Expert Opinion

Drug delivery to brain tumours: challenges and progress

Nagendra S Ningaraj
Curtis and Elizabeth Anderson Cancer Institute, Memorial Health University Medical Center, 4700 Waters Avenue, Savannah, GA 31403, USA

Nearly 12.5 million new cancer cases are diagnosed worldwide each year. Although new treatments have been developed, most new anticancer drugs that are effective outside the brain have failed in clinical trials against brain tumours, in part due to poor penetration across the blood–brain barrier and the blood–brain tumour barrier. This review will discuss the challenges of drug delivery across the blood–brain barrier/blood–brain tumour barrier to cancer cells, as well as progress made so far. This will include a biochemical modulation strategy that transiently opens the barrier to increase anticancer drug delivery selectively to brain tumours. It will also briefly discuss a quantitative non-invasive method to measure permeability changes and tumour response to treatment in the human brain.

Keywords: blood–brain barrier, blood–brain tumour barrier, brain tumour, drug delivery, magnetic resonance imaging, potassium channels

Expert Opin. Drug Deliv. (2006) 3(4):499-509

1. Introduction

Every year in the US, ~ 20,000 new primary and nearly 200,000 secondary (metastatic) brain tumour cases are reported. Worldwide numbers are more distressing. Even after surgical resection, brain cancer invariably recurs, severely shortening life expectancy [1]. Conventional treatment using radiation and intravenous chemotherapy often prove unsuccessful primarily because the anticancer drugs fail to cross the blood–brain barrier (BBB) in sufficient quantities [2]. Therefore, understanding the biochemical regulation of the BBB in its normal and abnormal states (in and around tumours) is of great importance as efforts continue to deliver therapeutic compounds to brain cancers. The focus is now on targeted cancer therapy by not only supplementing conventional chemotherapy and radiotherapy, but also by preventing toxicity in normal tissues and drug resistance. In particular, successful treatment of brain tumours involves efficient anticancer drug delivery to brain tumours across the blood–brain tumour barrier (BTB). Although the BTB is 'leaky' in the tumour centre, the established microvessels (capillaries) feeding the proliferating tumour edge and the brain tissue surrounding the tumour is nearly as impermeable as the BBB [3]. Therefore, the BTB still poses a major obstacle to anticancer drug delivery to tumours. This article reviews the challenges involved in and the progress made, especially in the past decade, towards delivering therapeutic drugs selectively to brain tumours.

The cerebral microvessels/capillaries that form the BBB protect the brain from toxic agents in the blood but also pose a significant hindrance to the delivery of small and large therapeutic molecules. Pardridge reported that the BBB blocks delivery of > 98% of CNS drugs [2,4]. The National Institutes of Health (NIH) cited as high priority goals to understand the function of the BBB and BTB, develop novel drug delivery approaches for molecular-targeted therapy, and to further develop methods to non-invasively image the response of brain tumours to treatment. Different strategies have been developed to circumvent the physiological barrier that is posed by the BBB, often based on a conception of the barrier as being controlled by

informa
healthcare

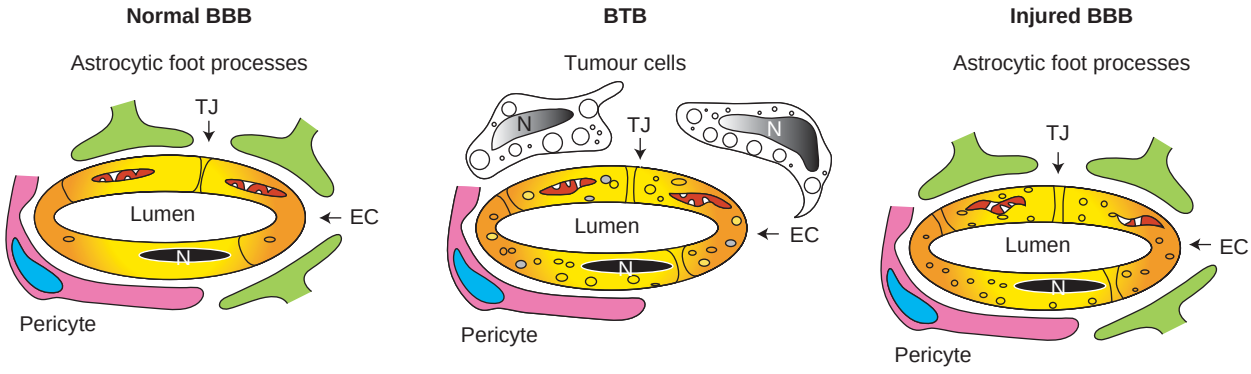

Figure 1. Representative structures of the BBB in normal and abnormal situations, such as brain tumour and ischaemia, are shown. Notice in the ischaemic brain, TJs are significantly altered, causing disruption of BBB integrity. The BTB lack astrocytic foot processes, which is important for reinforcing the BBB integrity. Therefore, a compromised BBB in stroke and brain tumour allows undesired molecules into the brain parenchyma, leading to severe neuropathology. From previous studies, it seems that the BTB and injured BBB are sensitive to potassium channel agonists, unlike the normal BBB, which is insensitive.
BBB: Blood–brain barrier; BTB: Blood–brain tumour barrier; EC: Endothelial cell; N: Nucleus; TJ: Tight junction.

what is called the neurovascular unit. This consists of endothelial cells (ECs), tight junctional proteins connecting the ECs, glia, pericytes and astrocytic foot processes, which interact with neurons (Figure 1). For the most part, research seeks to understand the interaction among the constituents of the BBB and neurons in normal and pathological conditions [5]. By using *in vitro* and *in vivo* models, researchers seek to achieve a better understanding of the effects of neurological disorders on the BBB and, thereby, improve our knowledge of BBB biology. The goal is to better comprehend the initiation and progression of neurological disease and to develop approaches to effectively treat brain diseases such as brain tumours.

Novel cancer therapies include antiangiogenic agents, immunotherapy, bacterial agents, viral oncolysis, cyclin-dependent kinases and receptor tyrosine kinase inhibitors, antisense agents, gene therapy and combinations of various methods. Amazing clinical success in treating some types of cancer has been achieved using immunotherapy-based anticancer agents, such as cytokines, monoclonal antibodies and cancer vaccines. For example, one promising treatment uses antisense oligonucleotides, such as small interfering RNAs, which have been used in various clinical trials for cancer, but only for cancers outside the brain [6]. Despite these promising approaches, the BBB still causes a significant complication to brain cancer treatment. As whole-brain gene microarrays have detected fewer BBB-specific transcripts [7], the focus of work carried out by Ningaraj and others is on cerebrovascular genomics and proteomic research. The ideal approach is to isolate brain capillaries in normal and diseased brain tissue and then to analyse genomic and proteomic differences. Generally, human brain tissue from a temporal lobectomy of a trauma or epilepsy patient is considered to be normal tissue in these studies as it is unethical to obtain normal, healthy human brain tissue [8].

2. Drug delivery to brain tumours: challenges

Drug delivery research focuses on several innovative methods, including nanoparticles [9], microparticles as carriers of anticancer agents, PEG technology, encapsulating anticancer drugs in liposomes, and monoclonal antibodies for the delivery of anticancer payloads [2]. Focusing on brain cancer, one area of research has focused on ECs, which are a major component of the neurovascular unit [10,11]. However, many issues that are related to ECs are still not well understood, including gene and protein profiling in normal brain and brain tumour capillary ECs [12,13]. This research is hampered due to the complexities that are involved in isolating pure ECs devoid of pericytes, neurons and tumour cell populations, as well as from the differences between and within brain tumours. For instance, significant differences were found between normal human brain and brain tumour capillaries, including differential expression of calcium-activated potassium (K_{Ca}) [14,15] and ATP-sensitive potassium (K_{ATP}) channels [16]. Recent progress in the molecular targeting of tumour-specific antigens with specific agents, however, can be exploited by identifying additional novel targets for modulating BBB/BTB permeability. Future studies will seek to determine whether there are significant differences in the expression levels (induced or suppressed) of certain genes and proteins between normal and brain tumour capillary ECs.

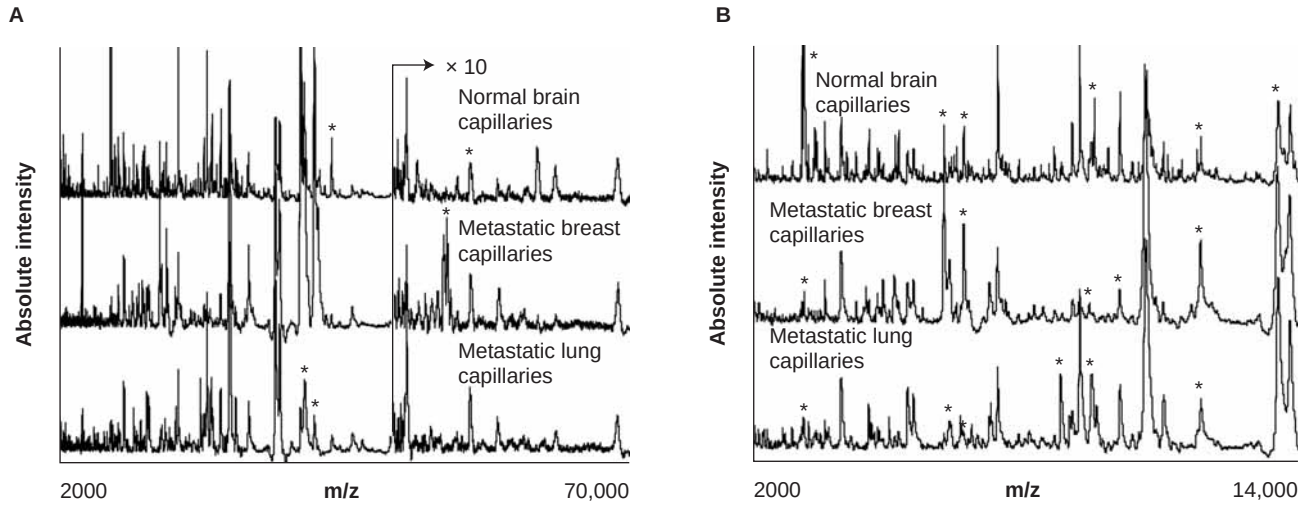

Figure 2. A. Unidentified protein profiles obtained from laser-captured microscopy-dissected blood vessels from human normal brain capillaries, human metastatic breast and lung tumours in the brain by MALDI-TOF mass spectrometry (Voyager DE-Star). Numerous differences are observed among the peptides and proteins whose molecular weight ranged from 2000 to 70,000 Da. **B.** Shows distinct differences in protein profiles in a smaller molecular range of proteins.
*Indicates differences in protein profiles that are clearly visible, although identity of these peaks is under evaluation.
MALDI-TOF: Matrix-assisted laser desorption ionising time-of-flight; m/z: Mass/charge ratio.

Studies were executed using cerebrovascular genomics and proteomics in a laser-captured microscopy-dissected pure capillary EC population isolated from human normal brain and metastatic breast and lung tumour tissues [17,18]. This approach may elucidate differences in gene clusters and their expression products between the capillary ECs of normal brain and brain tumours [19]. Known [2] and novel BBB/BTB-specific genes and proteins can then be used to better understand BBB/BTB permeability regulation in human brain tumour tissues. Developing novel drug delivery modalities to brain tumours across the BBB/BTB is crucial, especially now as receptor tyrosine kinase inhibitors have been shown to have clinical benefit in patients with cancers outside the brain [20-22], whereas their clinical efficacy against cancer in the brain is modest at best [22,23], mainly due to their failure to cross the BBB/BTB.

In a preliminary unpublished work, employing laser-captured microscopy technology, pure capillary ECs were isolated from human 'normal brain' (trauma brain tissue) and brain tumour tissues (Ningaraj *et al.*). An EC-rich sample was subjected to MALDI-TOF mass spectrometry proteomic analysis (Figure 2A and 2B) to compare capillaries from normal brain and cancer tissue and to highlight differences in protein expression between healthy and tumour tissue. Such pattern-specific tissue expression may provide the platform for further investigation into overall brain vascular biology as it pertains to conditions such as angiogenesis, cell adhesion, metastasis, cell–cell communication and local inflammation. Genomic and proteomic studies should facilitate the development of novel drug and gene delivery methods, including gene therapy via vectors or modified autologous cell transfer in the brain based on the unique properties of the BBB and BTB.

2.1 Drug delivery challenges

Permeability challenges in the context of enhanced anticancer drug transport across the leaky BTB and the intact BBB surrounding brain tumour is discussed in this review. For example, improving methods to overcome the permeability problem depends on understanding the mechanism of BTB permeability regulation in brain tumour capillaries (microvessels), as well as the interaction of primary and metastatic cancer cells with capillary ECs. By understanding the mechanism of tumour vascular transport across the BBB/BTB selectively to brain tumours, the effectiveness of anticancer drugs can be vastly improved. As large water-soluble molecules, such as therapeutic humanised monoclonal antibodies, are developed to treat neurological diseases, the challenge to deliver them across the BBB has assumed critical importance.

Brain tumour capillaries constitute the BTB, which has different structural and functional characteristics to that of the normal brain capillaries that form the BBB. Among many distinct differences [24,25], BTB capillaries are responsive to vasoactive agents [14-16,26,27]. The BTB, particularly at the tumour's leading edge, retains certain characteristics of the BBB, including the active efflux mechanism and some structural integrity [13]. Therefore, most anticancer drugs that are effective against cancers outside the brain are ineffective against brain tumours due to their failure to cross the BBB/BTB in effective quantities [13,27]. As translational models of brain cancer treatment, *in vivo* human brain cancer models in nude rodents were developed and characterised to study the unique features of the BBB and BTB. At present, researchers are examining genes and proteins that are uniquely expressed by the intact BBB and BTB, and mechanisms by which brain cells regulate EC gene

expression in normal and disease states [28]. These include changes that occur in response to oncogenesis, such as alterations in the signal transduction pathways of brain capillary endothelial transcytosis and the regulation of tight junctions. Several studies have characterised the endogenous influx and efflux properties of the BBB/BTB, including transporters in the luminal and abluminal membranes of brain endothelium and epithelium in normal [2] and cancer [29] states.

2.2 Biology of the BBB/BTB and drug transport: efflux system

Active efflux transporters at the BBB, include P-glycoprotein (Pgp), which actively exports the drug from the brain to the blood. In addition, there are many other BBB efflux systems that expel both small and large drug molecules [2]. Efflux pumps play a major role in restricting drug transport across the BBB. Studies are in progress to understand the structural domains that underlie the functional activity of efflux proteins in order to design improved inhibitors for drug efflux pumps. A recent symposium, 'Drug efflux pumps: challenges and opportunities' addressed the detection and functional characterisation of drug efflux pumps, and their role in BBB permeability [30]. Drug efflux pumps predominantly belong to the family of ATP-binding cassette (ABC) transporters such as breast cancer resistance protein [31], RLIP76/RALBP1 (a recently described non-ABC multi-specific transporters [32]), glycoprotein (Pgp), and multi-drug resistance-associated protein. These transporters are generally overexpressed in cancer cells, which lead to decreased drug retention, increased elimination and metabolism by CYP enzymes. These factors all contribute to increased drug resistance. Drug efficacy can be improved by rapid penetration, reduced drug binding to brain tissue or by increased passive permeability [33].

In the past decade, progress has been made in understanding the biology and transport properties of the BBB, including its efflux mechanism. For instance, transport of glucose and insulin across the BBB and BTB was achieved by a molecular approach [2]. These pathways are used to deliver therapeutic agents to the brain and brain tumours. New gene-targeting technology allowed the development of a non-viral, RNA interference-based gene therapy that encapsulated plasmid DNA inside receptor-specific, pegylated immunoliposomes. These carry plasmid DNA that expresses a short hairpin RNA directed against human EGFR [6]. Other promising drug delivery strategies involve the development of drugs that bypass the efflux system or use inhibitors to block efflux proteins.

2.3 Challenges of efflux mechanism to drug delivery

Drug delivery to malignant brain tumours and brain metastases present a formidable clinical challenge because multi-drug resistance proteins limit the penetration and accumulation of chemotherapeutics in cancer cells. There is ample evidence of the presence and critical role of Pgp in clinical resistance to chemotherapy in primary brain tumours [34]. For example, taxol is an antitumour agent that is a substrate for Pgp-mediated efflux from the BBB. Although taxol and many anticancer agents are highly lipophilic, they barely cross the BBB, largely due to active efflux by Pgp [35,36]. The amount of drug that arrives at the tumour site depends on BBB permeability, which varies considerably among brain cancer patients [36]. Drug concentrations in brain tissue usually drop with increasing distance from the tumour, and thus the drug concentration is fairly low in the peripheral parts of the tumour, where tumour cells infiltrate the normal brain. In these areas, where tumour proliferation is most rapid, the BBB is relatively intact [1]. Novel approaches for effective delivery through the BBB of anticancer agents that are substrates of Pgp would provide neuro-oncologists with additional and effective anticancer agents for the treatment of brain tumours in their efforts to increase patient survival rates.

2.4 Mechanism of drug transport across the BBB/BTB

Major transport routes across the BBB to brain parenchyma are via pinocytotic vesicles and endothelial tight junctions (Figure 3). However, BTB transport is altered due to rearrangement of the neurovascular unit by the tumour microenvironment. It has been shown by transmission electron microscopy that potassium channel agonists induce the accelerated formation of transport vesicles in both brain tumour capillary endothelium and tumour cells by the activation of their respective potassium channels [14,16]. Therefore, vesicular transport, rather than the opening of endothelial tight junctions, seems to be largely responsible for enhanced drug delivery across the BTB. A direct relationship was found between an increase in the number of brain tumour capillary endothelial vesicles and increased BTB permeability. Most significantly, it was observed that brain tumour capillary ECs form far more vesicles than normal brain capillary ECs without altering the endothelial tight junctions in response to vasomodulators, such as NS-1619 and minoxidil sulfate [14,16].

Compromised BBB is reported in several CNS diseases, including stroke, Alzheimer's disease, HIV, multiple sclerosis, vasogenic oedema, viral infection and brain cancer. Although the mechanism of BBB breakdown in CNS diseases is not clearly understood, long-term BBB breakdown causes astrocytic dysfunction followed by epileptogenesis [37], whereas traumatic brain injury causes the upregulation of VEGF, VEGF receptors and dense vascularisation in and around the lesion [38]. Therefore, understanding the biochemical regulation of the BBB and BTB is crucial to developing more effective methods to deliver therapeutic agents to the CNS, including anticancer drug delivery selectively to brain tumours.

Research carried out by Ningaraj *et al.* has shed light on the properties of the abnormal BBB, which results from damage caused by cerebral ischaemia and other neurological disorders, yet it is still somewhat different from the BTB (Figure 1) [27]. The infiltrative nature of brain tumour cells, particularly glioma cells, which invade normal brain cells surrounding the tumour, makes the delivery of anticancer drugs to all tumour cells extremely difficult [39]. A strategy to address this obstacle is to

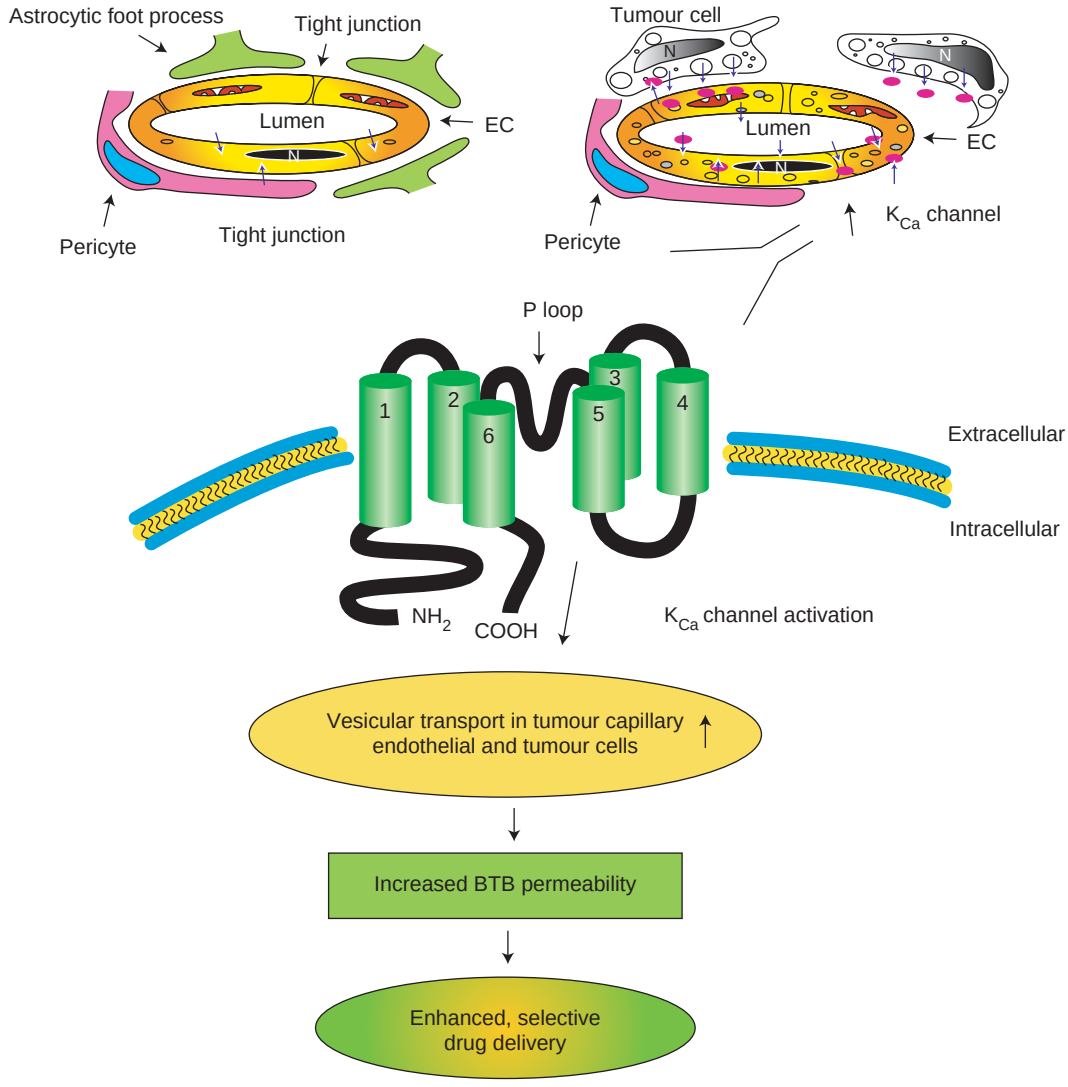

Figure 3. BTB capillaries are distinct from BBB capillaries: increasing evidence suggests that vascular endothelial cells from cerebral blood vessels overexpress ion channels, and these channels play an important role in modulating endothelial cell functions including regulating BBB permeability [56]**.** In gliomas, it was demonstrated that BTB capillaries respond differently than normal BBB capillaries to vasomodulators due to distinct differences in expression levels and the functional activities of brain tumour capillaries.

BBB: Blood–brain barrier; BTB: Blood–brain tumour barrier; K_{Ca}: Potassium channel; N: Nucleus.

seek to modify systemic drug delivery through cerebral microvessels/capillaries to be more effective in delivering anticancer agents to the often dispersed pockets of tumour cells that remain after aggressive therapy, including surgery, radiation and chemotherapy [1].

BTB capillaries have different structural and functional characteristics compared with normal BBB capillaries, and they even exhibit heterogeneity of gene and protein expression in a given tumour area. Among many distinct differences, K_{Ca} and K_{ATP} channels are overexpressed in brain tumour and brain tumour vascular ECs. Furthermore, these channels are highly responsive to vasoactive agents *in vivo*. These differences were exploited in the BTB to enhance anticancer drug delivery to brain tumour with little or no drug delivery to normal brain tissue. Ningaraj *et al.* found that in human glioma xenografts, BBB and BTB capillaries respond differently to K_{Ca} channel agonists due to their morphological and biochemical differences (Figure 3). It was also shown in a co-culture of glioma cells with human brain microvessel ECs, that glioma cells induce the overexpression of K^+ channels in ECs [16]. Capillary ECs may be induced to overexpress and/or to possess elevated K_{Ca} and K_{ATP} channel activity, depending on the tumour microenvironments produced by the particular brain tumour. In the author's investigation, the altered expression of K_{Ca} and K_{ATP} channels in other cell types was not apparent. Nevertheless, the propensity of a tumour microenvironment

to alter potassium channel expression in other cells can not be ruled out.

Advances in antiangiogenic drugs targeting brain tumour vascular genes and proteins have highlighted the importance of understanding the differences in gene and protein expression between normal BBB and abnormal BTB capillary ECs. Recently, Kallmann *et al.* showed the brain-specific gene expression in human normal cerebral ECs [40]. However, gene and protein profiling in an EC population isolated from gliomas have not been demonstrated. Such profiling is critical because it was shown that brain tumour capillary ECs overexpress K_{Ca} [27] and K_{ATP} [16] channels, which regulate BTB permeability. A similar altered potassium channel expression was observed in brain lesions caused by middle cerebral artery occlusion in rat stroke moels (MK Rao, B Konda, A Das, NS Ningaraj, unpublished observations). It remains to be studied whether altered expression of potassium channels in diseased brain capillaries occurs in other CNS diseases.

3. Progress in drug delivery to brain tumours

3.1 Common strategies: BBB/BTB disruption

There are several modalities for drug and gene delivery through the BBB, including BBB disruption; the use of endogenous transport systems; carrier-mediated transporters such as glucose and amino acid carriers; receptor-mediated transcytosis, such as insulin or transferrin receptor systems; and active efflux transporters, such as Pgp and the associated antiporters. Tight junctions lining the ECs may be transiently opened by the intra-carotid arterial infusion of hyperosmolar solutions. BBB/BTB disruption causes global changes in brain microvasculature permeability that allow therapeutic molecules to reach the brain. The problem with this approach is that the BBB stays open for only a short time and may allow plasma proteins, which are toxic, to enter the brain leading to chronic neuropathology.

3.2 Surgical drug delivery

Invasive strategies circumvent the BBB/BTB but require either a craniotomy or insertion of catheters into the carotid artery. An anticancer drug is injected into the brain directly or into the CSF via an intracerebroventricular infusion, which relies on controlled release of the drug. Besides the high cost of this approach, the drug is not delivered to the entire diseased area of the brain and, given its invasiveness, the procedure can lead to neuropathological changes. Although effective in delivering anticancer drugs to the tumour, the drugs in this delivery strategy are not targeted specifically at brain tumour cells, so potentially noxious anticancer agents are also delivered to healthy brain cells, resulting in undesirable side effects.

Contrary to the claims made by some studies in relation to developing a method to deliver drugs across the BTB, the drugs infused via the intracerebroventricular strategy may not necessarily cross the BBB or the BTB. Such a strategy may deliver drugs to CSF via circumventricular brain regions, which surround the ventricular system but are not protected by the BBB or the BTB. The BBB and the blood–CSF barrier are anatomically and functionally distinct. Therefore, the entry of a drug into the CSF via circumventricular brain regions does not essentially mean that the drug has crossed the BBB but is only a measure of blood–CSF barrier permeability. For instance, a recent study concluded that temozolomide crossed the intact BBB by showing the presence of this drug in the CSF after systemic administration [19]. Such an observation, however, does not demonstrate that the drug crossed the BTB or BBB because circumventricular brain regions lack a BBB or BTB. Furthermore, the level of temozolomide or its metabolite, methyl-triazenyl imidazole carboxamide was not quantified in the brain tumour following systemic administration. In contrast, it was found that [^{14}C]-temozolomide scarcely crossed the BBB and, in fact, only a very small amount of [^{14}C]-temozolomide was taken up by human brain tumour implanted in rat brain (MK Rao, B Konda, A Das, NS Ningaraj, unpublished observations). Therefore, drug entry into the CSF is not an index of BTB permeability unless drug levels in the tumour are quantified by quantitative autoradiography (QAR) [41,42], or by the detection and identification of a drug or its metabolites in brain tumour tissue by a quantitative assay, such as microdialysis or the high performance liquid chromatography-mass spectrometry method [43].

3.3 Intracerebral infusion and convention-enhanced diffusion

Intracerebral implants have a limited, narrow effect that is only effective against very small tumours. They are ineffective against larger tumours because they do not allow anticancer agents to diffuse and reach the small pockets of tumour cells well away from the tumour core. For instance, the diffusion of 1,3-bis(2-cholorethyl) 1-nitrosourea, a commonly used anticancer biodegradable implant, is limited to 500 mm from the implanted site [44]. This is sometimes clinically efficient but not when brain tumours are > 500 mm, which is often the case, especially for those types of tumours that are highly diffused, such as gliomas [45]. A recent clinical trial showed that convention-enhanced diffusion (CED) is an effective drug delivery strategy to treat human non-diffused brain tumours with intratumoural injection of anticancer agents [46]. However, CED, although effective, is highly invasive and is also best used against very small solid tumours, as it, as with intracerebral infusion, does not allow drug molecules to reach outlying pockets of diffuse tumour tissue (for a review of CED see [45]).

4. Non-invasive drug delivery

4.1 Transient opening of the BTB for selective drug delivery

In an effort to improve the methods discussed in Section 3, certain vasomodulators, such as potassium channel agonists, for targeted and enhanced delivery of chemotherapeutics selectively

to brain tumour in rodents were employed. This biochemical approach increased BTB permeability, enhanced delivery of therapeutic drugs to brain tumours selectively with little or no drug delivery to normal brain, and can deliver small- to large-sized substances, including contrast-enhancing agents, antitumour compounds, therapeutic proteins and viral vectors [14-16]. The authors of this paper have employed selected vasomodulators such as bradykinin, nitric oxide donors, soluble guanylate cyclase activator, a K_{Ca} channel agonist (NS-1619), and K_{ATP} channel agonist mionoxidil sulfate [16] to increase BTB permeability, resulting in significantly enhanced tracer/drug delivery specifically to brain tumour.

The modification of BBB/BTB permeability is being studied using K_{Ca} and K_{ATP} channel agonists to enhance the delivery of receptor tyrosine kinase inhibitors, which are effective outside the brain [20-23], but not within the brain [22,23], to human glioma xenografts in murine brains. Concurrently, dynamic contrast-enhanced (DCE) MRI is being used to develop novel translational strategies that will allow for high-throughput screening of various anticancer drugs delivered selectively to brain tumours.

As a case in point, the intactness of the BBB/BTB by QAR in animal glioma models was tested (Figure 4). The BTB in and around the brain tumour seems to be intact. In rodent brain tumour models, increased delivery of small and large anticancer agents to brain tumours by biochemical modulation of the BTB was achieved [14,15]. K_{Ca} and K_{ATP} channel modulation does not require modification of the drug to be delivered and can deliver multiple drugs selectively to the tumour, which is crucial as most brain tumour patients are treated with multiple drug combinations. The duration of BTB opening can be tightly controlled and the effect can be achieved with a simple intravenous or possibly oral infusion. Increased delivery of [^{14}C]-carboplatin [16] and [^{14}C]-temozolomide (MK Rao, B Konda, A Das, NS Ningaraj, unpublished observations) selectively to brain tumours following BTB modulation by K_{Ca} and K_{ATP} channel agonists was also shown.

5. BBB/BTB barrier permeability measurements

A DCE-MRI preclinical study helped support the clinical usefulness of the imaging strategy to measure the effectiveness of various approaches to open the BBB/BTB. DCE-MRI was used to obtain quantitative non-invasive, high-resolution measurements of blood perfusion and vessel permeability (usually denoted as K^{trans}), the extravascular extracellular volume fraction (denoted by v_e), blood volume (v_b), and vessel size index. All of these measures have been shown to correlate with disease progression and treatment response [47]. A better understanding of the mechanism of BTB permeability regulation in brain tumour capillaries (microvessels) and how glioma cells in the brain interact with capillary ECs should lead to improvements in methods to increase BBB/BTB permeability to allow the delivery of anticancer drugs selectively to brain tumours. Measuring BBB/BTB permeability is also critical in a clinical situation. BBB permeability can be quantified by inserting a dialysis probe into brain tissue but the probe causes brain injury and BBB disruption. Therefore, BBB permeability is artificially increased with this approach, such as in the case of morphine and morphine 6-glucuronide [2]. When measured by standard physiological methods, the BBB permeability of morphine is ~ 50-fold higher than that of morphine 6-glucuronide. However, when measured by the dialysis method, the brain uptake of morphine and morphine 6-glucuronide is comparable, owing to the disruption of the BBB.

The QAR method of measuring drug delivery across the BBB/BTB, although effective, is invasive, lacks a real-time capability, and requires radio-labelling of an investigative anticancer drug. Moreover, the method may not be used in a clinical setting to determine the efficacy of drug delivery. However, the DCE-MRI method can measure BBB/BTB permeability changes in response to modulating agents in real-time and can be used for high-throughput screening of investigational drugs without any modification. BBB disruption either due to tumour-induced necrosis or biochemical modulation of BTB capillaries results in increased permeability to contrast-enhancing reagents and, therefore, enhanced signals. Thus, high-speed DCE-MRI allows for the non-invasive, quantitative measurement and visualisation of vascular permeability, BBB permeability changes and allows for monitoring of the delivery and therapeutic efficacy of anticancer drugs to brain tumours [48].

DCE-MRI is already used to detect brain tumours before, during and after treatment in patients [48,49], and is extremely useful in monitoring treatment outcomes with novel anticancer drugs in experimental rodent brain tumour models [47-50]. DCE-MRI was used to monitor tumour responses to treatment with anticancer drugs in human brain tumour xenografts (MK Rao, B Konda, A Das, NS Ningaraj, unpublished observations). Furthermore, DCE-MR images were obtained and then co-registered with QAR images of rat brain sections as shown in Figure 4. In addition, a reliable method using DCE-MRI was developed to measure cerebral blood vessel permeability [51], changes in BBB permeability following the administration of potassium channel agonists and to monitor the antitumour effect of anticancer drugs.

6. Conclusions

The BBB severely hampers delivery of therapeutic agents from circulation to the brain. Brain tumour capillaries that form the BTB also prevent delivery of most hydrophilic molecules and antitumour agents to brain tumour. During the past decade, a considerable research effort has been made and various strategies employed to increase BTB permeability to enhance the delivery of anticancer drugs selectively to brain tumours. Intracarotid infusion of a hyperosmotic agent opens the BBB and has enjoyed some clinical success but it can also increase delivery of potentially toxic drugs to healthy brain tissue [2], and patients often exhibit severe side effects that are associated with global disruption of the BBB. Another drug delivery strategy relies on

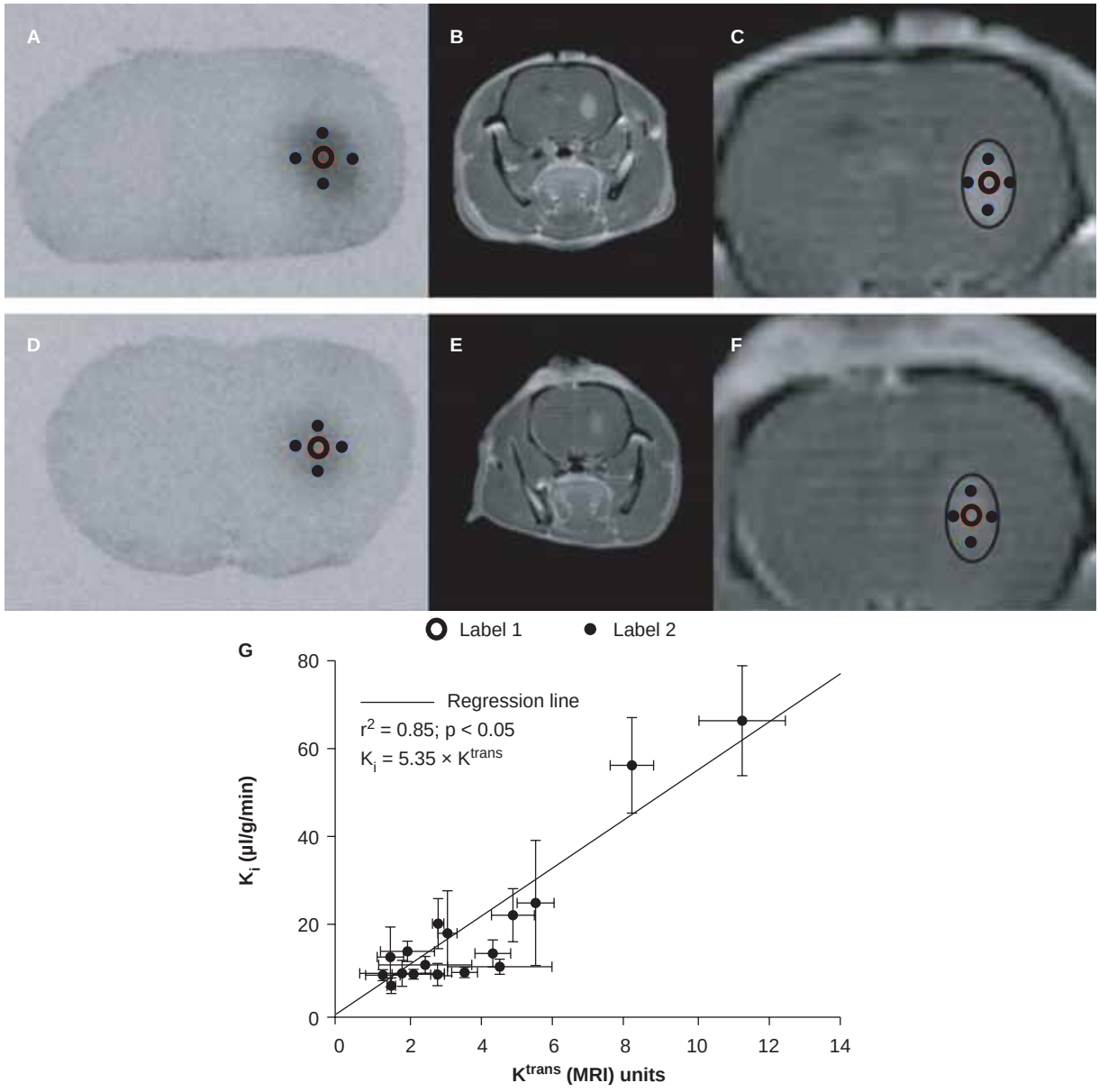

Figure 4. Demonstrates an example of applying DCE-MRI technique to a ^{9}L-glioma tumour model in a rat. The figure depicts an axial cut through the brain (left panel), K^{trans} (middle), and v_e (right) mappings. The tumour is in the right basal ganglia of the brain and the increased number of pixels (label 1) along the tumour border (in the K^{trans} map) indicates pixels that are well perfused and/or contain many leaky vessels. The pixels in the centre of the v_e map (label 1) indicate a tumour core that is highly oedaematous, as the extravascular extracellular volume fraction ranges from 0.40 to 0.50, whereas the pixels in normal brain (label 2) are ~ 0.04. These methods are readily applicable to assess tumour growth and treatment response. Furthermore, correlating these parameter maps to quantitative autoradiography methods will provide a much-needed validation of the DCE-MRI as demonstrated in G.

DCE-MRI: Dynamic contrast-enhanced magentic resonance imaging; K_i: Unidirectional transfer constant; K^{trans}: Blood perfusion and vessel permeability; v_e: Extravascular extracellular volume fraction; MRI: Magnetic resonance imaging.

using vectors, which carry modified proteins, peptides or monoclonal antibodies that undergo receptor-mediated transcytosis [28]. This strategy is complex and can allow delivery of therapeutic agents to normal brain tissue. Other drug delivery methods, such as the use of transport systems, intracerebral infusion, CED and intracerebroventricular infusion, all have some advantages but can potentially cause negative side effects.

In an effort to improve on these methods low doses of certain vasomodulators were used for targeted and enhanced delivery of chemotherapeutics selectively to brain tumour in rodents [14-16] and humans [52]. This biochemical approach increases BTB permeability, significantly enhances delivery of therapeutic drugs to brain tumours selectively with little or no drug delivery to normal brain, and can deliver small- to

large-sized substances, including contrast-enhancing agents, antitumour compounds, and therapeutic proteins and viral vectors [27]. Understanding the BBB drug transport mechanism is critical to developing more effective and efficient drug delivery strategies. It was demonstrated that the activation of potassium channels induces accelerated formation of transport vesicles in both brain tumour capillary endothelium and tumour cells [14,27,53]. These results provide evidence that vesicular transport is largely responsible for enhanced delivery of drugs across brain tumour capillaries to the tumour tissue. Most importantly, research has demonstrated that the activation of potassium channels by channel-specific agonists, and by agents that produce nitric oxide and cGMP *in situ*, can sustain enhanced drug delivery selectively to brain tumours. This or a similar strategy may improve the delivery of antineoplastic agents, including humanised monoclonal antibodies and therapeutic viral vectors selectively to brain tumours and neuropharmaceutics to diseased brain regions whilst leaving normal brain unaffected and, thereby, significantly increasing patient survival for those stricken with debilitating neurological diseases and tumours. A few clinical studies have shown increased drug delivery to human brain tumours by biochemical manipulation of the BBB/BTB opening [52]. For instance, a recent clinical study showed a beneficial effect of concurrent RMP-7 (a BTB permeabilising agent) [54]) and carboplatin with radiation therapy in children with newly diagnosed brainstem gliomas. However, further clinical studies, are essential to demonstrate the biochemical opening of BTB as a therapeutic strategy to facilitate anticancer drug transport to brain tumour.

It is important to understand how the expression of drug transport systems in the CNS controls the disposition and efficacy of anticancer agents. Pgp, an efflux transporter that confers the multi-drug resistance phenotype to many cells by extruding a broad range of anticancer drugs from the cancer cell, results in poor clinical outcomes. The focus of research is now on the cellular localisation, molecular expression and functional activity of Pgp and its relevance to pathogenesis and pharmacological treatment of CNS disorders [55].

Even though new strategies are being developed to enhance drug delivery to the CNS, methodologies to assess drug response and delivery to target tissues are lacking. Efficacy testing of new therapeutics in the clinical setting is time consuming because the standard end points used are radiographical response and clinical outcome measured by survival. Therefore, MRI offers a solution to this problem by offering real-time treatment response analysis. In an effort to standardise a non-invasive method to measure BBB/BTB permeability changes, Ningaraj *et al.* presented their preliminary study on the MRI technique at the annual meeting of the International Society for Magnetic Resonance in Medicine [51]. This represents a significant step towards a quantitative validation of the reference region model for the analysis of DCE-MRI data in, as a start, a rat glioma tumour model. There are, however, several significant limitations in this study, such as the inherent difficulty of co-registering the histological scale thickness (~ 20 μm) of the QAR images with that of the MR images (10^3 μm). To perform a truly quantitative comparison, Ningaraj *et al.* are now comparing the region of interests from exactly the same slice of tissue on the MRI and QAR images.

7. Expert opinion

Tumour-specific drug delivery has the potential to minimise toxicity to normal tissues and improve the bioavailability of cytotoxic agents to neoplasms. Existing site-specific drug delivery systems include delivery to the endothelial receptor $\alpha_v\beta_3$, and tumour-specific antigens. Antibody conjugation to cytotoxic agents has shown promise in achieving the goal of tumour-targeted cytotoxicity. This approach may be limited by the small subsets of tumours that can be targeted by these peptides and poor biodistribution of peptides into solid tumours. Alternative approaches to target all neoplasms exploit differences in human tumour blood vessel characteristics compared with normal brain blood vessels. Therefore, understanding the genomic and proteomic differences between the blood vessels of brain tumour and normal brain is extremely important.

The failure of conventional and novel anticancer drugs to treat brain cancers effectively inflicts a heavy toll in terms of human lives and healthcare costs around the world. The main stumbling block is the BBB/BTB, which prevents entry of most therapeutic hydrophilic molecules and anticancer agents to brain tumours. Among many methods to increase drug delivery to brain tumours, the biochemical approach may be a clinically useful strategy to increase BTB permeability and enhance delivery of therapeutic drugs to brain tumours selectively with little or no drug delivery to the normal brain. Small- to large-sized substances, including contrast-enhancing agents, antitumour compounds, therapeutic proteins and viral vectors may be delivered efficiently using this approach. Imaging tools such as MRI offer a means to effectively measure permeability changes in brain tumour and to provide early measurements about the usefulness of a therapy. The hope is that further studies will identify potential targets that can be used to improve anticancer drug delivery across the BBB/BTB selectively to brain tumours in patients, resulting in significantly improved control of brain tumours and longer lives for patients who are stricken with this deadly disease.

Acknowledgments and disclosure

The author appreciates the editorial help of S Macdonald (Cedars-Sinai Medical Center) and MRI expertise of T Yankeelov (Institute of Imaging Science). The author also acknowledges a grant support from the American Cancer Society.

The author has filed a new disclosure application on the method of using MRI to quantify BBB/BTB permeability measurements with the Technology Transfer Office, Vanderbilt University, Nashville, TN, USA The author is also a co-inventor (US and World patent) of the BTB modulation strategy using potassium channel modulators for increased drug delivery to diseased CNS.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. RICH JN, BIGNER DD: Development of novel targeted therapies in the treatment of malignant glioma. *Nat. Rev. Drug Discov.* (2004) 3:430-446.

•• This review article highlights the importance of EGFR as a molecular target for brain cancer treatment. It also reviews studies on EGFR amplification, overexpression or mutation in glioblastomas.

2. PARDRIDGE WM: Drug targeting, drug discovery, and brain drug development. In: *Brain Drug Targeting: the Future of Brain Drug Development*. Cambridge University Press, Cambridge, UK (2001):1-13.

3. STUPP R, MASON WP, VAN DEN BENT MJ *et al.*: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* (2005) 352:987-996.

4. PARDRIDGE WM, BOADO RJ, BLACK KL, CANCILLA PA: Blood–brain barrier and new approaches to brain drug delivery. *West J. Med.* (1992) 156:281-286.

5. GROOTHUIS DR: The blood–brain and blood–tumor barriers: a review of strategies for increasing drug delivery. *Neuro-oncol.* (2000) 2:45-59.

6. PARDRIDGE WM: Intravenous, non-viral RNAi gene therapy of brain cancer. *Expert Opin. Biol. Ther.* (2004) 4(7):1103-1113.

7. LI JY, BOADO RJ, PARDRIDGE WM: Blood–brain barrier genomics. *J. Cereb. Blood Flow Metab.* (2001) 21:61-68.

8. BECKER AJ, CHEN J, ZIEN A *et al.*: Correlated stage- and subfield-associated hippocampal gene expression patterns in experimental and human temporal lobe epilepsy. *Eur. J. Neurosci.* (2003) 18:2792-2802.

9. FERRARI M: Cancer nanotechnology: opportunities and challenges. *Nat. Rev. Cancer* (2005) 5:161-171.

10. BAZAN NG, MARCHESELLI VL, COLE-EDWARDS K: Brain response to injury and neurodegeneration: endogenous neuroprotective signaling. *Ann. NY Acad. Sci.* (2005) 1053:137-147.

11. DOOLITTLE ND, ABREY LE, BLEYER WA *et al.*: New frontiers in translational research in neuro-oncology and the blood–brain barrier: report of the tenth annual Blood–Brain Barrier Disruption Consortium Meeting. *Clin. Cancer Res.* (2005) 11:421-428.

12. FOLKMAN J: Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.* (1995) 1:27-31.

13. FIDLER IJ, YANO S, ZHANG RD, FUJIMAKI T, BUCANA CD: The seed and soil hypothesis: vascularisation and brain metastases. *Lancet Oncol.* (2002) 3:53-57.

14. NINGARAJ NS, RAO M, HASHIZUME K, ASOTRA K, BLACK KL: Regulation of blood–brain tumor barrier permeability by calcium-activated potassium channels. *J. Pharmacol. Exp. Ther.* (2002) 301:838-851.

15. NINGARAJ NS, RAO M, BLACK KL: Calcium-dependent potassium channels as a target protein for modulation of the blood–brain tumor barrier. *Drug News Perspect.* (2003) 16:291-298.

16. 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.

•• This research article describes a novel cellular mechanism for K_{ATP}-mediated BTB permeability increase. This novel mechanism involves the accelerated formation of pinocytotic vesicles, which can transport drugs across the BTB. This article also explains why the K_{ATP} channels are a potential target for biochemical modulation of BTB permeability to increase antineoplastic drug delivery selectively to brain tumours.

17. BALL HJ, MCPARLAND B, DRIUSSI C, HUNT NH: Isolating vessels from the mouse brain for gene expression analysis using laser capture microdissection. *Brain Res. Brain Res. Protoc.* (2002) 9:206-213.

18. XU BJ, CAPRIOLI RM, SANDERS ME, JENSEN RA: Direct analysis of laser capture microdissected cells by MALDI mass spectrometry. *J. Am. Soc. Mass Spectrom* (2002) 13:1292-1297.

19. AGARWALA SS, KIRKWOOD JM: Temozolomide, a novel alkylating agent with activity in the central nervous system, may improve the treatment of advanced metastatic melanoma. *Oncologist* (2000) 5:144-151.

20. GULLICK WJ: Update on HER-2 as a target for cancer therapy: alternative strategies for targeting the epidermal growth factor system in cancer. *Breast Cancer Res.* (2001) 3:390-394.

21. GOLDENBERG MM: Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer. *Clin. Ther.* (1999) 21:309-318.

22. COBLEIGH MA, VOGEL CL, TRIPATHY D *et al.*: Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* (1999) 17:2639-2648.

23. MENARD S, TAGLIABUE E, CAMPIGLIO M, PUPA SM: Role of HER2 gene overexpression in breast carcinoma. *J. Cell. Physiol.* (2000) 182:150-162.

24. RUOSLAHTI E: Specialization of tumour vasculature. *Nat. Rev. Cancer* (2002) 2:83-90.

25. FISHMAN RA, CHRISTY NP: Fate of adrenal cortical steroids following intrathecal injection. *Neurology* (1965) 15:1-6.

26. RAO M, NINGARAJ N, YAMAMOTO V, BLACK KL: Activation of calcium-dependent potassium channels elicits selective glioma cell death. *AACR-NCI-EORTC International Conference* (2001) 312:64.

27. BLACK KL, NINGARAJ NS: Modulation of brain tumor capillaries for enhanced drug delivery selectively to brain tumor. *Cancer Control* (2004) 11:165-173.

28. PARDRIDGE WM: Blood–brain barrier genomics and the use of endogenous transporters to cause drug penetration into the brain. *Curr. Opin. Drug Discov. Devel.* (2003) 6:683-691.

29. FELLNER S, BAUER B, MILLER DS *et al.*: Transport of paclitaxel (Taxol) across the blood–brain barrier *in vitro* and *in vivo*. *J. Clin. Invest.* (2002) 110:1309-1318.

30. AYALASOMAYAJULA SP, KOMPELLA UB: Drug efflux pumps: challenges and opportunities. *IDrugs* (2000) 3(2):148-149.

31. BREEDVELD P, PLUIM D, CIPRIANI G *et al.*: The effect of Bcrp1 (Abcg2) on the *in vivo* pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): implications for the use of breast cancer resistance protein and P-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. *Cancer Res.* (2005) 65:2577-2582.

32. AWASTHI S, HALLENE KL, FAZIO V *et al.*: RLIP76, a non-ABC transporter, and drug resistance in epilepsy. *BMC Neurosci.* (2005) 6:61.

33. LIU X, CHEN C: Strategies to optimize brain penetration in drug discovery. *Curr. Opin. Drug Discov. Devel.* (2005) 8:505-512.

34. REGINA A, DEMEULE M, LAPLANTE A *et al.*: Multidrug resistance in brain tumors: roles of the blood–brain barrier. *Cancer Metastasis Rev.* (2001) 20:13-25.

35. HEIMANS JJ, VERMORKEN JB, WOLBERS JG *et al.*: Paclitaxel (Taxol) concentrations in brain tumor tissue. *Ann. Oncol.* (1994) 5:951-953.

36. DONELLI MG, ZUCCHETTI M, D'INCALCI M: Do anticancer agents reach the tumor target in the human brain? *Cancer Chemother. Pharmacol.* (1992) 30:251-260.

37. KRIZANAC-BENGEZ L, MAYBERG MR, JANIGRO D: The cerebral vasculature as a therapeutic target for neurological disorders and the role of shear stress in vascular homeostatis and pathophysiology. *Neurol. Res.* (2004) 26:846-853.

38. SKOLD MK, VON GERTTEN C, SANDBERG-NORDQVIST AC, MATHIESEN T, HOLMIN S: VEGF and VEGF receptor expression after experimental brain contusion in rat. *J. Neurotrauma* (2005) 22:353-367.

39. LASSMAN AB: Molecular biology of gliomas. *Curr. Neurol. Neurosci. Rep.* (2004) 4:228-233.

40. KALLMANN BA, WAGNER S, HUMMEL V *et al.*: Characteristic gene expression profile of primary human cerebral endothelial cells. *FASEB J.* (2002) 16:589-591.

41. ASOTRA K, NINGARAJ N, BLACK KL: Measurement of blood–brain and blood–tumor barrier permeabilities with [14C]-labeled tracers. *Methods Mol. Med.* (2003) 89:177-190.

42. BAKER SD, WIRTH M, STATKEVICH P *et al.*: Absorption, metabolism, and excretion of ^{14}C-temozolomide following oral administration to patients with advanced cancer. *Clin. Cancer Res.* (1999) 5:309-317.

43. REYDERMAN L, STATKEVICH P, THONOOR CM, PATRICK J, BATRA VK, WIRTH M: Disposition and pharmacokinetics of temozolomide in rat. *Xenobiotica* (2004) 34:487-500.

44. FUNG LK, SHIN M, TYLER B, BREM H, SALTZMAN WM: Chemotherapeutic drugs released from polymers: distribution of 1,3-bis(2-chloroethyl)-1-nitrosourea in the rat brain. *Pharm. Res.* (1996) 13:671-682.

45. VOGELBAUM MA: Convection enhanced delivery for the treatment of malignant gliomas: symposium review. *J. Neurooncol.* (2005) 73:57-69.

46. LI C, HALL WA, JIN N, TODHUNTER DA, PANOSKALTSIS-MORTARI A, VALLERA DA: Targeting glioblastoma multiforme with an IL-13/diphtheria toxin fusion protein *in vitro* and *in vivo* in nude mice. *Protein Eng.* (2002) 15:419-427.

47. CHECKLEY D, TESSIER JJ, WEDGE SR *et al.*: Dynamic contrast-enhanced MRI of vascular changes induced by the VEGF-signalling inhibitor ZD4190 in human tumour xenografts. *Magn. Reson. Imaging* (2003) 21:475-482.

48. ROBERTS TP, CHUANG N, ROBERTS HC: Neuroimaging: do we really need new contrast agents for MRI? *Eur. J. Radiol.* (2000) 34:166-178.

49. KNOPP MV, RUNGE VM, ESSIG M *et al.*: Primary and secondary brain tumors at MR imaging: bicentric intraindividual crossover comparison of gadobenate dimeglumine and gadopentetate dimeglumine. *Radiology* (2004) 230:55-64.

50. ROBINSON SP, MCINTYRE DJ, CHECKLEY D *et al.*: Tumour dose response to the antivascular agent ZD6126 assessed by magnetic resonance imaging. *Br. J. Cancer* (2003) 88:1592-1597.

51. YANKEELOV TE, KASISOMAYAJULA K, NINGARAJ N, PRICE RR, GORE G. Correlation between quantitative autoradiography and the reference region model of DCE- MRI. *ISMRM Thirteenth Annual Meeting* 7 – 13 May, Miami Beach, FL, USA (2005):2749.

52. 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.

53. HASHIZUME K, BLACK KL: Increased endothelial vesicular transport correlates with increased blood–tumor barrier permeability induced by bradykinin and leukotriene C4. *J. Neuropathol. Exp. Neurol.* (2002) 61:725-735.

- The authors of this paper have for the first time shown that bradykinin and leukotriene C4 could increase molecular transport across the BTB. These agents trigger pinocytotic vesicular transport across the BTB, and these transport vesicles may enhance drug transport across the BTB.

54. WARREN K, GERVAIS A, AIKIN A, EGORIN M, BALIS FM: Pharmacokinetics of carboplatin administered with lobradimil to pediatric patients with brain tumors. *Cancer Chemother. Pharmacol.* (2004) 54:206-212.

55. LEE G, BENDAYAN R: Functional expression and localization of P-glycoprotein in the central nervous system: relevance to the pathogenesis and treatment of neurological disorders. *Pharm. Res.* (2004) 21:1313-1330.

56. FARACI FM, HEISTAD DD: Regulation of the cerebral circulation: role of endothelium and potassium channels. *Physiol. Rev.* (1998) 78:53-97.

Affiliation

Nagendra S Ningaraj
Research scientist, Curtis and Elizabeth Anderson Cancer Institute,
Memorial Health University Medial Center,
4700 Waters Avenue, Savannah, GA 31403, USA
Tel: +1 912 350 0958;
Fax: +1 912 350 8199;
E-mail: nningaraj@bellsouth.net