Minireview

Evaluation of Cancer Therapy Using Diffusion Magnetic Resonance Imaging[1]

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Abstract

Assessment of the effectiveness of cancer therapy traditionally relies on comparison of tumor images acquired before and after therapeutic intervention by inspection of gross anatomical images to evaluate changes in tumor size. The potential for imaging to provide additional insights related to the therapeutic impact would be enhanced if a specific parameter or combination of parameters could be identified that reflect tissue changes at the cellular or physiological level. This information could also provide a more sensitive and earlier indicator of treatment response in an individual animal or patient. Diffusion magnetic resonance imaging can detect relatively small changes in tissue structure at the cellular level and thus provides an opportunity to quantitatively and serially follow therapeutic-induced changes in solid tumors. This article provides an overview of the use of diffusion magnetic resonance imaging as a surrogate marker for quantitating treatment responsiveness in both preclinical and clinical studies.

Introduction

Conventional MRI[3] provides an opportunity to noninvasively follow gross tumor morphology and how it evolves over time. Conventional MRI exploits a variety of endogenous tissue properties that allow the investigator the ability to assess gross tumor extent on the resultant MRI contrasts such as T2-weighted and gadolinium-enhanced T1-weighted images. The actual image contrast values are rarely quantified because these are usually arbitrarily scaled and do not have a simple relationship to tissue properties. It is thought that there is significant untapped potential for MRI techniques designed to provide additional functional, structural, or molecular information related to tumor biology and physiology. Such information may be derived from quantitation of tissue properties that reflect, for example, perfusion dynamics, oxygenation levels, biochemistry/metabolism, cellularity, and levels of gene expression. Because the spatial information is retained, regional heterogeneity in these tissue properties and their change with therapy are also measurable.

Properties of tumor function actively under investigation using MRI include perfusion, oxygenation, and metabolism, however, this article focuses on the application of MRI to provide information related to the microscopic cellular environment in solid tumors. The use of water diffusion as a surrogate marker to probe tissue cellularity is compelling because this parameter is strongly affected by molecular viscosity and membrane permeability between intra- and extracellular compartments, active transport and flow, and directionality of tissue/cellular structures that impede water mobility. Therefore, diffusion MRI can be used to characterize highly cellular regions of tumors *versus* acellular regions, distinguishing cystic regions from solid regions, as well as detection of treatment response, which is manifested as a change in cellularity within the tumor over time.

Diffusion MRI pulse sequences incorporate two additional magnetic field gradients that makes the intensity of the MR signal dependent on the mobility of the signal source, *i.e.,* water molecules (1). Conceptually, the first of these two gradient pulses imparts a phase shift to each water molecule proportional to its initial location. The second gradient pulse will totally remove this phase shift if the water molecule remains at its original location. Any molecular movement between first and second pulses, however, leads to incomplete rephasing. The large number of water molecules and their respective random trajectories produce a net dephasing or signal loss. The amount of signal loss is a direct reflection on water mobility, *i.e.,* the greater signal loss implies greater molecular mobility. If the time interval between gradient pulses is sufficient to allow water molecules to migrate distances comparable with the size of and spacing between cells, then the apparent mobility will be reduced by the impediments of cellular membranes and tortuosity of the extracellular space. Thus, the water mobility within a tumor will increase over time after treatment, and the magnitude of the change will be related to the effectiveness of the therapy, which will result in membrane damage with a subsequent reduction in cell density as shown diagrammatically in Fig. 1. In addition, the directionality of cellular structures can be

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[3] The abbreviations used are: MRI, magnetic resonance imaging; ADC, apparent diffusion coefficient; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; XRT, X-ray therapy.

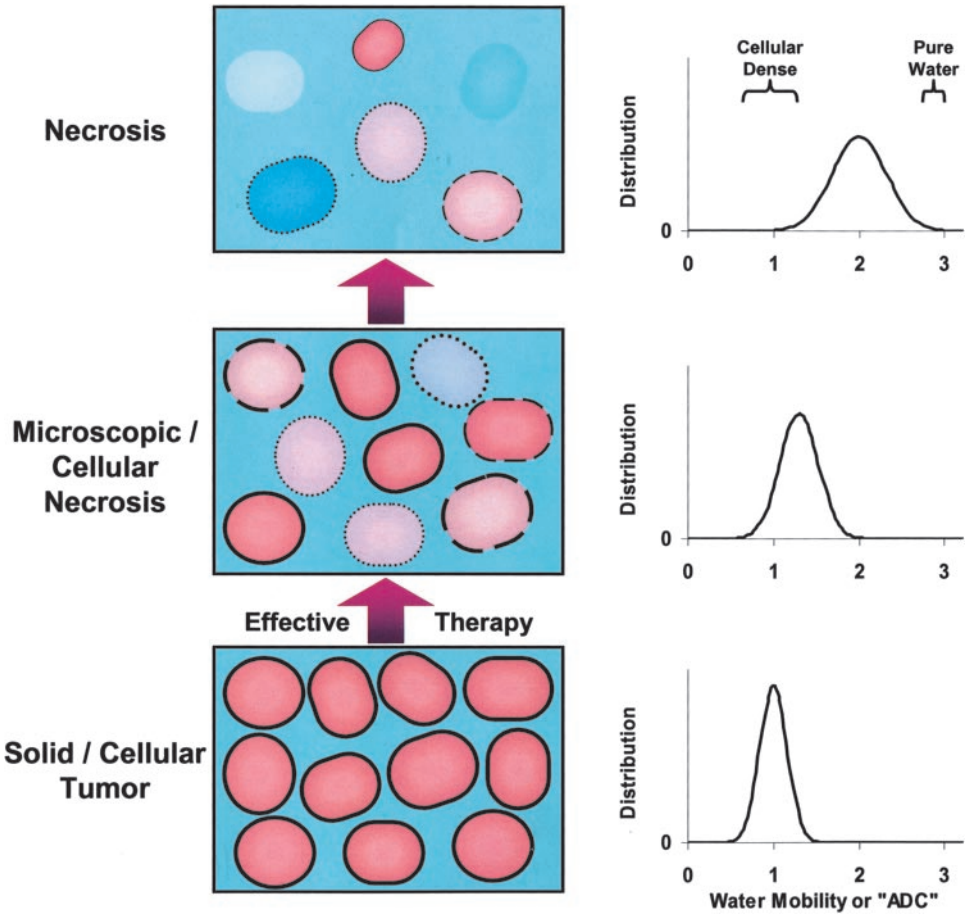

Fig. 1. Schematic representation of the relationship between change in tissue cellularity and molecular water mobility measured as an ADC. At *left* is the evolution toward necrosis after an effective therapy. The increase in extracellular space and membrane permeability allow greater water mobility as illustrated by distributions of diffusion on the *right*.

probed by controlling the direction of the applied diffusion pulses, however, we will avoid the issue of anisotropic diffusion because our diffusion measures are directionally independent via careful combination of data from several diffusion gradient directions (2, 3).

The raw diffusion-weighted image is used as a qualitative, diagnostic screen for acute ischemia in brain tissue in clinical practice (4–6). The clinical interpretation is that regions of conspicuously bright signal on diffusion-weighted MR images reflects restricted diffusion in cells swollen by cytotoxic edema secondary to ischemic insult. Acquisition of images at multiple diffusion sensitivities, however, allows the calculation of an ADC at each point in the image. Water mobility is reduced in the restricted environment of cellular-dense tissues relative to cellular-sparse tissues that exhibit high diffusion properties. Although it is an oversimplification of the biophysics involved, we will consider the ADC value to be inversely related to the cellularity of tumors (2, 7).

The Use of Diffusion MRI in Preclinical Studies

Successful treatment of a tumor with a cytotoxic agent will result in significant damage to the tumor cells in the form of loss of cell membrane integrity with a subsequent reduction in tumor cell density. This has a net effect of increasing the fractional volume of the interstitial space because of cell loss resulting in an increase in the mobility (diffusion) of water within the damaged tumor tissue (Fig. 1). The relative tissue contrast on an ADC image shows that high diffusion/mobility areas as bright in acellular/cystic tissues is actually reversed from the contrast of diffusion-weighted MRI. As such, ADC may be used for serial assessment of tumors in animals undergoing therapy. The sensitivity of diffusion MRI for detection of therapeutic-induced changes depends upon the possible overall dynamic range, which can be observed by ADC measurements. For example, relatively solid tissue such as normal adult brain has an ADC value of $0.6–0.8 \times 10^{-3}$mm^2/sec, whereas cerebral spinal fluid is $\sim 3.0 \times 10^{-3}$mm^2/s. Enthusiasm for the use of diffusion MRI for therapy assessment stems from previous animal studies that have reported that this approach can be used to monitor early events in tumor treatment in a variety of tumors models (7–11) along with a preliminary application to patients with primary central nervous system tumors (2, 12).

To demonstrate the utility of diffusion MRI for distinguishing a relatively ineffective treatment from an effective treatment, rats with orthotopically implanted gliomas were treated with a single low dose of BCNU (6.65 mg/kg) and a single high dose of BCNU (26.6 mg/kg). Data from the animal treated with the low-dose schedule is shown in Fig. 2. Anatomical T2-weighted images of the brain are shown before

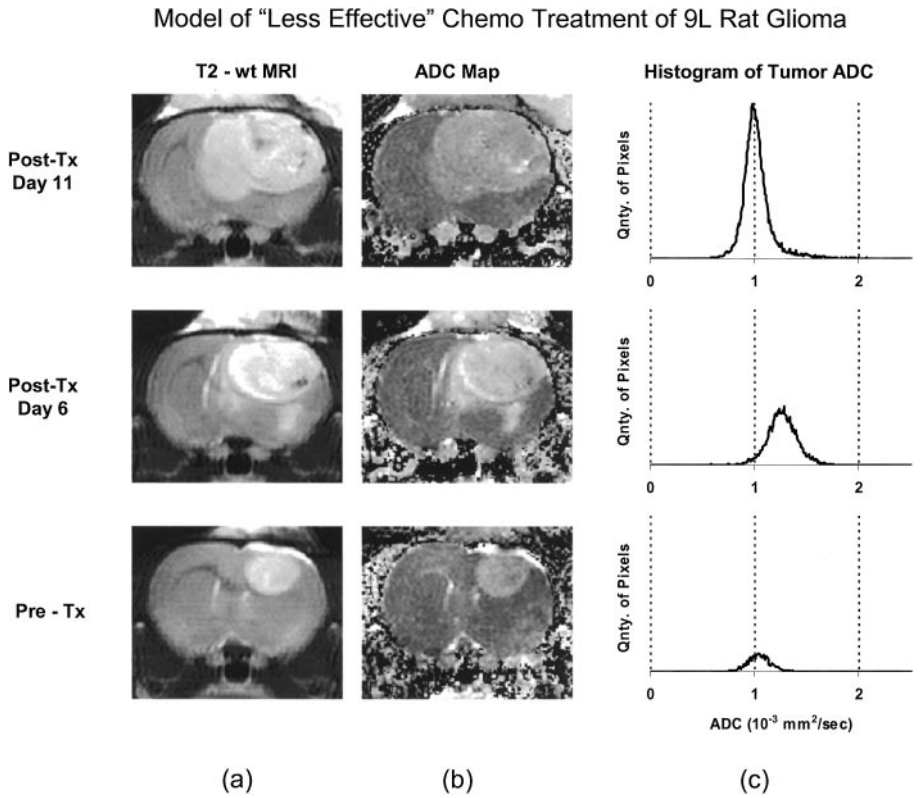

Fig. 2. Illustration of a 9L rat glioma treated with a single 6.65 mg/kg dose of BCNU chemotherapy. At this low dose, the 9L model mimics the course of a nonresponder because tumor growth rate is only slightly retarded. Serial T2-weighted MRI of this animal shown on the *left* indicates continued tumor growth. The corresponding ADC maps are illustrated in the *center*, where brighter pixels represent higher water mobility. The distributions of ADC values within the tumor are illustrated on the *right* for these three time points. Note, a slight increase in water diffusion at 6 days after treatment, but the tumor returns to a dense cellular state by day 11. The area under each histogram is proportional to tumor volume.

and at two time points after treatment along with the corresponding ADC maps and tumor ADC histograms. From the anatomical images, it is clear that the tumor continued to grow, and no regression of the mass was observed. The ADC maps of the brain revealed a slight increase in the tumor signal intensity, which reflects that a small amount of cell killing had occurred. These changes were quantified by circling the tumor regions from the ADC maps and plotting a histogram wherein the quantity of pixels (*y* axis) were plotted *versus* the ADC values (*x* axis) for the entire tumor mass over time. The area under the histogram curve is proportional to the volume of the tumor. As shown in the Fig. 2 tumor histograms, there was a slight shift to the right (higher mean diffusion values) at day 6 after treatment, indicating a transient loss of cells from the tumor mass, which recovered by day 11. For preclinical drug studies, the ability of diffusion MRI to detect a small therapeutic effect, especially when the anatomical images were unable to provide any clear evidence of therapeutic benefit, is in fact quite valuable. In this regard, detection of some therapeutic effect in a limited small number of animals could be quantified using diffusion MRI, and the decision could be made to increase the dose to determine whether the drug treatment could be improved. In this brain tumor model system, the dose of BCNU was increased by 4-fold, and the results from the anatomical images, ADC maps, and tumor diffusion histograms are shown in Fig. 3. This therapeutic dose of BCNU resulted in a significant regression of the tumor mass as observed on day 21 after treatment. A large increase in tumor diffusion values was observed at day 7 as evidenced by the bright tumor signal intensity in the ADC maps as compared with the pretreatment image. It is also important to note that the change in tumor diffusion values preceded regression, indicating that diffusion MRI can detect early therapeutic-induced changes to the tumor. Comparison of the histogram changes of the tumor treated with the high BCNU dose (Fig. 3) with the animal treated with the low dose (Fig. 2) revealed that the magnitude of the diffusion increase varied with therapeutic outcome, which is shown in Fig. 4. Plots of tumor volume *versus* time along with the corresponding changes in tumor ADC values for the low dose are shown in Fig. 4, *a* and *b*. At this dose of BCNU, the growth rate of the tumor remained essentially unretarded with a small, transient increase in the tumor ADC values. However, the high dose of BCNU produced a massive reduction in tumor volume (Fig. 4*c*) along with a dramatic increase in tumor ADC values (Fig. 4*d*). Overall, the magnitude of change in tumor diffusion values was significantly greater for the effective therapeutic intervention, which provides an important opportunity for using this approach in preclinical dose escalation studies of novel therapeutics.

The Use of Diffusion MRI in Clinical Studies

Studies exploring the potential for using diffusion MRI for the detection of early therapeutic-induced changes in tumors are ongoing. The goal of these studies is to determine whether diffusion MRI can provide early evidence of cancer treatment efficacy in an individual patient before the completion of the therapeutic regimen (12). Shown in Fig. 5 is an example of

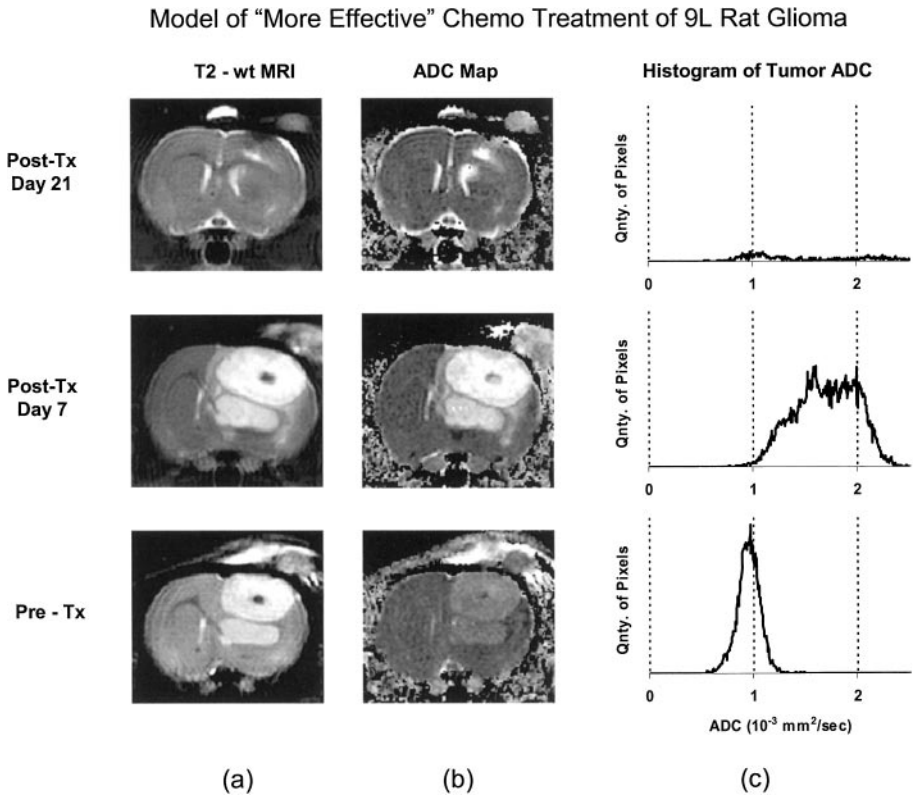

Fig. 3. Illustration of a 9L rat glioma treated with a single dose of 26.6 mg/kg BCNU chemotherapy. At this high dose, this rat mimics a responder because tumor shrinks substantially. Serial T2-weighted MRI on this animal, shown on the *left*, illustrated the degree of maximal tumor shrinkage on day 21. The corresponding ADC maps are shown in the *center*. Despite continued tumor growth on day 7, the increase water mobility is visually apparent on the ADC maps, which suggests necrosis. This is also illustrated graphically in the diffusion histograms on the *right*.

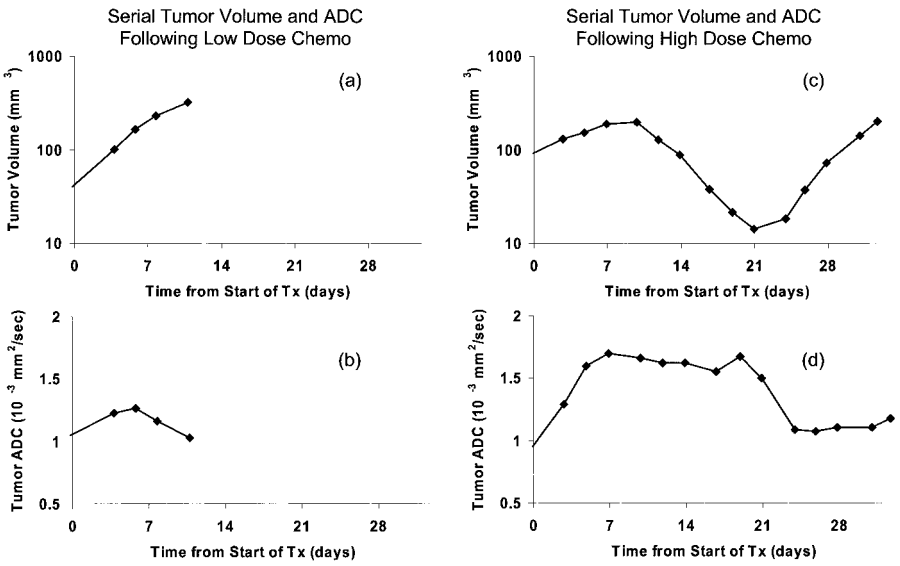

Fig. 4. Serial display of tumor volumes (*top*) and mean tumor ADC (*bottom*) averaged over the tumor at each time point. Curves *a* and *b* were derived from the low BCNU dose-treated animal (*i.e.,* nonresponder) shown in Fig. 2 and illustrate rapid tumor progression with moderate necrosis. Curves *c* and *d* are from the high BCNU dose animal (Fig. 3) and indicate significant necrosis (via an increase in ADC) and tumor shrinkage. There is eventual tumor regrowth and a drop in ADC beyond day 21, which indicated the mass returns to a cellular dense state.

how diffusion MRI can be used in the clinical setting. In this example, a 63-year-old woman diagnosed with an astrocytoma was treated with standard fractionated radiotherapy (70 Gy to the tumor in 35 fractions; Fig. 5). Shown are T1-weighted, gadolinium contrast-enhanced images along with corresponding ADC maps from pre-XRT 3 weeks into therapy and finally at 8 weeks after initiation of therapy, which ended on week 7. All images were coregistered to the pretreatment T1-weighted image for cross comparison over time (13). The radiological assessment on week 3 revealed an increase in size of the contrast enhancing area, which is believed to reflect the extent or boundaries of the primary tumor mass. The fact that the tumor diffusion value did not increase as displayed in the adjacent ADC histograms also suggested that the treatment produced no significant positive therapeutic benefit. In fact, the mean diffusion value decreased slightly throughout the treatment protocol, which could be interpreted as a lack of cell killing effect during this

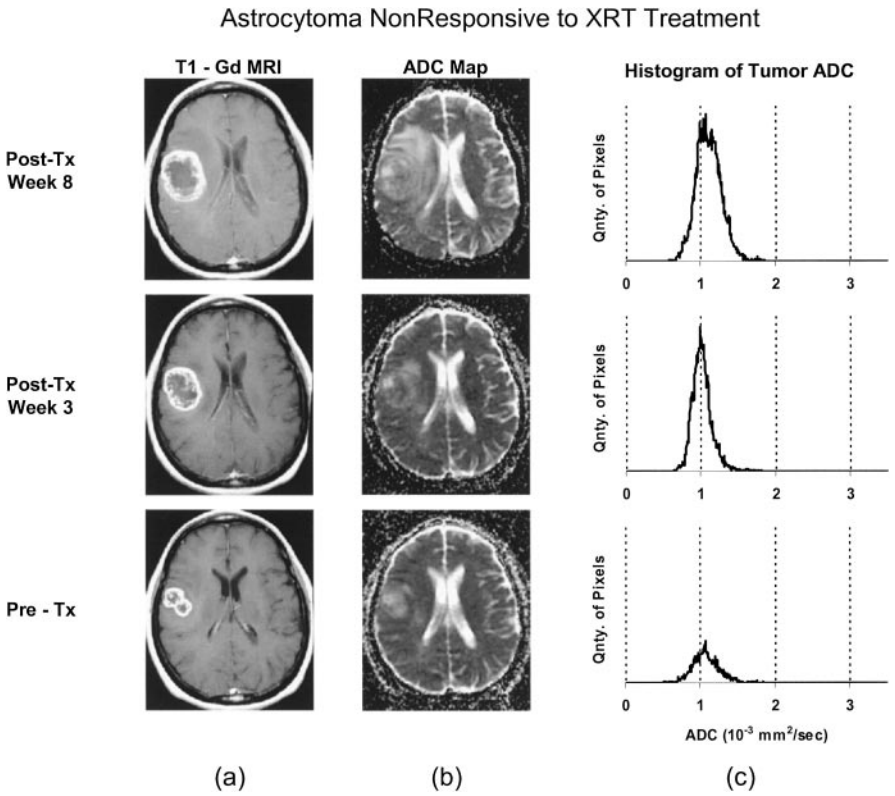

Fig. 5. Clinical example of a 63-year-old woman treated for an astrocytoma by standard fractionated radiotherapy (total dose, 70 Gy). Rapid disease progression on contrast-enhanced MRI (*left*) is apparent through week 8 after initiation of therapy. ADC maps (*center*) and diffusion histograms (*right*) suggest the cellularity of the tumor was not significantly altered over this period. This patient went on to receive chemotherapy but survived for only 18 weeks from initiation of radiation treatment.

treatment protocol. After completion of the fractionated radiation dosage schedule on week 7, a follow-up set of images was acquired on week 8, which revealed progressive disease. Thus, the lack of change in tumor diffusion values in this patient also correlated with the lack of therapeutic benefit.

In contrast to the nonresponsive patient shown in Fig. 5, a patient with a solid tumor, which responded to a fractionated therapeutic regimen, would be anticipated to show an increase in tumor ADC values over time during the therapeutic regimen. For example, a 56-year-old female diagnosed with an anaplastic oligoastrocytoma that failed chemotherapy was entered into a diffusion MRI study. This patient was subsequently treated with radiotherapy (week 0, 70 Gy in 35 fractions), which was completed 7 weeks later (Fig. 6). Shown are T1-weighted, gadolinium contrast-enhanced and ADC images from pre-XRT at week 3 and week 6. All images were coregistered to the pretreatment T1-weighted image. The clinical/radiological assessment revealed that radiotherapy provided a substantial benefit to this patient. This positive therapeutic effect was also detected by 3 weeks by a dramatic increase in the tumor diffusion values (Fig. 6). The early increase in observed diffusion values for the tumor mass of this patient is consistent with the subsequent clinical diagnosis of a partial therapeutic response (Fig. 6). The data obtained from this patient indicated that diffusion MRI was sensitive enough to detect tumor cell kill in patients with a brain tumor. It is possible that even a stronger diffusion shift might be anticipated in patients more responsive to treatment and at earlier time points such as within the first week of fractionated therapy.

The results presented from these two patients reveal that tumor diffusion values can be measured during treatment and appear to reflect dynamic therapeutic-induced changes (or lack thereof) in tumor tissue cytoarchitecture. A therapeutically nonresponsive tumor revealed no significant increase in diffusion values throughout the treatment protocol of the first patient (Fig. 5), which supports the hypothesis that the magnitude of change in tumor water mobility, as assessed using diffusion MRI, is related to the fraction of cells killed and hence therapeutic efficacy.

For all clinical studies, written informed consent was obtained from all subjects presented here, and all images and medical records were obtained according to protocols approved by the University of Michigan Medical School Institutional Review Board.

Potential Impact of Diffusion MRI for Oncology Studies

For preclinical drug studies using orthotopic animal tumor models, animal survival or animal moribundity are traditional therapeutic end points for quantitation of treatment efficacy. The use of diffusion MRI offers a significant improvement over these traditional approaches, which includes the ability to use the same animal as its own control, greater sensitivity to relatively small therapeutic effects, the ability to quantitate effects of varying the drug dosage and timing, and finally, the potential of requiring

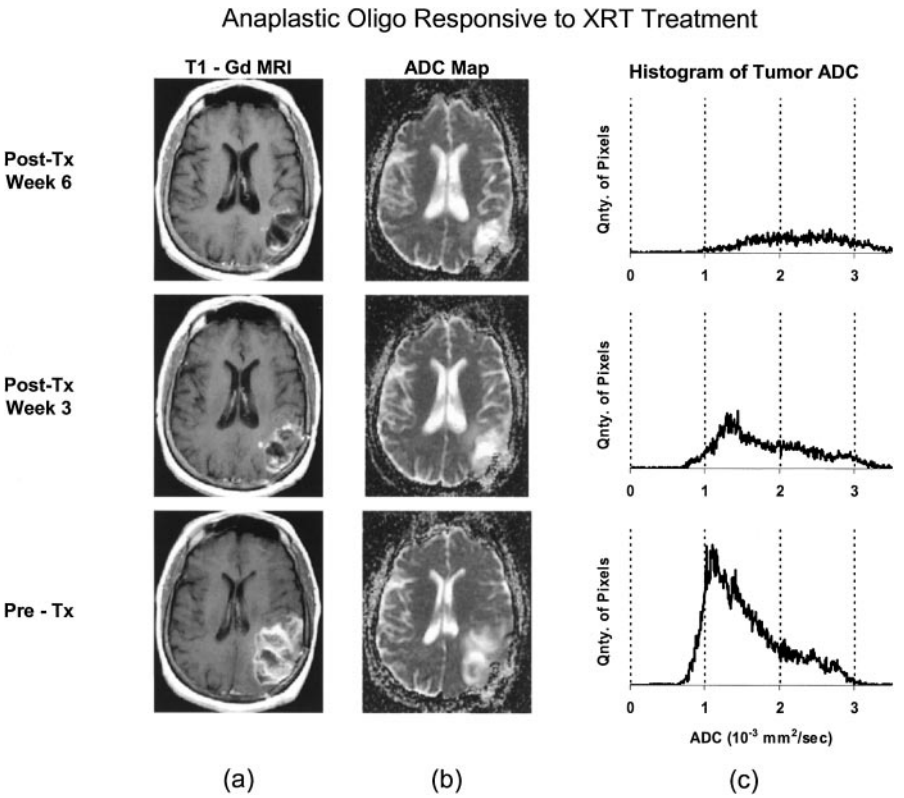

Fig. 6. Clinical example of a 56-year-old woman treated for an anaplastic oligodendroglioma by standard fractionated radiotherapy (total dose, 70 Gy). There was a reduction in contrast enhancement on T1-weighted MRI as shown on the *left* (times are relative to start of XRT). ADC maps (*center*) and diffusion histograms (*right*) indicate greater water mobility, which suggests tumor necrosis over this period. This patient went on to receive chemotherapy and survived for 73 weeks from the start of the radiation treatments.

smaller numbers of animals to obtain statistical significance. This latter issue can be especially important if variables such as heterogeneity of tumor take or growth rates are properties of the tumor model used. Another important aspect of the use of diffusion MRI is that it is translatable from the animal model into a clinical trial, which could thus provide a valuable quantitative and sensitive surrogate marker for therapeutic monitoring. Presently, a comparison of sequential MRI or computed tomography scans is the method of choice for monitoring the response of solid tumors to therapy, which compares the change in maximal diameter, cross-sectional area of the tumor via the product of the maximal perpendicular tumor diameters or full volume determination (14). For MRI, gadolinium-enhanced T1-weighted images are often used, but T2-weighted or other MR contrast strategies may be used. Comparisons of tumor burden are usually made between pretreatment scans and those obtained weeks to months after the conclusion of a therapeutic protocol (15). Methods such as diffusion MRI for assessing treatment response that are not dependent on relatively slow changes in tumor volume may be capable of providing earlier indications of therapeutic outcome because molecular and cellular changes typically precede observable macroscopic changes in gross tumor size. Therefore, the use of a quantitative MRI surrogate marker scheme such as water diffusion for determination of therapeutic-induced changes in tumor cellularity is an area of active research investigation (16, 17).

Summary

Diffusion MRI has tremendous potential for monitoring early changes in tumor cellularity that are thought to be reflective of treatment response. It is envisioned that diffusion MRI can be used for many preclinical tumor therapy studies in animal models followed by translation into subsequent Phase I/II clinical studies. Current applications also include the ability to potentially assist the physician in tailoring treatments for a individual patient and provide additional opportunities for applying alternative therapies in a more timely fashion if a tumor is found to be resistant to first-line therapies. Because the display of diffusion maps retains the anatomical spatial orientation of the diffusion values, this approach should also provide the possibility of assessing the regional/spatial heterogeneity of therapeutic response within a tumor. The heterogeneity of response may be accentuated in applications involving direct intratumoral administration of the therapeutic agent as is done in certain therapeutic protocols involving cancer gene therapy or radiosurgery. Additional work is under way to determine whether the observed changes in tumor diffusion are a universal response to tumor cell death via a variety of interventions and to more fully delineate the prognostic ability of diffusion MRI for application in both experimental and clinical oncology studies.

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