TUMORS AND THE BRAIN BARRIERS

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Introduction

The blood-brain barrier (BBB) is critical to the understanding and management of primary and metastatic brain tumors. This complex pathophysiologic structure has been extensively studied and manipulated in an effort to improve our understanding and management of brain tumors; however, many issues remain unresolved. Neuroimaging demonstrates clear disruption of the BBB in many tumors, yet available drug-based therapies are often excluded in whole or part from the tumor, possibly on the basis of efflux transporters and other restrictions. In this report we summarize the current state of the science and seek to address the areas which remain a barrier to progress and identify specific ways to move this field forward.

1. State of the Science - Progress Since 2000

Blood-Brain and Blood-Tumor Barriers

Since 2000, critical advances have been made at the cellular and molecular levels in the structure of the blood-brain barrier and how it changes with tumor invasion and growth. The transcriptome of the brain microvascular endothelium, which comprises the BBB, has been cataloged[1] and initial comparisons have been made between vascular endothelial cells in nonneoplastic and malignant brain tissue.[2] A number of proteins which comprise the endothelial tight junction, including claudin-3, -5 and -12, occludin, junctional adhesion molecule A and zonula occludens proteins, have been identified,[3] and initial studies have been conducted examining changes induced by selective gene knockout.[4] The brain microvascular endothelium has been shown to express a broad range of drug efflux transporter proteins that aggressively remove many chemotherapeutic drugs from the central nervous system. These transporters include ABCB1 (p-glycoprotein), ABCC1, 2, 4 and 5 (MRP), ABCG2 (BCRP), OAT-3, OATP1A2, and OATP2B1. Preliminary studies have demonstrated alterations in transporter expression in gliomas and the

blood-tumor barrier.[5-7] The role of these transporters in limiting brain and brain tumor drug uptake has been established for a number of important chemotherapeutic agents (i.e.., paclitaxel, imatinib, topotecan, vincristine, vinblastine, doxorubicin).[8-10] Together, the low passive permeability of the BBB, coupled with active extrusion by transporter proteins, forms a potent barrier for brain delivery of many chemotherapeutic drug agents.

Multiple factors have been identified that modify vascular endothelial function and barrier tightness in pathologic conditions, including brain tumors.[11] Significant strides have been achieved in understanding the factors that control metastatic cell invasion of the nervous system as well as tumor implantation and angiogenesis.[12-16] Novel imaging methods have also been introduced to map changes in *in vivo* blood-tumor barrier permeability to fluorescent probes[17] or paramagnetic probes.[18] Tumor seeding and growth in brain can be followed by MRI [19] as well as luminescent methods[20,21] that offer the ability to monitor the time course of drug efficacy and can be coupled with drug pharmacokinetics. Physiologic factors,[22] including tumor interstitial fluid pressure, have been shown to markedly influence drug penetration to tumor. Lowering interstitial fluid pressure can enhance the time-concentration exposure of drug to tumor tissue.[23]

Drug Delivery Strategies

A critical issue in the management of brain tumors is adequate delivery of chemotherapeutic agents to the tumor. This delivery may be impeded by the BBB (Figure 1), as well as osmotic pressure within the tumor itself. In order to better deliver drugs to brain tumors, multiple strategies to circumvent, disrupt or manipulate the BBB have been employed.

Convection enhanced delivery (CED), wherein chemotherapy is infused directly into the tumor via strategically placed catheters, has had significant progress from animal models to clinical trials in the last 10 years. CED is an attractive approach for agents that are thought to be too large to

cross the BBB or too toxic for systemic administration. This has been a particularly enticing approach for conjugated targeted toxin therapies and antibodies.[24,25] The initial results with cintredekin besudotox (CB) delivered via CED showed safety and promising efficacy.[26,27] However, the large phase 3 trial of CB versus carmustine impregnated wafers (PRECISE trial) was recently completed and did not meet its efficacy endpoint of a significant difference in the overall survival (OS). There was, however, activity with both CED of CB and carmustine impregnated wafers, resulting in overall survival rates of 14% at 24 months for recurrent or progressive GBM. Similarly, the TransMID (modified diphtheria toxin conjugated to transferrin) CED phase 3 trial was recently stopped at the interval analysis due to low probability of meeting efficacy endpoints. Final results from these studies are expected in the next year.

Challenges that may limit the success of CED are limited distribution of infused drug and selection of effective agents for infusion and neurotoxicities due to large volume infusions. To overcome these limitations, most clinical trials use $\geq$ 2 catheters for infusion to maximize distribution of drug to various regions of tumor. However, it is not yet clear that multiple catheters result in substantially improved distribution of drug throughout heterogeneous brain tumors. The ongoing phase II trial of chlorotoxin coupled with the radioisotope ^{131}I (^{131}I-TM-601) infuses agent into the tumor resection cavity via Ommaya reservoir.[28] This approach allows for repeat dosing, but remains limited in the potential volume of distribution. Reverse microdialysis in which drug is infused into tissue with small volumes has shown similar results to standard CED approaches, but may have even more difficulty achieving wide volumes of distribution. Recent studies have used liposomal drug preparations or solvent facilitated perfusion to increase the area of distribution of infused drug in preclinical and early clinical trials.[29] Increasingly, CED studies are employing imaging techniques such as FDG-PET, DWI MRI and SPECT to attempt to image the area of distribution of agent after infusion.[30] These techniques have not yet been validated with direct measures of drug concentration. However, this is an ongoing important area of research that may help in planning future trials to optimize CED and other local therapies.

Polymer-based local drug delivery to tumors has been part of standard care for select patients with malignant gliomas since the FDA approval of carmustine impregnated wafers for recurrent GBM in 1996. However, recent studies have shown that the maximal tolerated dose (MTD) of carmustine in polymers in patients with recurrent gliomas may in fact be 40 mg, rather than the 7.7mg dose currently approved and in clinical practice.[31] Efficacy studies at the high dose are needed to determine if there is improved benefit, but such trials are difficult to efficiently advance due to the FDA requirement for approval of each polymer and drug combination. There has also been advancement in the biotechnology of local drug delivery with increasing variety of media being tested for local implantation. One such agent is ReGel, which is a viscous liquid when cooled, but becomes solid at room or body temperature, conforming to the shape of the cavity it occupies.[32] Similar to polymers, it degrades over time with slow local release of the chemotherapeutic agent. Phase II trials using this technology with paclitaxel for patients with recurrent gliomas are now being planned. Other local delivery techniques, such as cisplatin-infused plates placed into tumor, have also shown initial promising results.[33] The benefits of these approaches are direct drug delivery to the tumor with minimal systemic or local toxicities and little added complexity or risk to surgical procedures. Similar to CED, limitations include uneven distribution of the drug to heterogeneous infiltrating tumors as well as variable release of the chemotherapeutic agent.

Blood-brain barrier disruption (BBBD), with or without intra-arterial (IA) chemotherapy, has been extensively investigated as a technique to permit agents to go through, rather than around, the BBB. Most commonly this approach utilizes osmotic BBBD with agents such as mannitol followed by IA chemotherapy. However, more recently, MRI-guided ultrasound-induced BBBD has been investigated.[34,35] Although relatively small numbers of patients have been treated, there have been promising results with BBBD and IA chemotherapy including in traditionally difficult tumors such as brainstem gliomas.[36] There also may be particular promise for long-term complete responses without the cognitive loss due to whole brain radiation therapy[37] when this technique is used with methotrexate based therapy for primary central nervous system lymphoma

(PCNSL).Delivery of antibodies such as Rituxan, is more difficult (Figure 2), than smaller molecules such as methotrexate [38] but now can be studied in PCNSL animal models.[39]

MRI techniques are increasingly being used with this approach in an attempt to quantify the degree of BBBD achieved. Interestingly, standard therapy such as conformal radiation therapy (XRT) has been demonstrated to alter the BBB permeability to gadolinium diethylenetriamine-pentaacetic acid (Gd-DTPA) in patients with high grade gliomas. Initially, non-enhancing tumor had significantly increased contrast uptake after 30 Gy, whereas initially contrast-enhancing tumor had a decrease in degree of contrast enhancement over time. The results suggest that XRT alone may induce BBBD potentially allowing increased entry of anti-cancer agents. This is particularly notable given the recent improvement in survival in GBM with the regimen of temozolomide given concurrently with XRT.

Modifying systemic agents to increase their permeability or enhance receptor mediated transport across the BBB is of increasing interest. Technological advances in the delivery of drugs via nanoparticles also continue to develop. Nanoparticles are particularly attractive as they can be engineered to maximize transport across the BBB and can be "loaded" with both therapeutic agents and agents to optimize various neuroimaging techniques.[40-44] Currently, this technology remains in pre-clinical development.

Tumor and Brain Drug Pharmacokinetics

Key questions for any delivery approach are what amount of drug is delivered and to which area of tumor. It is becoming increasingly apparent that determining intratumoral drug concentrations and local effects of investigational agents is an important step in early phase clinical trials. There are multiple techniques in development for assessing drug delivery to brain tumors.

Microdialysis uses small, flexible catheters with specialized permeable membranes placed within tumor to sample the extracellular fluid. This has been used extensively in preclinical trials to assess drug PK within tumor and can be used to measure brain concentrations after both systemic and local delivery. The first clinical trial to measure intratumoral drug concentrations in brain cancer patients with microdialysis catheters was recently completed through the New Approaches to Brain Tumor Therapy (NABTT) consortium.[45] Results demonstrated: 1) the feasibility of the technique for obtaining continuous intratumoral pharmacokinetic data in glioma patients, and 2) regional variability in tumor drug concentration relative to BBB integrity. Similar feasibility has been shown using this technique to measure p-boronophenylate in tumors for glioma patients undergoing boron neutron capture therapy.[46] Additional studies in brain cancer patients to measure differential concentrations of investigational agents within tumor are currently under way. This technique is valuable in early phase clinical trials not only to determine investigational drug PK, but also to assess local effects of the agent. Such information may be particularly meaningful for pharmacodynamic determinations of optimal doses of small molecular-based therapies that are unlikely to have the toxicities on which traditional MTD studies depend. Although microdialysis is a powerful and readily available tool, it is limited by regional sampling of the extracellular fluid, requiring multiple catheters to assess heterogeneous portions of tumors, inability to sample substances > 20kD, and technical considerations regarding recovery.

Another approach to determining intratumoral drug concentration and effect is tissue sampling. This approach is preferable for assessment of large molecules and membrane bound proteins such as receptors. An increasingly popular clinical trial design is to treat patients with the investigational agent for some period prior to a planned resection and then sample the tumor at the time of resection for drug concentration and targeted effect.[47] The limitations of this approach are that it requires the patient have surgery and gives only one time point regarding drug concentration and activity, The trauma of surgery can also impact blood-tumor and blood- brain pharmacology. Accurate MR assessment of tumor blood volume, and flow without the artifact of

gadolinium leakage requires blood pool agents such as nanoparticles[48] to complement changes in permeability using gadolinium following anti- angiogenesis therapy

Imaging

Brain imaging technologies have been applied with increasing success to measure the delivery and local effects of anti-cancer agents in brain tumors. Batchelor et al. recently applied a variety of MRI sequences for quantification of tumor vessel response to a systemic pan-VEGF receptor tyrosine kinase inhibitor (AZD2171) in patients with recurrent GBM.[49] They were able to show that with antiangiogenic therapy, there was progressive normalization of microvessel size, volume of contrast enhancement and edema. Moreover, the MRI measures were correlated with circulating markers of angiogenesis, such as FGF2 and SDF1, as well as viable circulating endothelial cells. Although this approach does not give specific intratumoral PK data, it does show the activity of the agent non-invasively over time, providing a new technique for serial monitoring of drug effect on the tumor.

Techniques to quantify cerebral blood volume are under intense investigation and may add additional sensitivity to existing imaging sequences for evaluation of tumor vasculature in response to various interventions. Investigation of molecular imaging agents is also under intense development for important tumor targets such as EGFR. MRI can also be used to measure delivery of drug to tumor directly, particularly with agents known to shorten T1 such as the radiation enhancing agent motexafin gadolinium. Single photon emission computed tomography (SPECT) has been increasingly used in clinical trials to measure delivery of labeled drug brain. Interestingly, SPECT has also been successfully applied in conjunction with PET to assess the activity of multidrug resistance (MDR1) P-glycoprotein (Pgp) in cell culture, suggesting that non-invasive monitoring of key BBB transporters in response to various chemotherapeutics may be possible *in vivo*.

It is critical to note that delivering drug directly to tumor is often associated with pathologic imaging changes that may confound assessments of tumor progression. This has been demonstrated for CED, polymer-based therapies, immunotherapies, and BBBD approaches. In particular, immune therapies can increase inflammatory events dramatically. A flare phenomenon has been observed in MRIs performed post-immunotherapy. Interpretation of increased mass effect and edema have resulted in inadvertent placement of patients off protocol. Better discernment of tumor progression and inflammation are needed. Novel applications of PET, SPECT and new MRI sequences are being investigated to better distinguish tumor growth from treatment-induced injury. These imaging changes remain a complicating factor in the clinical management of brain tumors after drug delivery across the BBB.

In an NIH-sponsored conference, "a reality check" of tumor delivery, such as those discussed above, has been published.[50]

2. Barriers to Progress

Brain tumors are heterogeneous both genotypically and phenotypically. The BBB-brain tumor interface may be similar or quite different in different types of tumors. Current *in vitro* and animal models are inadequate for studying the BBB-brain tumor interface. Brain metastases are by far the most common brain tumor and yet the least studied. By sheer number it makes sense to focus on brain metastases which may provide unique insights into the molecular pathogenesis of tumors that home to the brain.

There is a lack of information regarding pharmacokinetic and pharmacodynamic endpoints in the brain. Most studies to date have largely focused on whether or not drug can be detected in tumor or even CSF as a surrogate for brain/brain tumor concentration. It will be critical to consider issues such as effective target tissue concentrations, dose and duration of exposure, timing of

measurements, receptor saturation and the variability of drug levels at different regions within the tumor. The ability to directly measure drug delivery to tumors is difficult. Current techniques, such as tissue drug levels and microdialysate drug levels, require surgical intervention and demand intensive time and effort on the part of a multidisciplinary team to coordinate. Small variations, such as the location of catheter placement, can have a significant impact on results. Further research is needed to define the efflux transporters and their activity as associated with various antitumor agents. Surrogate measures of the BBB-brain tumor interface, such as imaging surrogates, are difficult to validate and are often expensive.

There are interdisciplinary barriers to advancing the understanding of BBB-brain tumor interface. The groups that need to be able to interact include neurosurgeons, neurologists, neuro-oncologists, medical oncologists, neurobiologists, neuroradiologists and pharmacologists. This is a barrier in establishing effective collaborations and communicating existing data. Interdisciplinary grants may have a difficult time in the review process for a number of reasons including standard committee review criteria or being submitted to the wrong institute/program. This is exacerbated by an oversimplified understanding or appreciation of the importance and role of the BBB by many professions involved in the care of brain tumor patients.

Although a number of different strategies have been developed to technically improve the delivery of various agents to brain tumors, phase 3 trials, albeit difficult, are needed to show incremental therapeutic benefit for the patient. On the other hand, increasing delivery as reviewed by a statistician appears to impact efficacy.[51] An important goal moving forward would be to target both effective delivery strategies and to pair these with agents that have the most promise if delivered efficiently. The expense and regulatory requirements related to the conduct of clinical trials is a significant barrier to progress.

3. Specific Recommendations to Advance the Field

Key Basic Science Research areas:

- Develop representative tumor model systems that can be used in animals to monitor tumor growth and drug action noninvasively.
- Characterize the proteome and genome of the BBB-brain tumor interfaces and how they change with tumor progression and treatment. Included in this would be factors that influence tumor cell metastasis and invasion of brain, including growth and angiogenesis.
- The factors that determine drug transport across the BBB so that “brain” or “brain tumor” available drugs can be designed de novo.
- The role of tumor invasion and micrometastases, particular those tumors which develop in the setting of an intact BBB.
- Delivery of targeted molecules, including large molecular weight antibodies to treat primary and metastatic CNS tumors.

Key Clinical Research areas:

- Develop surrogate measures of the BBB-brain tumor interface (including permeability, drug delivery, etc). In particular, new imaging modalities are likely to be critical and it will be important to encourage interactions with expert radiologists and the imaging branch of the National Institutes of Health to optimize these modalities. However, it is also felt to be critical that these imaging surrogates be validated or confirmed with actual tissue assays.
- Develop nanotechnology-based drug delivery, receptor-mediated drug delivery, and carrier-mediated drug delivery to target and enhance drug uptake into tumors and surrounding brain tissue.
- Develop Phase 0 clinical trials to optimize drug and tissue assays appropriate to validate the delivery of novel agents or novel delivery strategies before proceeding to larger more traditional trials.
- Future clinical trials should include:
    - Tissue studies of major efflux transporters in newly diagnosed patients
    - Brain metastasis

- Pre-irradiation studies
- Microdialysis studies in low grade-gliomas where the BBB is presumably intact could be valuable
- Drugs that inhibit the major efflux transport systems

Education

- To educate and integrate medical professionals involved in brain tumor research at all levels about the importance of the BBB. This includes but is not limited to clinicians caring for brain tumor patients, pharmaceutical companies and laboratories.
- Lobby American Society of Clinical Oncology or Society of Neuro-Oncology to dedicate an education session to the relevance of the BBB in the understanding and treatment of metastatic and primary brain tumors.

Specific Resources

- Funding initiatives targeted to BBB and blood tumor barrier research. These initiatives should clearly emphasize and truly support interdisciplinary research. This funding is critical both for the basic science as well as the clinical research areas.
- In addition to specific research funding, it is critical to have adequate insurance coverage for standard procedures to allow the conduct of clinical trials.

4. Single Most Important Issue

Multiple topics were discussed during the meeting that are of importance to brain barrier systems and tumors. The consensus of the group at the meeting was that imaging was the single most important issue. Currently, *in vivo* imaging of brain tumors is limited by a number of factors. The skull and current methods restrict the accurate assessment of tumor size, location and type as

well as response to therapy. In most studies, information obtained is constrained to a small subset of properties, such as barrier passive permeability, blood volume, blood flow, and glucose metabolism. Improved imaging techniques, which offer the opportunity to accurately monitor tumor invasion, growth, angiogenesis, cellular apoptosis, necrosis, inflammation, edema, and gene expression, would greatly aid studies of brain tumor biology. Such may be combined with continuous assays of tumor drug delivery to more appropriately assess *in vivo* response to therapy. A need exists for quantitative imaging methods that can be used in humans and small experimental animals to characterize tumors and their response to therapeutic interventions.

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Figure Legends

Figure 1. Relationship between BBB permeability and octanol/water partition coefficient for chemotherapeutic agents. The solid line is the least-squares fit to the data for agents that are not actively taken up by brain or pumped out by the BBB (adapted from Smith, 2003) The permeability to specific chemotherapeutic agents is shown.[52] Many chemotherapeutics show limited uptake into brain because they are either polar (low octanol/water distribution coefficient), have molecular weight >500, bind extensively to plasma proteins, or are transported out of brain by active efflux pumps.

Figure 2.

Ibritumomab (Zevalin™) delivery and efficacy in PCNSL. Panels A and B: SPECT images at 24 hours (A) or 120 hours (B) after IV administration of 5.2 mCi ^{111}In-ibritumomab in a single case study. Panel B shows questionable uptake at the arrow. At 45 hours after ^{111}In-ibritumomab administration, decay-corrected radioactivity was determined in CSF (1579 dpm) and serum (572,443 dpm), giving a CSF/plasma ratio of 0.0028. Panels C-E: Magnetic resonance imaging of tumor response in the same case study. T1-weighted with gadolinium contrast axial scans are shown for (C) Pre-^{90}Y-ibritumomab showing enhancing tumor (i.e. high signal) around the occipital horn of the ventricle; (D) complete response 2 months after ^{90}Y -ibritumomab; (E) Relapse around the opposite occipital horn 3 months after ^{90}Y -ibritumomab, with continuing complete response at the site of the original tumor. Figure previously published in the Journal of Clinical Oncology.[50]

Figure 1

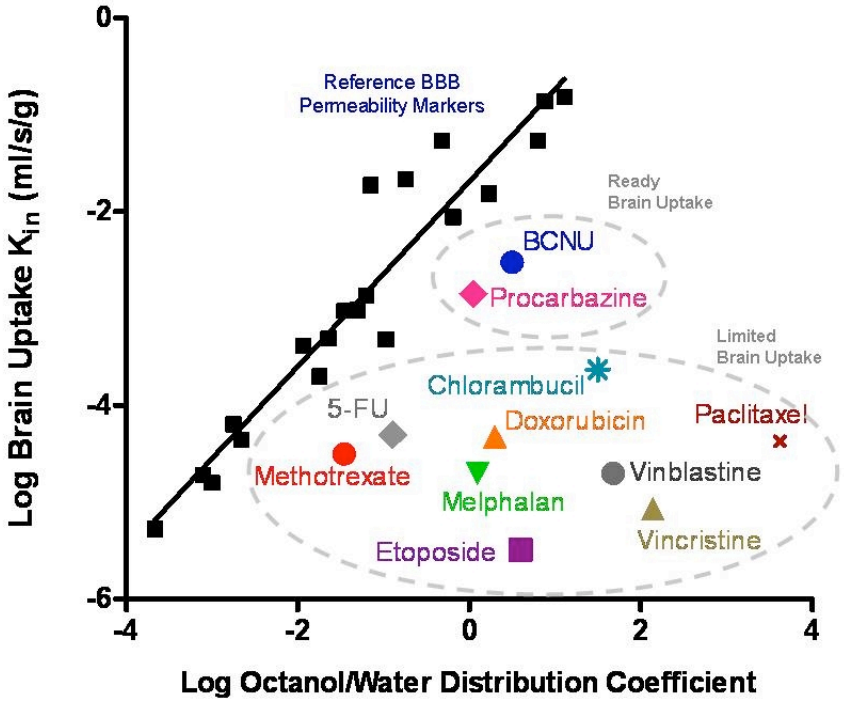
Log Brain Uptake K_{in} (ml/s/g)
0
-2
-4
-6
-4
-2
0
2
4
Log Octanol/Water Distribution Coefficient
Reference BBB
Permeability Markers
Ready
Brain Uptake
BCNU
Procarbazine
Limited
Brain Uptake
Chlorambucil
5-FU
Doxorubicin
Paclitaxel
Methotrexate
Melphalan
Vinblastine
Vincristine
Etoposide

Figure 2