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Monitoring Early Response of Experimental Brain Tumors to Therapy Using Diffusion Magnetic Resonance Imaging

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Abstract
Quantitative magnetic resonance imaging was performed to evaluate water diffusion and relaxation times, T1 and T2, as potential therapeutic response indicators for brain tumors using the intracranial 9L brain tumor model. Measurements were localized to a column that intersected tumor and contralateral brain and were repeated at 2-day intervals before and following a single injection of 1,3-bis(2-chloroethyl)-1-nitrosourea (13.3 mg/kg). Tumor growth was measured using T2-weighted magnetic resonance imaging to determine the volumetric tumor doubling time (Td) before (Td = 64 ± 13 h, mean ± SD, n = 16) and after (Td = 75 ± 9 h, n = 4) treatment during exponential regrowth. Apparent diffusion coefficient of untreated tumors was independent of tumor volume or growth time, whereas relaxation times increased during early tumor growth. Diffusion displayed the strongest treatment effect and increased before tumor regression by 55% 6–8 days following treatment. Changes in relaxation times were also significant with increases of 16% for T1 and 27% for T2. Diffusion and relaxation times returned to pretreatment levels by 12 days after treatment. Histological examination supports the model that the observed increase in diffusion reflects an increase of extracellular space following treatment. Furthermore, the subsequent apparent diffusion coefficient decrease is a result of viable tumor cells that repopulate this space at a rate dependent on the surviving tumor cell fraction and recurrent tumor doubling time. Serial tumor volume measurements allowed determination of log cell kill of 1.0 ± 0.3 (n = 4). These results suggest that diffusion measurements are sensitive to therapy-induced changes in cellular structure and may provide an early noninvasive indicator of treatment efficacy.

Introduction
The management of high-grade malignant tumors of the central nervous system is problematic, and despite the use of multimodality therapy, malignant gliomas remain uniformly fatal (1). Furthermore, quantitation of the response of brain tumors to therapy is more difficult than in systemic cancers because an improvement in patient function is multifactorial, and changes in neurological deficits may be unrelated to changes in tumor volume (2–5). MRI+ and X-ray CT scans of malignant human brain tumors do not readily allow quantitation of the actual tumor volume. Administration of a contrast agent allows estimates of tumor areas from the largest cross-sectional area of contrast enhancement indicating a compromised blood-brain barrier. The blood-brain barrier, however, may be altered by chemotherapeutic agents (5) or corticosteroids; thus, measurements of tumor dimensions by the boundaries of contrast enhancement is an indirect estimate of tumor size. Moreover, not all tumors exhibit contrast enhancement (6).

Because of the difficulty in obtaining accurate measurements of intracranial tumor volumes, brain tumor therapy trials usually report median survival time and median time to progression as a quantitative measure of response (2–5). Improved methods that would allow for earlier and accurate quantitation of the therapeutic response in individual patients are still needed. Because diffusion MRI is sensitive to tissue structure at the cellular level, we believe that this technique has the potential to detect important quantitative information about the tumor cellular changes that would occur following successful therapeutic intervention.

Cellularity and the integrity of cellular membranes that impede water translational mobility can affect the diffusion of water within tumor tissue. For example, water diffusion measurements have been shown to be sensitive to tissue cellular size, extracellular volume, and membrane permeability (7–9). In addition, it has been shown that structural anisotropy is manifest as diffusion anisotropy (10–12). Diffusion MRI applications include imaging of cerebral stroke (13–16) and as a probe in the study of central nervous system tumors in humans (17–19). Diffusion studies on human (17–20) and animal (21–23) tumors have demonstrated strong differences between solid tumors relative to high diffusion within necrosis and cysts. As both solid tumor and peritumor edema can have a continuum of diffusion values that are elevated relative to normal brain, the diffusion coefficient alone does not readily distinguish tumor from edematous tissue (19, 23). However, structurally anisotropic tissues, such as white matter, can exhibit persistent diffusion anisotropy.
despite a diffusion coefficient elevated by edema. This could potentially provide contrast between edema and an isotropic solid tumor (18–21).

The consistent observation of high diffusion in necrotic tissue relative to solid tumor provides a rationale for the use of diffusion to monitor cellular changes following anticancer therapies. Preliminary studies in gliomas revealed that the evolution toward necrosis that occurs following successful therapy could be detected as an increase in ADC relative to pretreatment levels in the intracranial 9L model after administration of a chemotherapeutic agent (24, 25). The observed increase in water mobility was anticipated to be the result of increased interstitial water volume and cellular permeability as the tumor cells necrosed, thus indicating the potential of diffusion MRI for predicting therapeutic efficacy. More recently, others have made similar observations in a s.c. tumor model using diffusion-weighted spectroscopy (26).

In this study, we sought to determine if quantitative MR diffusion measurements could be used as a predictor of treatment outcome through early detection of cellular changes before tumor regression in an intracerebral tumor model. In addition, NMR relaxation times, T1 and T2, have been shown to be sensitive to extracellular volume (23, 27) and thus may also demonstrate changes in response to treatment. In this regard, diffusion and T1 and T2 relaxation times were obtained from rat intracranial 9L gliosarcomas before and following treatment with BCNU. We found that of the MR parameters evaluated, diffusion MRI was the most sensitive to changes in membrane integrity affecting intra- and extracellular water volumes following treatment. Furthermore, MRI diffusion values varied among x, y, and z directions, indicating the presence of diffusion anisotropy near tumor boundaries that correlated with histologically distinct features. These results should help motivate future experiments correlating diffusion changes in human brain tumors with clinical outcome following therapeutic intervention.

Materials and Methods

**Glioma Model.** Rat 9L tumor cells were grown as monolayers in sterile plastic flasks in modified Eagle’s MEM with 10% fetal bovine serum. Cells were cultured in an incubator at 37°C 95% air, 5% CO2 atmosphere until confluent. Cells were then harvested by trypsinization, counted, and resuspended in serum-free medium for intracerebral injection.

Adult male Fischer 344 rats weighing approximately 150 g were anesthetized via i.p. injection of a ketamine (87 mg/kg body weight) and xilazine (13 mg/kg) cocktail. A small incision over the right hemisphere was made, and a high-speed drill was used to create a 1-mm diameter burr hole through the skull. The rat head was affixed in a stereotactic holder for inoculation of 106 9L tumor cells in 5 μl in the right forebrain at a depth of 3 mm via a 25-μl Hamilton syringe. The surgical region was irrigated with 70% ethanol, and the burr hole was filled with bone wax to reduce extracerebral extension of the tumor and loss of cerebrospinal fluid. The skin incision was sutured closed, and the rats were allowed to recover. Tumors were produced in 20 rats for this experiment.

**MRI Measurements.** *In vivo* MRI was initiated 10–12 days after tumor inoculation and performed serially on average every 2 days. A rigid bite-bar was used to hold the anesthetized rat within a 35-mm diameter transmit-receive slotted-resonator radiofrequency coil (28). Gradient-recalled-echo MRI interleaved in orthogonal planes was used to position the animal rapidly and reproducibly. Quantitative *in vivo* measurements included tumor volume, localized water diffusion, and localized T1 and T2 relaxation times. The 9L glioma appears hyperintense on T2-weighted MRI and has relatively distinct tumor margins with only moderate peritumoral edema. Therefore, tumor volume was assessed using multislice T2-weighted MRI acquired with repetition time TR = 2850 ms, echo time TE = 80 ms, 32 coronal 0.8-mm slices, 128 × 128 matrix, two-signal average, and a 30-mm field of view. These images were acquired as two interleaved 16-slice sets collected near the beginning and at the end of the NMR session. This indicated whether the animal had moved due to failed sedation, in which case only the initial 16-slice set was used for tumor volume estimation. Tumor volume was quantified as the product of slice-to-slice separation and the sum of areas from manually drawn tumor ROI on images.

Volumetric tumor doubling time, \( T_d \), and constant, \( \kappa \), related to initial viable tumor volume, were calculated using a two-parameter, least-squares-fit of measured tumor volume versus time by:

\[
\text{Tumor volume} (t) = \kappa \cdot 2^{\kappa t / T_d}
\]  

**A**

Data during exponential pretreatment and tumor regrowth were fit independently to yield \( T_{d,\text{pre}} \) and \( T_{d,\text{post}} \), respectively. Pretreatment tumor volume, \( V_{\text{pre}} \), and the effective volume of tumor that survives treatment, \( V_{\text{post}} \), were derived by extrapolation of fitted pretreatment and regrowth curves to the time of treatment. If the cellular density of the tumor at time of treatment and during exponential regrowth are approximately the same, then the following provides an estimate of log cell kill:

\[
\text{Log cell kill} = \log_{10} \left( \frac{V_{\text{pre}}}{V_{\text{post}}} \right)
\]  

**B**

T2-weighted images were also used to prescribe subsequent water diffusion and relaxation time measurements. The diffusion pulse sequence used orthogonal 90° and 180° slice-selective pulses that defined a right-to-left oriented, 2 × 2-mm column through the most homogeneous tumor region and contralateral brain (11, 21). Diffusion measurement is susceptible to errors due to tissue motion. These artifacts were lessened by using a frequency encode gradient along the column axis for spatial encoding and magnitude processing of Fourier transformed echoes. As a result, phase cancellation effects commonly associated with tissue motion in the presence of diffusion gradient pulses were greatly reduced (11). Spatial resolution along the one-dimensional image of the column was 0.23 mm. Potential directionality of water mobility is probed by controlling diffusion gradient direction. To account for potential diffusion anisotropy, paired diffusion sensitization gradient pulses were trapezoidal, 15 ms each, and applied independently on x, y, and z axes. Diffusion gradient direction and amplitude were interleaved during the scan and stepped from 14 to 60 miliTesla/meter for a total of 42 "b-factors" (17) on each axis, ranging from 87 to 1669 s/mm². ADCs were calculated for all
Fig. 1 Serial coronal T2-weighted MR images of a rat brain with time after tumor implantation. Images were acquired immediately before treatment with BCNU (a) and at 167 h (b), 319 h (c), and 442 h (d) following BCNU treatment. The full 12 time point series on this animal spanned 2 weeks. The column used for obtaining localized diffusion, T1 and T2 data, and ROI of tumor and brain are overlaid on each image.

points along the column and each direction via least-squares-fit to the model:

\[ S(b_i) = S_0 e^{-b_i ADC_i} ; i = x, y, z \]  

Disparate ADC versus gradient direction is an indication of diffusion anisotropy (10, 11). Although a 3 x 3 tensor is required to fully characterize diffusion in an anisotropic system such as tissue (29), the quantity \( ADC_0 = [ADC_x + ADC_y + ADC_z]/3 \) is useful because it represents a scalar invariant of the diffusion tensor. That is, \( ADC_0 \) is more closely related to a tissue-inherent property rather than one dependent on the relative orientation of gradient axes and the rat brain.

An IR experiment was used for T1 measurements (T1IR). Orthogonal slice-selective 90° and 180° pulses following non-selective inversion and variable inversion recovery delay were used for rapid T1IR data collection along the same frequency encoded column probed for diffusion. Data at 16 inversion times between 10 and 5000 ms were acquired within 4 min. T1 relaxation times were estimated from a three-parameter T1IR model fit for each 2 x 2 x 0.23-mm volume element along the column (30).

Unlike diffusion and T1 measurements, additional echoes can be collected without a time penalty; therefore, localized T2 measurements were derived from full two-dimensional multiecho images of a 2-mm coronal slice acquired with TR = 2000 ms, TE = n x 20 ms (n = 1, 2, ..., 8), 128 x 128 matrix, and 30-mm field of view. Pixels were averaged within a 2-mm vertical range to synthesize a one-dimensional column at each echo time equivalent to that probed for T1 and diffusion measurements. A least-squares-minimization fit to a monoexponential decay was used to estimate T2 for each point along the column. This approach is consistent with the observations of others that noted monoexponential T2 behavior in experimental brain tumors (23).

All NMR data were acquired on a two Tesla, 20-cm bore system equipped with actively shielded 18-cm gradient coils (Omega console; GE NMR Instruments). Tumor volume, diffusion, and T1 and T2 data reduction were performed on a SPARC10 workstation (SUN Microsystems, Mountainview, CA) using custom software developed within the AVS software environment (Advanced Visual Systems, Inc., Waltham, MA). As indicated above, diffusion and relaxation time data were reduced to one-dimensional plots of each NMR observable versus right-to-left position through the tumor and brain. Points within a linear segment through a relatively homogeneous region of the solid tumor were averaged for each one-dimensional parametric image. That is, the right-to-left extent of an ROI was selected using the corresponding T2-weighted image to include apparent tumor while excluding grossly necrotic or cystic areas that exhibit high ADC and T2. A separate linear ROI was defined for contralateral brain.

Treatment Protocol. Seven of the 20 rats did not receive chemotherapy and served as controls; the other 13 rats received a single i.p. injection of 13.3 mg/kg of BCNU given between 14 and 19 days after tumor cell inoculation. BCNU was administered without anesthesia at least 1.5 days following the last anesthetic injection and at least 1 h before the next anesthetic injection to minimize induction of liver enzymes that could accelerate the clearance of BCNU as described previously (31). The average tumor volume at the time of treatment was 101
mm$^3$. Four of the treated rats were sacrificed at selected time intervals following treatment for histological analysis.

**Histology.** Rat brains were removed and immersed for 3 days in 10% neutral buffered formalin solution. Several coronal sections corresponding to those studied by MRI were cut from each brain and embedded in paraffin. The 6-µm sections were cut, stained with H&E, and evaluated microscopically.

**Results**

**Dependence of MR Parameters on Untreated Tumor Volume and Treatment Efficacy.** Intracranial 9L tumor doubling times ($T_d$) were determined using Eq. A and three or four pretreatment MRI tumor volume measurements in BCNU-treated animals and all available volumetric points for untreated animals. The average doubling time of untreated 9L tumor was $T_d = 64 \pm 13$ h ($n = 16 \pm SD$), which is in close agreement with earlier studies using this model (31).

A series of coronal T2-weighted images from a single animal harboring an intracerebral 9L tumor before and following BCNU treatment is shown in Fig. 1. Each T2-weighted image was selected to intersect the largest cross-sectional area of the tumor and is the section used to prescribe quantitative acquisitions. The tumor is clearly evident as a hyperintense lesion in the right hemisphere. At 167 h after treatment, an area of peritumoral edema is observed followed by tumor shrinkage and regrowth at 319 and 442 h, respectively. Overlaid on each image are the locations of the column and ROI representative of where diffusion and T1 and T2 measurements for tumor and brain were acquired.

Fig. 2 displays the mean values for the MR-observable parameters for untreated and BCNU-treated 9L tumors. Data from treated rats acquired before or within 1 day of treatment were combined with control rats to improve statistical estimates of untreated tumor. Data are grouped into 2-day bins. For control rats, “day 0” corresponds to when its tumor volume was closest to that of treated rats on the day of treatment (mean volume, 101 mm$^3$). As shown in Fig. 2a, there is a clear increase from baseline diffusion values 2–4 days after treatment, which peaked at 6 days after treatment, then a return to pretreatment values. The therapeutic effect is accentuated by the fact that untreated tumor diffusion values remained essentially unchanged during the entire growth period of the 9L tumors. Of measured parameters, diffusion exhibited the greatest independence of untreated tumor volume or growth time, whereas T1, and to a lesser extent T2, tended to increase with untreated tumor growth. Despite this evolution unrelated to treatment, significant therapeutic changes in tumor T1 and T2 values were still apparent (Fig. 2b and c). Tumor NMR quantities were higher in treated rats on day 6 by 53% for diffusion, 10% for T1, and 47% for T2 relative to the three control rats that survived to day 6.

Summarized in Table 1 are the average values for tumor volume, ADC, T1, and T2 for 9L tumor and contralateral brain. Baseline values represent control rats (when tumor volume was closest to 101 mm$^3$) combined with treated rats using the last pretreatment time point or within 24 h of treatment. Only data collected at the point of peak change in diffusion for the 8 of 13 treated animals that survived 6–8 days after BCNU are repre-
Table 1  Peak change in NMR parameters 6–8 days following BCNU treatment

<table>
<thead>
<tr>
<th>Pretreatment baseline (n = 15)</th>
<th>Posttreatment (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid tumor</td>
<td>Contralateral brain</td>
</tr>
<tr>
<td>ADC0 (×10^{-3} \times \text{mm}^2/\text{sec})</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>T1 (ms)</td>
<td>1665 ± 109</td>
</tr>
<tr>
<td>T2 (ms)</td>
<td>98 ± 8</td>
</tr>
<tr>
<td>Tumor volume (mm³)</td>
<td>98 ± 45</td>
</tr>
<tr>
<td>Solid tumor</td>
<td>Contralateral brain</td>
</tr>
<tr>
<td>ADC0 (×10^{-3} \times \text{mm}^2/\text{sec})</td>
<td>1.41 ± 0.12</td>
</tr>
<tr>
<td>T1 (ms)</td>
<td>1935 ± 66</td>
</tr>
<tr>
<td>T2 (ms)</td>
<td>124 ± 17</td>
</tr>
<tr>
<td>Tumor volume (mm³)</td>
<td>182 ± 57</td>
</tr>
</tbody>
</table>

* Data are presented as means ± SD.

b  P comparing baseline and posttreatment groups using two-sample t test.

a  Not significant to P = 0.05 level.

Fig. 3  a, plot of intracerebral 9L tumor volume obtained from MR images versus time for one animal. The initial four-tumor volume measurements determined the pretreatment tumor doubling time (Td, 55 h) before BCNU (13.3 mg/kg) administration on day 0. Immediately after treatment, the tumor growth rate began to deviate from initial exponential growth, followed by a period of shrinkage. Apparent exponential regrowth occurred between 12 and 15 days for a posureatment Td of 86 h. Extrapolation of the regrowth line back to the time of treatment provides an estimate of treatment log cell kill in this animal.  

b, plot of diffusion for the 9L tumor and contralateral brain tissue for the animal shown in a. The animal died during the last NMR session, accounting for the final drop in diffusion values.

Table 1  Peak change in NMR parameters 6–8 days following BCNU treatment. The observed treatment change in tumor diffusion was highly significant. Treatment effects on relaxation times were also significant, whereas no significant changes were observed in contralateral brain values. The relative increase in treated tumor values from baseline (i.e., pretreatment) were 55% for diffusion, 16% for T1, and 27% for T2.

Determination of treatment log cell kill in individual animals was accomplished using serial tumor volume data collected over a period long enough to exhibit exponential regrowth. Extrapolation of the fitted exponential regrowth line back to the time of treatment provides an estimate of log cell kill by Eq. B. This method is illustrated for one animal in Fig. 3a, with the corresponding diffusion time course in Fig. 3b. A total of four treated rats survived long enough to exhibit exponential regrowth (Td = 75 ± 9 h, n = 4) for an estimate of efficacy: log cell kill = 1.0 ± 0.3 (mean ± SD, n = 4). These data suggest that a single LD_{10} dose (13.3 mg/kg) of BCNU kills approximately 90% of the 9L tumor cells. At 6–8 days following BCNU treatment, tumor diffusion values increased markedly by over 50% and returned to pretreatment values during 8–14 days after treatment. A direct comparison of tumor volume (Fig. 3a) and diffusion over time following treatment (Fig. 3b) from one animal reveals that as the tumor growth begins to slow, diffusion is increased and peaks just before regression of the tumor mass occurs. Furthermore, the tumor volume exhibits exponential regrowth at the same time when the diffusion value drops to pretreatment levels. The relatively high diffusion environment suggests a significant increase in extracellular water or an increase in permeability between intra- and extracellular water spaces. For reference, unrestricted water at 40°C has a diffusion coefficient of ≈3.0 × 10^{-3} \text{mm}^2/\text{s}. Observed peak tumor diffusion values are well below that of pure water, indicating that although 90% of the tumor cells present at time of treatment ultimately die, the fractional volume of extracellular space does not approach 90% at any one time. Upon mass shrinkage and then regrowth, there is a radical shift back toward a low diffu-
Therapy Assessment via Quantitative Diffusion MRI

Histopathology of treated animals sacrificed at selected intervals following BCNU treatment support the model of a therapy-induced increase of extracellular space. Relative to a control tumor (Fig. 4a), tumors 6 days following BCNU treatment revealed a marked increase in tumor extracellular space, which was filled with mucosubstance (Fig. 4b). The lymphocyte-predominant mixed inflammatory response was most intense 6 days after treatment. There was also a noticeable increase in pleomorphism, large cells, and in cells with structural features of apoptosis. Peritumoral edema was also most extensive 6 days after treatment. Nine days after BCNU treatment, the extracellular space diminished (Fig. 4c). At 12 days, the number of inflammatory cells decreased, and regions of reduced diffusion were associated with dense regrowth of tumor cells (Fig. 4d, left) that was similar in appearance to untreated tumors (Fig. 4a). Geographic regions of coagulative necrosis appeared in the same specimen as shown in Fig. 4d, right. Fig. 5a illustrates the T2-weighted MRI of the animal sacrificed for the histology 12 days after treatment. Associated diffusion plots (Fig. 5b) clearly demonstrate tumor heterogeneity, with cellular dense and necrosed regions as low and high diffusion environments, respectively. These findings underline the importance of spatially resolved diffusion measurements because even relatively homogeneous tumors, such as the 9L gliosarcoma, may become heterogeneous following therapy.

**Diffusion Anisotropy.** Diffusion anisotropy is known to exist in highly ordered structures such as myelinated white matter fiber tracts (10–12). It was interesting to note that significant diffusion anisotropy also exists within and near the edges of the 9L tumor mass. The most conspicuous and consistent anisotropy pattern was that of low diffusion perpendicular to the mass surface (ADCx in these experiments) and relatively high diffusion in orthogonal directions. Although it was anticipated that tumors would have a highly disorganized cellular pattern (i.e., isotropic), histological inspection revealed a significant cellular directionality at the tumor boundary as shown in Fig. 6. Note there is an apparent lamination of tumor and adjacent brain cells that are radially compressed by mass effect. As a result, diffusion perpendicular to the tumor surface is impeded relative to diffusion with the cellular "grain," i.e., parallel to the tumor surface. This pattern of strong diffusion anisotropy at the tumor edges was observed in all rats studied and is exemplified in Fig. 7a. Anisotropic diffusion in the...
Fig. 5  
(a) coronal T2-weighted image of a rat with a 9L tumor that was treated 12 days earlier with BCNU. Tissue regions corresponding to recurrent tumor, coagulative necrosis, and cerebrospinal fluid in the ventricle are shown, which were positively identified by histology (shown in Fig. 4d). The location of the column used for obtaining diffusion data is enscribed on the image.  
(b) plot of water diffusion as a function of position along the column shown in (a). Note the high contrast in diffusion between solid recurrent tumor and the necrotic region.

Fig. 6  
H&E-stained coronal histological section of the 9L tumor/brain interface from an untreated rat. Note the anisotropic pattern of cellular shape and distribution at the brain/tumor interface.

central tumor was apparent in some rats, but this pattern was not observed consistently. The degree of anisotropy varied with time toward more isotropic high diffusion 6–8 days after treatment and was isotropic in areas of coagulative necrosis. Presumably, this is a result of less directional cellular order because as cells necrose, the mass “loosens” with increasing extracellular volume leading to a more isotropic environment. This is illustrated in Fig. 7a at 30 and 151 h after treatment (Fig. 7b).

Discussion

Human brain tumors are diverse in response to treatment. Hence improved methods for reliable quantitation of treatment response in individual patients would provide additional time for applying alternative treatments to tumors unresponsive to the initial therapeutic regimen. A common approach used in estimating tumor “size” is by measuring two major axes of the tumor from clinical CT or MR images. A positive treatment response is typically defined as a substantial decrease in cross-sectional tumor area from the pretreatment size. This approach requires substantial shrinkage of the tumor mass that may proceed relatively slowly due to the time necessary for absorption of cellular debris. Further complications in the estimation of treatment efficacy and tumor size often arise from malignant brain tumors that have ill-defined margins between viable tumor and brain tissue due to peritumoral edema, as observed by T2-weighted MR images. This edema may be secondary to tumor involvement or represent regional alterations in noncancerous brain parenchyma in response to treatment. Contrast-enhanced CT and MR imaging offers additional sensitivity, but enhancement characteristics may not always be directly related to the presence or absence of tumor cells. Consequently, although morphological imaging is relied upon as the principle clinical tool, it often leads to only crude estimates of tumor size, and pronouncement of treatment response can be made only relatively late following treatment initiation through evidence of substantial tumor regression or progression of growth.

The main objective of this preclinical study was to evaluate quantitative diffusion and relaxation time MRI measurements as early therapeutic response indicators using the 9L brain tumor model. This widely used model has been shown previously to be responsive to BCNU treatment (31, 32). Serial tumor volume measurements provided a classic indicator of treatment response for comparison with quantitative NMR parameters. As is commonly observed with some tumor models and human gliomas, volume determinations are sensitive to error from ill-defined tumor boundaries, particularly when the ROI are manually defined. The 9L model was chosen for these studies because it is reported to have well-demarcated borders and minimal peritumoral edema, features that allow for noninvasive quantitation of tumor volumes and cell kill in individual animals using MRI.
Fig. 7 Overlaid plots of $ADC_x$, $ADC_y$, and $ADC_z$ 1.25 days (a) after BCNU treatment and 6.3 days (b) after treatment in the same animal when diffusion changes are maximal. Note that the high diffusion anisotropy decreases with time following treatment, particularly at the tumor edges.

Even in the 9L model, however, tumor boundaries become indistinct during the period following treatment but prior to substantial regrowth. Indeed, it is during this time that cellular density changes are greatest as indicated by diffusion. If, however, survival is long enough for most of the cellular debris to be removed, then tumor regrowth will appear exponential, thus allowing an estimate of doubling time. Note knowledge of pretreatment tumor doubling time is not essential if volume is directly measured at the time of treatment. If one assumes the tumor cellular density during regrowth is the same as prior to treatment, then Eq. B provides an estimate of log cell kill. In this regard, histology from control 9L and posttreatment tumor dur-
ing regrowth appear qualitatively similar in terms of cellular density. A quantitative measure of cell density from histology was not performed because the fixation process is known to alter extra- and intracellular spaces. We can consider the theoretical impact of reduced cellular density (i.e., number of cells/unit volume) during regrowth on calculated posttreatment $T_d$ and cell kill. If upon regrowth the cellular density is constant but lower than before treatment and tumor cell division rate is unchanged, then the apparent posttreatment tumor doubling time will be reduced. This is an unlikely scenario given that, empirically, $T_d$ during regrowth is not reduced. Alternatively, if there is a transient elevation in extracellular volume fraction due to an incomplete removal of necrotic debris, then there will be an apparent increase in $T_d$, leading to an underestimation of cell kill. Although the potential of this error cannot be excluded, the estimation of cell kill using only animals that exhibited significant regrowth minimized it. In these animals, the final tumor volume was greater than 4-fold the volume at the time of treatment such that the necrotic volume was a relatively small fraction of the mass.

Diffusion provides further evidence that cellular density during late regrowth is similar to untreated tumor. Using a mathematical model of the dependence of diffusion on cellular properties, others have argued that the extracellular fluid volume fraction is the main determinant of measured diffusion (9). The observation of diffusion returning to pretreatment levels during tumor regrowth suggests that the extracellular volume fraction, and thus cellular density, is similar to untreated tumor. Therefore, the effects of treatment on MR-observable parameters can be quantitated and compared with treatment efficacy in the same animal. This provides a powerful approach for evaluating the dynamic changes in MR parameters of interest following treatment for correlation with the effects on tumor growth and histopathology.

Our results revealed a substantial increase in tumor water diffusion that occurred early following administration of BCNU and before tumor regression. Although relaxation times were also affected by the treatment, diffusion values were found to be a more robust predictor of treatment-induced changes to cellular tissue integrity. In fact, histological comparisons of 9L tumors treated with BCNU suggest that treatment-induced changes in tumor diffusion values are reflective of the changes in microscopic water environment that occurred following treatment and during repopulation of the tumor from the surviving clonogenic tumor cells. Based on this data, we believe the increased diffusion is primarily a result of a proportional increase in extracellular water because a large fraction of tumor cells necrose with dissolution of cell membranes and secondarily from vasogenic edema and an inflammatory response. An alternative scenario is that observed diffusion changes are primarily due to vasogenic edema and an inflammatory response to treatment, with a coincident arrest of tumor growth (in contrast to tumor cell death), followed by resolution of edema and regrowth. This scenario, however, is not supported by colony-forming assays that yield yet greater cell kill in the 9L model at the same BCNU dose (32). This suggests the our log kill of 1 is not an overestimate.

Consistent with studies of untreated tumor (17–23), we have observed that necrosis is clearly evident by high diffusion. Furthermore, recurrent tumor could be distinguished from therapy-induced regional necrosis by localized MR diffusion measurement, thereby providing important diagnostic information not easily obtainable by other nonlocalized or low-resolution spectroscopic methods. The ability to detect diffusion anisotropy within tumors prior to treatment and progression to isotropic diffusion following treatment reveals the sensitivity of this approach to obtain potentially important information related to patterns of organized cellular distribution.

In conclusion, in this present study we extended our previous work by quantitation of cell kill following treatment and identified treatment-induced histological changes that may be important factors in affecting the observed changes in water mobility. Furthermore, diffusion anisotropy was observed near the boundaries of intracranial 9L tumors, which reflected directionality in water mobility that decreased upon successful treatment. Although growth retardation of treated 9L tumors was observable by serial MR volume measurements, precise tumor volume measurements are not readily obtainable from human brain tumor patients for reasons discussed above. Therefore, the results of this study suggest that quantitative water diffusion offers significant potential in the early assessment of antineoplastic treatment response and should provide the motivation for validating this quantitative noninvasive approach for monitoring tumor treatment in the clinical setting.

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