C). In contrast to the tumor center, MS with or without glibenclamide did not significantly affect BBB permeability in normal brain surrounding tumor (2-mm area outside tumor margin) or in normal contralateral brain (Fig. 2C). Similarly, when infused alone or before MS infusion, glibenclamide did not affect BBB or BTB permeability (data not shown).

**Time Course.** The present QAR study in rats with implanted RG2 tumor showed that i.c. infusion of MS (30 μg/kg/min) significantly (P < 0.001) enhanced sustained delivery of [14C]-AIB to the tumor for 15-, 30-, and 60-min infusions (Fig. 3A). We found that the ability of MS to sustain BTB permeability increase up to 60 min was consistent with our reported data on NS-1619 in a similar model (4).

In contrast, a 30- or 60-min infusion of BK failed to sustain the initial increase of K_i (P < 0.001) attained at 15 min (Fig. 3A). For comparative purpose, we infused a similar molar concentration of BK and NS-1619.

**K_ATP Channel Activation Elicits Increased Delivery of Molecules of Various Sizes.** Intracarotid infusion of MS (30 μg/kg/min) increased BTB permeability in implanted intracerebral RG2 tumor to various-sized radiotracers that normally fail to cross the BTB, including hydrophilic compounds such as [14C]-labeled AIB, dextran, and a chemotherapeutic agent, CPN. Coinfusion with MS significantly enhanced delivery of [14C]-labeled AIB, dextran, and CPN (Fig. 3B). In contrast, [14C]-CPN delivery was negligible in vehicle-treated rats. These studies further suggest that K_ATP channel-mediated increases in BTB permeability allow delivery of drugs of various molecular sizes, including AIB (M_r 103,000), CPN (M_r 361,000), and dextran (M_r 70,000), suggesting that the effect is independent of molecular size (Fig. 3B).

**Synergistic Effect of K_Ca and K_ATP Channel Agonists on BTB Permeability.** To investigate whether K_ATP and K_Ca channel agonists exert a synergistic effect on BTB permeability increase, MS and NS-1619 were coinfused i.c. for 15 min. This combination signifi-
investigated whether MS increases Neu, Her-2 MAb, and GFP-Adv delivery to rat brain tumor \textit{in vivo}. In this study, we used RG2 (Neu positive) tumor-bearing Wistar rats and GBM (Her-2 positive) tumor-bearing athymic nude rats. MS infusion enhanced delivery of Her-2 MAb selectively to GBM and of Neu to RG2 (Fig. 5A) tumor tissues without any delivery to contralateral brain tissues (Fig. 5A). In contrast, very little Her-2 MAb or Neu was delivered to tumor tissue in vehicle-treated rats (Fig. 5, Aa and Ab). MS also enhanced adv-GFP delivery to brain tumors. Abundant GFP expression was seen in brain tumor cells in rats coinfused with MS and adv-GFP (Fig. 5Bb) but not in the tumor periphery (TP). In addition, GFP expression was observed predominantly on the tumor cells because GFP colocalized with GFAP (Fig. 5Bi). In contrast, adv-GFP infused alone failed to cross the BTB and, therefore, negligible GFP expression was detected on tumor cells. Furthermore, GFP expression was observed in the cerebral blood vessels but not in the tumor cells because the GFP did not colocalize with GFAP (Fig. 5Bl).

\textbf{K}_{\text{ATP}} Channel-mediated BTB Permeability Modulation and CBF.} Previously, we showed that i.c. infusion of low doses of \(K_{\text{ATP}}\) channel activators such as BK and NS-1619 did not alter CBF in tumor and normal brain (4). In the present study, using a laser-operated Doppler, we showed that i.c. administration of 30 \(\mu\)g/kg/min MS did not significantly affect CBF (Table 1), although BTB permeability increased in the tumor area. Mean arterial blood pressure in the drug-treated groups was not significantly different from the vehicle-treated group (Table 1).

\textbf{Tumor Cells Increase \(K_{\text{ATP}}\) Channel Expression in Endothelial Cells.} The specific binding of \(^{3}H\)-glibenclamide in the membranes of tumor cells and tissues was significantly higher than in membranes prepared from normal endothelial cells and brain tissue (Fig. 6A). When cocultured with tumor cells, endothelial cells showed increased \(^{3}H\)-glibenclamide binding compared with endothelial cells and tumor cells alone (Fig. 6A), suggesting an increase in \(K_{\text{ATP}}\) channel density distribution in the cocultured cells, possibly influenced by signals arising from the tumor cells. An increased \(^{3}H\)-glibenclamide binding in GBM compared with normal brain tissue was also observed (Fig. 6A). RT-PCR analysis also showed the influence of tumor cells on \(K_{\text{ATP}}\) channel mRNA expression in endothelial cells cocultured with RG2 and GBM primary cells (Fig. 6B). Western blot analysis (Fig. 6C) and \(K_{\text{ATP}}\) channel activity assay by potentiometry (Fig. 6, D and E) confirmed the RT-PCR results and also indicated that tumor cells may induce overexpression of \(K_{\text{ATP}}\) channels in endothelial cells.

\textbf{\(K_{\text{ATP}}\) Channel Activity.} The functional activity of putative \(K_{\text{ATP}}\) channels in a monolayer of HBMVECs and GBM cells was determined by measuring their membrane potential in response to MS at various concentrations. The depolarization action of MS was highly pronounced in GBM when compared with HBMVECs (Fig. 6D). The membrane potential decreases in HBMVECs and GBM cells in response to the addition of MS, and a return to resting membrane potential with the addition of glibenclamide was measured spectrophotometrically using potassium ion-fluorescent dye. These results demonstrate \(K_{\text{ATP}}\) channel activity in HBMVECs and GBM cells in response to MS. Furthermore, endothelial cells, when cocultured with tumor cells, exhibited higher activity than endothelial or tumor cells alone (Fig. 6E). This finding suggests the presence of higher \(K_{\text{ATP}}\) channel density distribution on endothelial cells that were grown with brain tumor cells than on endothelial cells alone. This observation confirmed similar results obtained by RT-PCR, Western blot, and \(^{3}H\)-glibenclamide binding experiments (Fig. 6A). In other experiments, we observed that MS caused a dose-dependent decrease in \(K^{+}\) dye-specific fluorescence intensity in RBECs, HBMVECs, and RG2.

\textbf{ErbB-2 Antibody and GFP-Adv Delivery across the BTB.} ErbB-2 expression was demonstrated in RG2 and GBM \textit{in vitro} and \textit{in vivo} (Fig. 4). Low Her-2-expressing MCF-7 (human breast tumor cells) were used as a negative control. Furthermore, the glial origin of RG2 and GBM was demonstrated by GFAP expression \textit{in vitro} and \textit{in vivo} (Fig. 4B). After studying the ability of MS to increase BTB permeability to allow the delivery of various-sized molecules, we
and GBM cells that was reversed by glibenclamide administration (data not shown).

**Immunolocalization of K\textsubscript{ATP} Channels.** We next asked why and where K\textsubscript{ATP} channel modulators selectively induce BTB permeability without affecting BBB permeability. Our hypothesis was that such a selective effect might be because of increased expression of K\textsubscript{ATP} channels in tumor capillaries and tumor cells compared with normal brain. To address this issue, we used anti-K\textsubscript{ir}6.2 subunit (the pore-forming) antibody to immunolocalize K\textsubscript{ATP} channels in paraformaldehyde perfusion-fixed GBM and RG2 tumor-bearing rat brain sections. This analysis of rat brain or human brain tumor sections for expression of K\textsubscript{ATP} channels and endothelial cell marker vWF by two-color immunocytochemistry indicated that vWF-positive tumor vessels (red) were also positive for K\textsubscript{ATP} channels (green). We demonstrated K\textsubscript{ATP} channel expression on the plasma membrane of endothelial, RG2, and GBM cells. We also observed more intense immunostaining for K\textsubscript{ATP} channels in tumor cells (Fig. 7Ab) compared with normal endothelial cells (Fig. 7Aa). Microvessels positive for both antigens are shown in yellow (Fig. 1 and Fig. 7, B and C). We also sought to determine whether K\textsubscript{ATP} channels are expressed differentially and more abundantly on tumor cells than in normal brain, which might explain the MS-induced-selective BTB permeability increase. The immunolocalization study of normal brain sections showed some positive expression for K\textsubscript{ATP} channels in noncapillary cells, whereas no positive K\textsubscript{ATP} channel expression was observed in normal human brain endothelial cells (Fig. 1). However, a robust expression of K\textsubscript{ATP} channels in GBM cells (Fig. 7Ab), tumor capillary endothelium (Fig. 7, Bb and Bc), and rat brain tumor capillary endothelial cells (Fig. 7, Cb and Cc) compared with low K\textsubscript{ATP} channel colocalization in normal brain capillaries (Fig. 1) was observed. These
results strongly suggest that the selective BTB permeability effects of MS and MS-induced BTB permeability increase attenuation by glibenclamide is attributable to the increased density distribution of $K_{\text{ATP}}$ channels on brain tumor capillary endothelium and tumor cells compared with normal brain capillary endothelium and normal brain cells.

**Vesicular Transport.** We further investigated whether vesicular transport is largely responsible for enhanced delivery of drugs and macromolecules across the BTB. We used transmission electron microscopy to demonstrate that no changes occurred in the normal capillary endothelium of contralateral brain tissue after i.c. MS infusion alone. Abundant Her-2 MAb binding was observed in the tumor center (TC) and tumor periphery (TP), suggesting that infiltrating tumor cells overexpress Her-2 receptors. Enhanced delivery of Neu was observed in TC when coinjected with MS (e and f), whereas a small amount of Neu was delivered when infused alone. B, the ability of MS to increase delivery of adenoviral-green fluorescent protein (Adv-GFP) across the blood-tumor barrier was studied in nude rats with intracranial GBM xenografts. Abundant GFP expression was seen predominately in the TC and to a small extent in the TP (g). GBM cells are shown expressing glial fibrillary acidic protein (GFAP; h), and GFP was colocalized with GFAP, suggesting that GFP was predominantly expressed on tumor cells (i). In vehicle- and Adv-GFP-infused rat, however, hardly any GFP expression was detected on tumor cells but was observed trapped in blood vessels (j), possibly because of the inability of Adv-GFP to cross the blood-tumor barrier. Furthermore, GFP did not colocalize with GFAP on tumor cells, indicating that Adv-GFP did not infect tumor cells.
The physiological parameters were determined either before or during vasomodulator infusion. The values are mean ± SD of n = 4 rats, except for cerebral blood flowmetry (CBF), in which three representative rats per group were used. A dose of MS and NS-1619 (30 μg/kg/min) that did not appreciably alter mean arterial blood pressure (MAP) in rats was selected for BTB permeability studies by quantitative radiographic method.

<table>
<thead>
<tr>
<th>Groups</th>
<th>pH</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>CBF (change vs. vehicle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7.3 ± 0.02</td>
<td>87 ± 4</td>
<td>42 ± 2</td>
<td>90 ± 5</td>
<td>0</td>
</tr>
<tr>
<td>MS-treated (30 μg/kg/min)</td>
<td>7.38 ± 0.01</td>
<td>90 ± 5</td>
<td>45 ± 1</td>
<td>83 ± 4</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>MS + Glb (30 + 10 μg/kg/min)</td>
<td>7.35 ± 0.02</td>
<td>88 ± 5</td>
<td>49 ± 3</td>
<td>81 ± 5</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>MS + NS-1619 (both 30 μg/kg/min)</td>
<td>7.30 ± 0.02</td>
<td>92 ± 5</td>
<td>44 ± 4</td>
<td>75 ± 5</td>
<td>12 ± 2</td>
</tr>
</tbody>
</table>

*pH, arterial pH; PaO₂, arterial oxygen; PaCO₂, arterial carbon dioxide; MS, minoxidil sulfate; Glb, glibenclamide.

**Table 1 Physiological measurements during blood-brain tumor barrier (BTB) permeability determination**

**DISCUSSION**

**Therapy for Brain Tumors.** The prognosis for patients with GBM is extremely poor, with a median survival of 9 months, primarily because of a paucity of effective treatment options. Although some agents have proved effective against tumors outside the brain, impaired drug delivery across the BTB limits drug delivery to primary (27) and metastatic brain tumors (28). Usually, primary brain tumors are intrinsically resistant to drugs, only adding to the problem of inadequate drug delivery across the BTB. In contrast, metastatic brain tumors that spread to the brain are sensitive to anticancer drugs. The BTB, however, prevents the delivery of anticancer drugs in sufficient amounts to achieve any therapeutic benefit. Therefore, improved delivery of anticancer agents to brain tumors may greatly improve the prognosis of patients with metastatic brain tumors. Breast and lung cancers, which together account for 70% of brain tumor metastases, are the most frequent tumors to spread to the brain. Some 70% of patients with non-small cell lung cancers respond to chemotherapy using CPN/etoposide. The response rate, however, drops to 10–30% for non-small cell lung cancer patients with brain metastases, because the anticancer drugs fail to reach the tumor in the brain. Delivering...
anticancer drugs to the brain is far more difficult than delivering such drugs to elsewhere in the body. For example, 88% of patients with Her-2-positive breast cancer develop bone metastasis, and 33% develop brain metastasis. When breast cancer patients receive Trastuzumab, however, only 4% develop bone metastasis but 28% still develop brain metastasis. The difference, for the most part, is because of the difficulty of delivering anti-cancer drugs across the BBB/BTB to brain tumor.

**BBB Capillaries Differ from BTB Capillaries.** We demonstrated that brain tumor capillaries overexpress \( K_{Ca} \) channels (4, 5). These findings are consistent with other studies that showed overexpression of vascular proteins (29, 30), such as angiogenic vascular endothelial growth factors, fibronectin, and \( \alpha \beta \) integrins in tumor capillaries. The BTB is structurally and functionally different from the BBB (3, 10, 31, 32). Recent studies (4, 5, 32), however, have shown that certain proteins are specifically expressed or overexpressed in tumor capillaries. We showed that tumor cells overexpress BK type 2 receptors (14), whereas both tumor and tumor capillary endothelial cells overexpress \( K_{Ca} \) channels (4, 5) and protein kinase-G (32) and \( K_{ATP} \) channels, rendering them potential targets for biochemical modulation of BTB permeability. Although, the magnitude of differences in protein expression between normal and tumor capillaries is qualitative, using a series of complimentary studies, we quantitatively showed that tumor capillaries have increased \( K_{ATP} \) channel density, activity, and/or responsiveness to MS.

**Biochemical Modulation of BTB Permeability.** Vasomodulators, such as BK, nitric oxide donors, soluble guanylate cyclase activators, and NS-1619, increase BTB permeability via \( K_{Ca} \) channels (4). MS

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Fig. 7. Immunocytochemistry. A, confocal microscopic immunolocalization of ATP-sensitive potassium (\( K_{ATP} \)) channels (green) and von Willebrand factor (red) in human brain microvascular endothelial cells (HBMVECs; \( a \)), glioblastoma multiforme (GBM) cells (\( b \)), and rat glioma cells (\( c \)). B, colocalization of \( K_{ATP} \) channels in GBM tissue section. Representative image (×20) of a GBM tissue section showing the number of capillaries immunostained with von Willebrand factor (vWF; \( Ba \)), the abundant expression of \( K_{ATP} \) channels (green) in tumor microvessel as well as in tumor cells (\( Bb \)), and these \( K_{ATP} \) channels colocalizing (\( Bc \)) with vWF on capillary endothelial cells (yellow). C, Similarly, colocalization of \( K_{ATP} \) channels in rat glioma tumor capillary endothelial cells was also observed. Control experiments were performed with secondary antibody but without primary antibody.
K<sub>ATP</sub> Channels Regulate BTB Permeability.

**K<sub>ATP</sub> Channels in Cerebral Vasculature.** The presence and role of K<sub>ATP</sub> channels in normal cerebrovascular endothelium have been described (34, 35), but their role in BBB/BTB permeability has not been elucidated. We demonstrated (Figs. 6, A and B, and 7) abundant expression of K<sub>ATP</sub> channels in tumor cells (in vitro and in vivo) in contrast to normal astrocytes and endothelial cells. Confocal images clearly revealed K<sub>ATP</sub> channel overexpression on tumor and endothelial cells compared with normal brain. Importantly, the tumor capillaries (Fig. 7, B and C) showed abundant expression of K<sub>ATP</sub> channels as the K<sub>ATP</sub> channels colocalize with vWF in tumor capillary endothelial cells. Increased presence of K<sub>ATP</sub> channels (Fig. 6, A–C) and their activity in endothelial cells (Fig. 6, D and E), possibly by tumor cell-induced signaling, was observed when endothelial cells were cocultured with tumor cells. Others also reported increased K<sub>ATP</sub> channel activity in pathological conditions such as hypoxia (36), which might also be true in tumors, because tumors thrive in hypoxic environments. In normal brain capillaries, however, K<sub>ATP</sub> channels were barely detectable even when overexpressed K<sub>ATP</sub> channels were detected in tumor capillaries (Fig. 1). This unique feature of tumor capillaries offers a mechanism that can be exploited to alter tumor capillary permeability selectively without concomitant effects on normal brain.

**Brain Tumor Cells Induce K<sub>ATP</sub> Channel Overexpression.** In rat and human brain tumor cells, we demonstrated the functional activity of K<sub>ATP</sub> channels in normal and tumor cells alone and in cocultures. MS elicited higher membrane potential changes in tumor cells than in normal cells, suggesting greater density distribution or sensitivity of K<sub>ATP</sub> channels in tumor cells than in normal cells. Furthermore, in vitro [H]<sub>3</sub>glibenclamide binding studies showed that K<sub>ATP</sub> channel density is significantly higher in tumor than normal cells/tissue (Fig. 6A). We concluded that K<sub>ATP</sub> channels are overexpressed both on tumor cells and tumor capillary endothelial cells compared with those of normal brain, which might explain why MS selectively increases BTB permeability while leaving the BBB unaffected. Because of the observed synergistic effect of K<sub>Ca</sub> and K<sub>ATP</sub> channel agonists on BTB permeability, K<sub>ATP</sub> channels are an additional target besides K<sub>Ca</sub> channels (4, 5) for BTB permeability modulation to enhance drug delivery to brain tumors.

**Mechanism of Increased Transport: Pinocytotic Vesicles or via Tight Junctions?** Our results demonstrate that MS induces accelerated formation of transport vesicles in both brain tumor capillary endothelium and tumor cells by MS-induced activation of K<sub>ATP</sub> channels (Fig. 8A). Therefore, vesicular transport is largely responsible for enhanced delivery of drugs across the BTB rather than via the opening of endothelial tight junctions. This finding is consistent with our previous study (4), in which we reported that a K<sub>Ca</sub> channel agonist, NS-1619, increased the density of rat brain tumor microvessel endothelial vesicles. A slight increase in basal BTB permeability might be attributable to a small increase in the number of pinocytotic vesicles and the tight junctional cleft index (37, 38). We found a direct relationship between an increase in the number of brain tumor capillary endothelial vesicles and increased BTB permeability. Importantly, we observed that rat brain tumor capillary endothelial cells form far more vesicles (Fig. 8B) than normal brain capillary endothelial cells without altering the endothelial tight junctions in response to vasomodulators, such as MS (Fig. 8C) or NS-1619 (4).

**Enhanced Survival.** In a previous study, we showed that CPN enhanced survival when rats with intracranial glioma were cotreated with NS-169 (5) or BK (13). In the present study, we showed that i.c. MS infusion selectively enhanced [14]C carboplatin delivery to tumor tissue without increasing delivery to normal brain (Fig. 3B). We also

Can also biochemically modulate K<sub>ATP</sub> channels, resulting in a significant BTB permeability increase, allowing the delivery of hydrophilic tracers, GFP-Adv vectors, and Her-2 antibodies specifically to brain tumor tissue. Glibenclamide coinfusion with MS attenuated MS-induced BTB permeability. The efflux of glibenclamide may be a biochemical target besides K<sub>ATP</sub> channels for BTB permeability modulation to enhance drug delivery to brain tumors.

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showed that MS coinfusion with CPN in rats resulted in tumor regression, significantly increasing survival (Fig. 8, A and B). Primary brain tumors, particularly GBM, frequently have altered genes resulting in tumor cell proliferation, as well as poor prognosis and patient survival (39–41). In particular, Her-2, which is overexpressed in 17–20% of GBMs (20), facilitates tumor cell proliferation (41). Trastuzumab and 2C4, developed by Genentech Inc., are potential Her-2 receptor-based therapies for gliomas. The molecular sizes of these MAbs, however, prevent their efficient delivery across the BTB to tumor. We demonstrated that MS-induced biochemical modulation of K<sub>ATP</sub> channels enhanced delivery of macromolecules, including Her-2 MAb, selectively to tumors without increasing MAb delivery to normal brain in a GBM xenograft model (Fig. 5A). This finding suggests that therapeutic antibodies could be efficiently and selectively directed to glioma cells in vivo. In addition to MAb therapy, gene therapy for GBM is emerging as a potential treatment strategy. Efficient and selective delivery of adeno viral vectors to tumor across the BTB, however, is difficult when viral products are administered through an intravascular route because of the difficulty of getting such vectors across the BTB. In this study, we demonstrated enhanced and selective GFP-Adv delivery across the BTB after i.c. coinfusion with MS (Fig. 5B). This strategy may be useful clinically to deliver therapeutic antibodies with a chemotherapeutic drug or gene product selectively to brain tumor while leaving healthy brain intact.

**Summary.** Taken together, our findings demonstrate that K<sub>ATP</sub> channels are effective targets for BTB permeability modulation. It is conceivable that other types of potassium channels may play a role in BTB permeability regulation, which remains to be thoroughly investigated. This study presents evidence that activation of K<sub>ATP</sub> channels by specific agonists, such as MS, can sustain enhanced drug delivery selectively to tumors. Specifically, we showed that CPN delivery to brain tumors can be increased using MS, resulting in enhanced survival in rats with intracranial tumors. Furthermore, MS-induced BTB permeability modulation allows delivery of macromolecules (such as dextran, Her-2 MAb, and GFP-Adv) selectively to brain tumor. In conclusion, our results confirm that selective and enhanced delivery of
small and macromolecules across brain tumor microvessels after \( K_{\text{ATP}} \) channel activation can be exploited to increase BTB permeability and enhance drug delivery to brain tumor. This study may have significant implications for improving targeted delivery of antineoplastic agents to brain tumors and neuropharmacuetics to diseased brain regions while leaving normal brain unaffected.

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Nagendra S. Ningaraj, Mamatha K. Rao and Keith L. Black


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