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The Brain Tumor Window Model: A Combined Cranial Window and Implanted Glioma Model for Evaluating Intraoperative Contrast Agents

Daniel A. Orringer, M.D.¹, Thomas Chen², Dah-Luen Huang, MS¹, William M. Armstead, Ph.D.³, Benjamin A. Hoff², Richard F. Keep, Ph.D.¹, Martin Philbert, Ph.D.⁴, Raoul Kopelman, Ph.D.⁵, and Oren Sagher, M.D.¹

¹Department of Neurosurgery, University of Michigan Health System, Ann Arbor, Michigan

²University of Michigan, Ann Arbor, Michigan

³Department of Anesthesiology and Critical Care, University of Pennsylvania, Philadelphia, Pennsylvania

⁴Department of Toxicology, University of Michigan, Ann Arbor, Michigan

⁵Department of Chemistry, University of Michigan, Ann Arbor, Michigan

Abstract

Objective—Optical contrast agents for brain tumor delineation have been previously evaluated in *ex vivo* specimens from animals with implanted gliomas and may not reflect the true visual parameters encountered during surgery. This study describes a novel model system designed to evaluate optical contrast agents for tumor delineation *in vivo*.

Materials and methods—Biparietal craniectomies were performed on 8-week-old Sprague-Dawley rats. 9L glioma cells were injected intraparenchymally. A cover slip was bonded to the cranial defect with cyanoacrylate glue. When the tumor radius reached 1mm, coomassie blue was administered intravenously while the appearance of the cortical surface was recorded. Computerized image analysis of the red/green/blue color components was used to quantify visible differences between tumor and non-neoplastic tissue and to compare delineation in the brain tumor window (BTW) model to the conventional 9L glioma model.

Results—The tumor margin in the BTW model was poorly defined prior to contrast administration, but readily apparent post-contrast administration. Based on red component intensity, tumor delineation improved four-fold at 50 minutes post-contrast administration in the BTW model ($P<0.002$). The conventional 9L glioma model overestimated the degree of delineation compared to the BTW model at the same dose of coomassie blue ($P<0.03$).

Conclusions—Window placement overlying an implanted glioma is technically possible and well tolerated in the rat. The BTW model is a valid system for evaluating optical contrast agents designed to delineate brain tumor margins. To our knowledge, we have described the first *in vivo* model system for evaluating optical contrast agents for tumor delineation.

Correspondence: Oren Sagher, M.D., Department of Neurosurgery, University of Michigan Health System, 1500 E. Medical Center Drive, Room 3552 Taubman Center, Ann Arbor, MI 48109-5338, Phone: 734-936-9593; Fax: 734-936-9294, osagher@umich.edu.

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Keywords

Cranial window; Intraoperative imaging; brain tumor

Primary brain tumors are diagnosed in approximately 51,000 individuals per year in the United States¹. In the majority of cases, the treatment of brain tumors begins with maximal surgical resection. It is increasingly apparent that the extent of surgical resection correlates with patient outcome for all types of brain tumors². Numerous methods for improving the extent of resection including stereotactic navigation, intraoperative ultrasound and intraoperative MRI have been developed. However, the common limitation of each of these methods is that the images they generate are physically separated from the operative field, requiring the surgeon to correlate imaging data with what is visually observed through the operating microscope. To bridge the imaging gap between radiographs and the operating field, investigators have long proposed the use of fluorescent and visible dyes that would optically delineate tumor margins³⁻¹⁰.

Dyes including fluorescein⁸ and fluorescent porphyrins⁹ have been used, with varying success, in brain tumor patients to improve the extent of resection. However, research aimed at developing better visible contrast agents for brain tumor surgery has yielded a number of promising candidates that have yet to be tested clinically. Because they could be visualized under the normal lighting conditions of the operating room, bromophenol blue⁷ and indocyanine green^{3,4} are among the most attractive candidate dyes currently proposed for intraoperative tumor delineation. Experimental studies in implanted 9L gliomas demonstrate that in *ex vivo* specimens, both indocyanine green and bromophenol blue provide clear visual contrast with the surrounding tissues at high doses. However, clinical trials of these agents have been limited due to the fact that the doses of contrast required to visibly delineate the margins of implanted tumors have unacceptable or lethal side effects.

Due to the visual differences between perfused and non-perfused tissue, we hypothesized that the properties of candidate optical contrast agents could be best characterized using *in vivo*, rather than *ex vivo* models. Therefore, we aimed to create an animal model to allow real-time, *in vivo* visualization of the tumor brain interface. In this study we describe a combination of the conventional 9L implanted glioma model with the chronic closed cranial window model to create the brain tumor window (BTW) model, a new system for evaluating the visual appearance of experimental brain tumors *in vivo*.

Materials and Methods

Cell culture

The 9L gliosarcoma cell line was cultured under standard cell culture conditions in RPMI media with 10% fetal bovine serum (Invitrogen, Carlsbad CA).

Brain Tumor Window Model

Approval from the University Committee on Use and Care of Animals at the University of Michigan was obtained prior to all experiments. Sprague-Dawley rats weighing 250-350g were anesthetized with isoflurane, injected with buprenorphine 0.1mg/kg, intubated with a 16 gauge intravenous catheter and placed in a stereotactic headholder. Vancomycin (25 mg/kg) was administered intraperitoneally prior to incision. A midline incision was carried out over the skull. 4-0 Neurolon sutures were placed to retract the scalp (Figure 1A). The periosteum was detached from the skull bluntly using a cotton-tipped applicator and excised using bipolar electrocautery. The bregma and lambda were identified as landmarks for placement of a craniectomy (Figure 1A). Bleeding during exposure was controlled with

bipolar electrocautery and retraction. A high-speed drill was used to perform a nearly full thickness craniectomy of the medial aspect of the parietal bones bilaterally (Figure 1B). Special care was taken to avoid damaging the bone overlying the superior sagittal sinus. The final layer of bone was removed with a fine forceps and curved Penfield retractor. A linear rent in the dura in the coronal plane, towards the caudal end of the craniectomy was created using a bent 30-gauge needle. The caudal-most dura was then incised along the base of the cranial defect and reflected cranially so that it could be removed in a single flap. Care was taken to avoid disrupting the large cortical veins entering the superior sagittal sinus. A dissecting forcep with curved tines was helpful in lifting and tearing the dura without causing damage to the underlying structures. Once the dura was maximally removed (Figure 1C), the brain was copiously irrigated. 10^5 9L cells were injected at a depth of 1mm into the right or left frontal portion of the exposed brain in an area free of large blood vessels. A thin round glass microscope cover slip (10mm diameter) was placed over the cranial defect (Figure 1D). The edges of the cover slip were covered with cyanoacrylate glue (Figure 1E). The glue was allowed to dry over a period of 1-2 hrs. A plastic ring was sewn into the scalp with 3-0 Ethilon suture to enable continuous in vivo visualization of the implanted window (Figure 1F). Animals were extubated after window implantation, treated with buprenorphine and returned to their cages. They were monitored in the postoperative period for signs of pain and treated with buprenorphine as needed.

Serial Monitoring

Oral intake, grooming behavior, neurologic status, urine output and fecal production of the animals was monitored daily. Animals were weighed daily. Windows were monitored daily and the surface of the cortex was inspected for signs of tumor in the region of the injection. Signs of tumor growth observed include hypervascularity, pinkish color change in the region of tumor injection and elevated cell mass at the site of injection. To minimize the chance of infection, all animals were treated daily with 25 mg/kg of vancomycin intraperitoneally.

Conventional 9L implanted glioma model

Gliomas were created based on previously published descriptions of the conventional implanted glioma model¹¹. Animals were anesthetized with isoflurane, intubated and placed in a stereotactic head holder. A linear incision was made over the right coronal suture. The right coronal suture was identified. A burr hole was placed immediately posterior to the right coronal suture. The dura was incised. 10^5 9L cells were implanted 2mm deep to the inner table of the skull. The skin was closed with 3-0 nylon suture. Oral intake, grooming behavior, neurologic status, urine output and fecal production of the animals was monitored daily.

MRI Imaging

All rats were anesthetized with 2% isoflurane/air mixture throughout MRI examination. Rats lay supine, head first in a 7.0T Varian MR scanner (183 mm horizontal bore, Varian, Palo Alto, CA) with body temperature maintained around 37C using a heated air system. A linear volume radio-frequency coil was used to scan the heads of the rats. Axial T2-weighted images were acquired using a fast spin-echo sequence with the following parameters: repetition time (TR) / effective echo time (TE), 4000/60 ms; echo spacing, 15 ms; number of echoes, 8; field of view (FOV), 35×35 mm; matrix, 256×128; slice thickness, 1 mm; no gap; number of slices, 15; 2 averages; 2 dummy scans. T1-weighted images were acquired both before and after intravenous injection of 0.15 mmol/kg Gadopentate dimeglumine (Bayer HealthCare Pharmaceuticals Inc. Wayne, NJ) using a spin-echo sequence with the following parameters: repetition time (TR) / effective echo time (TE), 510/15 ms; field of view (FOV), 35×35 mm; matrix, 128×128; slice thickness, 1 mm; no gap; number of slices, 15; 2 averages; 4 dummy scans.

Images were assessed for lesions consistent with tumor growth. T2 images were assessed for round hyperintensity and peri-lesional edema. T1 pre and post-contrast images were compared to evaluate blood-brain barrier breakdown, appearing as a round hyperintensity, in the region of cell injection.

Assessment of Tumor Delineation by Optical Contrast Agent

When the radius of tumors reached 1mm, and the tumors were clearly visible adjacent to normal brain tissue, they were used for evaluation of optical contrast agents. Only animals where there was obvious visible tumor adjacent to normal appearing brain were used for analysis. When an animal met criteria for analysis, femoral venous access was established by placing PE50 surgical tubing (0.25" inner diameter, Dow Corning, Midland, Michigan) into the right femoral vein. Animals were then placed into a stereotactic head holder and any scar tissue that had accumulated near the window was removed. Continuous video recording with a high-definition camcorder was then initiated. Coomassie blue (CB), an optical contrast agent, was administered intravenously over 5 minutes using a Medfusion 3500 syringe pump (Medex, Dublin, OH) infusion syringe pump. Animals were euthanized after 2hrs of video recording via anesthetic overdose and decapitation.

Image Analysis

Video collected during the experiment was imported and processed with Final Cut Express (Apple, Inc.). Processed video was examined qualitatively to evaluate the difference in appearance between normal cortex and tumor tissue. Still images were generated from the video and analyzed colorimetrically with Image J as previously described to quantify the degree of color change in the tumor. The difference in red hue was found to be the best method to reflect the visual difference between tumor and normal brain.

MRI images were also assessed with Image J. Rather than evaluating the red hue as a method of distinguishing tumor from normal brain, gray scale values were calculated from various regions of interest. On T1 weighted post-contrast images the tumor generally appeared hyperintense to brain, and, therefore had lower values on the gray scale. Photographic images were compared to MR images by creating a normalized scale based on percentage change in either red hue or MR intensity from a region of cortex remote from the tumor.

Tissue processing

Tissue was imaged following each experiment on both a macroscopic and microscopic level. At the conclusion of each experiment, the brain was removed from the skull and photographed digitally on a standard white background. Tissues were not perfused with paraformaldehyde in the conventional cranial window model to enable an accurate comparison between the two models. All harvested brains were then sectioned using a brain mold and razor blades to generate coronal cross sections of 2mm thickness. Gross coronal sections were photographed digitally. Coronal sections were then immersed in 4% paraformaldehyde for 15 minutes then placed in 40% sucrose solution for 24 hours. Subsequently, coronal sections were frozen in Tissue-Tek® O.C.T.™ Compound (Sakura Finetek, The Netherlands). Routine hematoxylin and eosin staining was performed on 15 µm coronal sections created with a cryotome.

Statistical Analysis

Colorimetric data was evaluated using the Microsoft Excel data analysis statistics package (Microsoft Corp., Redmond, WA) to run 2-tailed t tests, with statistical significance set at $P < 0.05$. Paired t-tests were used to compare color change over various time points during the

experiments. Unpaired t-tests were used to compare data points between BTW animals and conventional glioma model animals.

Results

Technique Development

The BTW model was refined using 50 animals. One of the animals did not thrive after window implantation, demonstrated hunched posture, irregular respirations and was euthanized on the second post-operative day. The remaining 49 animals demonstrated a two to three day period of approximately 7% body weight loss, but did not show any behavioral abnormalities. All animals returned to their preoperative weight and to the expected rate of growth for rats of their age by the 5th post-operative day. There were no focal deficits noted in any of the BTW model rats. One of the rats removed his window on the seventh post-operative day and, while he did not show any signs of neurologic deficit or infection, was euthanized. Eight rats developed purulent appearing material under the window that prevented visualization of the cortical surface and those rats were euthanized. Extensive hemorrhage obscuring the window to the point where the cortical surface could not be visualized was noted in three of the rats. In each of these rats, hemorrhage cleared within days, but these animals were excluded from analysis due to a subtle yellowish tinting of the cortex, likely due to hemosiderin staining. Animals demonstrating hemorrhage under their windows did not demonstrate any signs of behavioral change, neurologic deficit or weight loss.

Tumor growth was evaluated serially whenever possible. The stages of tumor growth observed are demonstrated in Figure 2A. Assuming a spherical growth pattern, tumor growth followed an exponential curve (Figure 2B). The average latency before tumor detection was four days. The average time for animals to meet criteria for evaluation of contrast agents (tumor radius of 1mm) was 12.8 days. Six animals exhibited no tumor growth. Three animals exhibited tumor growth diffusely along the entire cortical surface of the injected hemisphere and were excluded from further analysis. Tumor tissue appeared slightly redder than the normal cortical surface. Tumor tissue was void of the large draining veins and major arteries that could be observed coursing along the surface of the normal cortex. However, small diameter, convoluted vessels were noted within the tumor tissue and their density increased with increasing tumor size. Tumor growth began at the injection site and expanded in a radial fashion.

Animals with visual evidence of tumor growth were evaluated with MRI to confirm the presence of tumor. MRI provided three-dimensional definition of tumor geometry. Most tumors were spherical with a small wedge shaped component at the injection site (Figure 3). Tumor tissue was isointense on T1 weighted imaging and hyperintense on T2 weighted imaging. Tumor tissue enhanced maximally on T1 weighted imaging 6 minutes after administration of gadolinium contrast.

BTW Model Characterization

After the BTW model could be reliably and consistently created, 6 BTW animals were used to evaluate the ability of contrast agents to delineate neoplastic tissue in the BTW model. Each of the six animals chosen for these experiments animals were selected for based on ideal, uniform tumor size, spherical tumor geometry and window clarity. Prior to CB administration, it was difficult to visibly delineate the margins or implanted tumor in comparison to adjacent normal cortex (Figure 4A). Subjectively, a contrast dose of 70mg/kg caused a marked improvement in our ability to distinguish tumor from normal brain (Figure 4B). The average red hue of normal brain was 187.9 prior to contrast administration and

140.6 at 25 minutes after contrast administration. The average red hue of the tumor was 207.2 prior to contrast administration and 65.6 at 25 minutes after contrast administration. There was a significant difference ($P<0.0002$) in red hue between normal brain and tumor after contrast administration (47.3 vs. 141.4). The red hue of normal brain and tumor were quantified and followed for multiple time points over the course of the two-hour experiment (Figure 4C). Color change was most rapid between 0 and 25 minutes and varied little between 25 and 60 minutes (Figure 4C).

Whole brains harvested from animals following experiments were evaluated histologically. Grossly, there was evidence of a superficial tissue mass at the site of the injection. Prior to sectioning, the mass appeared spherical and raised from the adjacent normal brain (Figure 5A). Coronal sectioning confirmed that the tumor had grown in a largely spherical shape (Figure 5B). Microscopic examination of specimens revealed evidence of a sphere-shaped implanted glioma adjacent to normal cortex. Glioma tissue appeared hypercellular in comparison to normal brain. The nuclear to cytoplasmic ratio in the neoplastic appearing tissue was much greater than that of cells identified in the normal cortical tissue. The nuclei of the implanted tumor appeared highly polymorphic. Mitotic figures were easily identified. Fronds of tumor cells could be observed infiltrating into normal cortex adjacent to tumor (Figure 5D).

Additionally, the ability of CB to delineate tumor from normal brain was evaluated in both the BTW model and the conventional implanted 9L glioma model (Figure 6). Twenty minutes after CB injection, the average difference in red hue between tumor tissue and normal brain was significantly smaller ($p<0.03$) in BTW model animals (75.0) than in the conventional implanted glioma model animals (101.8).

Finally, tumor borders defined by CB injection in the BTW and conventional implanted glioma models were compared to MRI-defined tumor borders. A significant change in red hue value occurred at the visually apparent tumor margin in the BTW model ($p<0.01$) and the conventional implanted glioma model ($p<0.02$). A significant change in gray scale value occurred within 0.2mm of the visually apparent tumor margin at MRI ($p<0.01$) (Figure 7).

Discussion

Achieving gross total resection remains the cornerstone of current treatment of gliomas². An accumulating body of data demonstrates that extent of resection correlates with outcome in almost all brain tumors studied^{2, 12, 13}. Technologies such as intraoperative ultrasound, intraoperative MRI, and stereotactic navigation have improved the ability of neurosurgeons to achieve more complete brain tumor resections, but they carry significant limitations. To address these limitations, investigators have long suggested the use of visible contrast agents to assist in delineating neoplastic tissue within the operative field⁴. Visible agents such as fluorescein⁸, indocyanine-green³ and bromophenol blue⁷ have been proposed. More recently, 5-aminolevulinic acid⁹ and 5-aminofluorescein labeled albumin¹⁴ have been demonstrated to be useful in delineating glioma tissue intraoperatively with fluorescent microscopy. We have recently suggested that dye-loaded nanoparticles may hold promise as visible contrast agents for brain tumor delineation¹⁵.

Given the longstanding, continuous interest in developing better ways to visualize tumor at the time of surgery, we sought to design a novel animal model to recreate the common difficulty surgeons face in distinguishing glioma from normal brain. If successful, we hypothesized that the model would be well suited to evaluating and developing visible contrast agents for tumor delineation. Our approach was to combine existing models for studying gliomas as well as cortical surface architecture.

Originally described in 1973, the 9L glioma model is one of the oldest and most well studied animals models used in brain tumor research¹⁶. Although it has been criticized on a number of accounts, it remains widely used for a variety of investigations in the field of brain tumor research both *in vitro* and *in vivo*¹⁷. For example, the blood brain barrier breakdown in implanted 9L gliomas¹⁸ makes them a favorable model for studying optical contrast agents which delineate tumor by crossing fenestrations found within 9L vasculature that are not present in normal cerebral vasculature. The cranial window model has been widely used to study cortical architecture and cerebrovascular physiology and pathophysiology¹⁹⁻²². One investigation has detailed a combination of the implanted glioma and cranial window models using nude rats and human tumor xenografts to study microvascular properties in experimental brain tumors²³. Another has described implantation of mammary adenocarcinoma in a rat cranial window model²⁴. To circumvent the need to maintain nude rats, obtain and prepare reliable human xenografts for implantation, or use a non-glial based tumor cell line, we created the BTW model using implanted 9L cells implanted in immunocompetent rats.

Among the challenges encountered in the creation of the BTW model were stroke due to herniation of brain through the craniectomy in one animal, spontaneous superficial cerebral hemorrhage in three animals and purulent infection under the cranial window in eight animals. We concede that these complications are unlikely to happen in the conventional 9L model. However, once our surgical protocol was optimized, and we became more experienced with the operative procedure, we were able to reliably produce animals bearing 9L tumors, whose growth could be followed through direct visual observation and confirmed by MRI. Moreover, while animals exhibited mild anorexia and weight loss in the immediate post-operative period, they tolerated chronic window implantation (some for up to four weeks) without any observable behavioral or physiologic abnormalities.

The BTW model allows direct observation of tumor formation from the earliest visible stages of tumorigenesis. We followed tumor growth in a subset of our animals to reveal an exponential increase in tumor volume as previously described in the 9L model¹⁶. In most cases, tumor grew at a rate that allowed us to create a subset of animals with tumor sizes that were ideally suited to our objective of evaluating the appearance of tumor tissue adjacent to normal cortex.

Because it was somewhat challenging to distinguish normal brain from tumor, we used MR to confirm the presence of tumor. As expected, 9L gliomas in the BTW model behave as conventional 9L gliomas on MR: isointense on T1, hyperintense on T2 and strongly contrast enhancing. With visual and radiographic confirmation of the presence of tumor, we were able to create a subset of BTW model animals for use in evaluating CB, a candidate molecule for intraoperative tumor delineation.

Prior to contrast administration it was difficult to determine the margins or tumor spread in the BTW model despite the fact that 9L gliomas have a generally non-invasive growth pattern. A subjective difficulty in distinguishing tumor margins is a requirement in an animal model designed to test candidate optical contrast agents. The BTW model recreates the commonly encountered clinical challenge of definitively judging tumor margins based on visual cues alone. While tumor tissue has a microvascular pattern that is distinct from normal brain, there is little difference in the appearance of the tumor parenchyma and the cortex.

However, after CB administration, there is a clear difference between tumor tissue and normal cortex. Although this change was readily apparent to the operator (see supplemental data, Figure 4), we sought to quantify the visible difference between tumor and normal brain

to provide an objective means to evaluate color change. We also felt that quantification would allow a more standard description of visual color changes, within and between animals. We examined the change in red, green, blue color system as well as changes in the hue, saturation and brightness color systems. We found that measuring the red component of the regions of interest in our images provided the best representation of tumor delineation both before and after contrast administration. Determining a sound method for quantifying color change allowed us to perform statistical analysis to evaluate the significance of color change in the BTW model.

In addition to selecting specific time points for analysis, we quantified color change in tumor and normal brain serially throughout the course of our two-hour experiments to identify the kinetics underlying the observed color change. Previous studies designed to characterize contrast agents at multiple time points have required individual animals euthanized at various times after contrast administration⁷. Therefore, we feel that future studies following the kinetics of candidate contrast agents may be performed with fewer animals than previously required with other tumor models.

In addition to reducing the number of animals required for contrast evaluation, we feel that the BTW model may offer a more accurate assessment of candidate contrast agents. Unlike the conventional implanted model, the BTW model allows the assessment of candidate contrast agents in a living animal. We designed an experiment to compare the conventional 9L model with the BTW model. Contrast between BTW and normal brain was subtler in the BTW model than the conventional 9L glioma model. The difference in the magnitude of color change in the two models is likely due to the lack of blood in the microcirculation in an *ex vivo* specimen. Both normal brain and implanted tumor appear redder *in vivo* than when they are removed for *ex vivo* analysis. Irrespective of the cause of the difference in the magnitude of color change between the two models, we feel that an *in vivo* model is more likely to accurately reproduce the visual characteristics encountered during human brain tumor resection than an *ex vivo* model.

Evaluating color change as a function of distance from the visible interface between tumor and normal brain allowed us to judge how sharply the BTW model could approximate the true tumor margin. A significant change in red hue occurred at the visually apparent tumor margin. Interestingly, a significant change in the grayscale value occurred within 0.2 mm of the visually apparent tumor margin on contrast-enhanced T1 weighted MR images. This analysis suggests that the tumor margin in the BTW model closely corresponds to the MRI-defined tumor margin. Therefore, the BTW may be a relevant model system for studying visible contrast agents capable of delineating contrast-enhancing portions of brain tumors.

While we have demonstrated the utility of the BTW model for evaluating optical contrast agents, it is possible that the model could also be used for evaluating fluorescent and near-infrared contrast agents that are under development for human use. For example, as agents such as 5-aminolevulinic acid (5-ALA) are developed for human use, their optical and pharmacokinetic properties could be optimized in the BTW model. If 5-ALA was metabolized by 9L cells to fluorescent porphyrins and the appropriate lighting conditions were present, we would expect the margins of implanted tumors to be well delineated in the BTW model. In addition, we have found that the BTW model can be used for imaging near-IR contrast agents as well using an operating microscope designed for ICG imaging (unpublished data).

Moreover, since the cranial window model has been applied previously to the study of cortical microarchitecture, it is possible that transgenic tumor cells expressing a fluorescent protein, such as green fluorescent protein, might be able to be tracked within this model. If a

fluorescent dye was used in an animal bearing GFP-labeled tumor cells, it might be possible to dynamically evaluate, with accuracy at the cellular level, the extent of uptake of a given candidate dye within an tumor.

We also feel that the BTW model may have application outside of development of novel contrast agents. Because tumor growth can be directly observed, the possibility of using the BTW model to follow tumor regression in response to experimental therapies also exists. Rather than following treatment response in terms of appearance of a tumor on MRI or survival of an animal, the effect of treatment on an experimental glioma could be followed directly.

The utility of the BTW model could be further expanded if it were possible to implant or induce tumor formation with a more invasive pattern of growth than 9L gliosarcomas. Our histologic analysis of tumors from the BTW model suggests that implanted 9L tumors are substantially less invasive than the typical human glioma. A number of conventional implanted brain tumor models in rodents could be combined with the cranial window system to evaluate their patterns of growth and invasion. Newer genetically-engineered brain-tumor models mimic the observed invasive pattern of glioma growth and would likely yield more-accurate models for the assessment of visible tumor delineation by candidate contrast agents.

Conclusion

By combining the conventional 9L model with the closed cranial window model, we have created the BTW model. Visual observation, MRI data and gross and microscopic pathology confirm that the BTW model accurately recreates the appearance of glioma tissue adjacent to normal brain *in vivo*. Moreover, administration of optical contrast agents in the BTW model significantly accentuates the subtle visible differences between tumor and normal brain tissue with greater accuracy than *ex vivo* models. In summary, by providing an accurate means of evaluating candidate optical contrast agents, the BTW model may be helpful in predicting the clinical success of experimental compounds designed to improve the extent of brain tumor resection by visibly delineating tumor margins during surgery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Key operative steps in the brain tumor window model: A. reflection of scalp flaps laterally with placement of retracting sutures; B. biparietal craniectomy; C. biparietal, semilunar durotomy; note how the appearance of the arachnoid, pia and surface vessels are clearly visualized here; D. subcortical tumor injection; E. bonding of glass coverslip to margins of cranial defect; F. attachment of plastic ring to skin flap edges.

Figure 2A

Figure 2B

Figure 2.

A. Photographs of tumor growth following window and tumor implantation. Postoperative day is listed below each image. The white circle indicates the general region where tumor growth is noted. B. Average tumor volume following implantation. The calculated average tumor doubling time is 1.2 days. Values indicate $\pm$ SEM (n=6).

Figure 3. MRI of brain tumor window model 12 days following implantation A. T2 weighted MRI B. T1 weighted MRI without gadolinium C. T1 weighted MRI post gadolinium. Tumor is highlighted with a white circle. MRI shown is representative of all animals used for evaluating delineation in the BTW model.

Figure 4C

Figure 4.

Photographs of a representative brain tumor window model before (A) and 25 minutes after coomassie blue administration (B). Sample regions of interest are indicated by the black boxes. Quantification of red hue within regions of interest over the course of the 2 hour experiment (C). Values indicated are averages from three regions of interest. Error bars indicate $\pm$ SEM (n=6).

Figure 5. Gross (A,B) and microscopic pathology (C,D) of specimens from brain tumor window model animals. Note the generally spherical shape of the implanted tumor on gross and microscopic specimens. Also note the fronds of tumor tissue infiltrating into the surrounding cortex (D).

Figure 6. Photographs of conventional implant model (A) and brain tumor window model (B) 25 minutes after coomassie blue administration. Note the effect of physiologic cerebral perfusion on the appearance of the normal cortex and brain tumor in the BTW model. Quantification of red hue within tumor and normal brain (C). The significant difference between red hue of the tumor and normal brain is also depicted ($p < 0.0002$, indicated by dagger). This difference in the appearance between normal brain and tumor tissue is significantly smaller in the brain tumor window model than the implanted model ($p < 0.03$, indicated by asterisk). Values indicate $\pm$ SEM ($n=6$).

Figure 7.

Normalized analysis of change in red hue on post coomassie blue administration images from BTW and conventional implanted glioma animals and in gray scale on post-contrast MRI. The asterisk indicates the point where a significant difference in red hue or gray scale value occurs in comparison to normal brain ($p < 0.02$, $n = 6$). All points to the left of the asterisk are also statistically different in red hue or gray scale from normal brain. The visually apparent tumor margin is indicated by the vertical dashed line; all points to the left of the line are within the tumor, while all points to the right of the line are within normal brain.