Functional diffusion map: A noninvasive MRI biomarker for early stratification of clinical brain tumor response

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Communicated by George J. Todaro, Targeted Growth, Inc., Seattle, WA, February 25, 2005 (received for review November 24, 2004)

Assessment of radiation and chemotherapy efficacy for brain cancer patients is traditionally accomplished by measuring changes in tumor size several months after therapy has been administered. The ability to use noninvasive imaging during the early stages of fractionated therapy to determine whether a particular treatment will be effective would provide an opportunity to optimize individual patient management and avoid unnecessary systemic toxicity, expense, and treatment delays. We investigated whether changes in the Brownian motion of water within tumor tissue as quantified by using diffusion MRI could be used as a biomarker for early prediction of treatment response in brain cancer patients. Twenty brain tumor patients were examined by standard and diffusion MRI before initiation of treatment. Additional images were acquired 3 weeks after initiation of chemo- and/or radiotherapy. Images were coregistered to pretreatment scans, and changes in tumor water diffusion values were calculated and displayed as a functional diffusion map (fDM) for correlation with clinical response. Of the 20 patients imaged during the course of therapy, 6 were classified as having a partial response, 6 as stable disease, and 8 as progressive disease. The fDMs were found to predict patient response at 3 weeks from the start of treatment, revealing that early changes in tumor diffusion values could be used as a prognostic indicator of subsequent volumetric tumor response. Overall, fDM analysis provided an early biomarker for predicting treatment response in brain tumor patients.

diffusion MRI | therapeutic response

It is projected that in 2004, $\approx 18,400$ new cases of primary brain cancer will be diagnosed in the United States and 12,690 people will die of the disease (1). Malignant gliomas are the most common of these brain tumors and have a high mortality rate and short median length of survival (40.9 weeks) (2). In terms of biology, treatment, and prognosis, brain tumors are a heterogeneous group of neoplasms (3). The early identification of tumors responsive to therapy versus those that are not would greatly facilitate modifying an ineffective treatment regimen in a more timely fashion than is usual with standard measurements of tumor response.

Current assessment of CNS tumor treatment response relies on evaluating changes in the maximal cross-sectional area of the tumor or the product of the maximal perpendicular tumor diameters (4, 5) weeks to months after the conclusion of a therapeutic protocol (6, 7). Several noninvasive imaging methods [positron-emission tomography, single-photon emission computerized tomography, magnetic resonance (MR) spectroscopy and diffusion MRI] are being evaluated for assessing early therapeutic response that are independent of late changes in tumor volume (8–13). Diffusion MRI detection of cancer treatment response was first successfully reported in a rodent brain tumor model treated with chemotherapy (13). The hypothesis underlying this approach is that changes in tumor water diffusion

occur after successful treatment that can be attributed to changes in cell density (13–19), resulting from necrosis and/or apoptotic processes (Fig. 1A). Moreover, initial regions of high extracellular water content (e.g., intratumoral edema, necrosis, and/or cysts) may decrease in volume because of dynamic reorganization of the heterogeneous tumor structure after treatment (Fig. 1B). The change in cell density due to cell kill along with tissue reorganization may lead to heterogeneous changes in the underlying tissue morphology (e.g., ratio of intra- to extracellular water), resulting in spatially varying changes in tumor apparent diffusion coefficient (ADC) values.

Changes detected in mean tumor ADC values after treatment in rodent tumor models revealed that this approach has merit for preclinical drug development studies as a sensitive and early predictor of therapeutic efficacy (13, 20–27). However, clinical utility of this approach has been hampered because of tumor heterogeneity and suboptimal methods of digital image analysis (28–32). Furthermore, although these preliminary studies have shown correlations between ADC change and tumor response, a definitive study demonstrating the ability of diffusion MRI to predict response has not been reported. Prediction of tumor response is critical for diffusion MRI to become a validated clinical biomarker of early treatment response.

We investigated whether diffusion MRI could be used to provide early detection of the therapeutic response of malignant brain tumors in humans as was observed in rodent brain tumors (13, 28). In this study, we prospectively compared tumor diffusion values at 3 weeks after initiation of therapy with pretreatment images to quantitate therapy-induced changes in ADC. A diagrammatic representation of this approach is shown in Fig. 1C, where the two image datasets are coregistered and computationally analyzed (see *Methods*) to yield functional diffusion maps (fDMs), which consist of a color overlay image of therapeutic-induced ADC change within the tumor. The fDM provides the ability to objectively segment the tumor into three color regions based on the magnitude and direction of ADC change. The volumes of these regions are also displayed as a scatter plot and were found to correlate with subsequent tumor response (post-treatment change in tumor size by using standard radiographic criteria). Statistical analysis of this data revealed that this fDM

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Abbreviations: ADC, apparent diffusion coefficient; fDM, functional diffusion map; MR, magnetic resonance; PD, progressive disease; PR, partial response; SD, stable disease; V_b , blue voxels; V_g , green voxels; V_r , red voxels.

^{††}T.L.C., A.R., and B.D.R. have a financial interest in the underlying technology (patent no. 6,567,684). Furthermore, this technology is licensed to a company in which A.R. and B.D.R. have a financial interest.

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Fig. 1. Biological processes proposed to be involved in therapeutic-induced changes in tumor ADC values along with a pictorial description of the fMRI analytical process. (A) A schematic representation of the dynamic biological processes associated with changes (increase or decrease) in tumor water diffusion values. Tumor cells within an image voxel have several fates during treatment. Cells can be resistant to therapy (unaltered ADC, green) or can undergo necrosis initiated by a transient cell swelling (decreased ADC, blue). Cell enlargement (swelling) can also be associated with mitotic catastrophe or a reduction in tumor blood flow resulting in focal ischemia/hypoxia (decreased ADC, blue). These processes can eventually progress to cell lysis and necrosis (increased ADC, red). Cells can also undergo apoptosis involving cell shrinkage and blebbing followed by phagocytosis (increased ADC, red). (B) The concept that necrotic or cystic regions of a tumor can undergo drainage (displacement) of water as cells move into the region resulting in a drop in diffusion values (decreased ADC, blue) is summarized. (C) Diffusion MRI data undergo digital image postprocessing and analysis that involves coregistration of images before and during treatment. Data are used to generate a three-color overlay representing regions in which tumor ADC values are unchanged (green voxels), significantly increased (red voxels), or significantly decreased (blue voxels). This data can also be presented in a scatter plot and percentages assigned to the three defined ADC regions, allowing quantitative assessment of overall changes in tumor ADC values.

approach could be used as a biomarker to predict early tumor response to therapy.

Methods

Patients. Patients with unresectable primary brain tumors were recruited to participate in a longitudinal clinical imaging trial to test the efficacy of the fDM approach for assessment of treatment response. To be eligible for the trial, patients were required to have a malignant brain tumor confirmed by histology as glioblastoma multiforme, astrocytoma, anaplastic astrocytoma, anaplastic oligodendroglioma, germ cell, or primitive neuroectodermal tumor, either at the initial diagnosis or at the time of tumor relapse. In addition, patients were only included if they were to receive radiation, chemotherapy, or a combination of both. Twenty patients were recruited into the study and underwent pretreatment diffusion MRI as well as the standard MRI (fluid attenuated inversion recovery, T2-weighted MRI, and gadolinium-enhanced T1-weighted MRI). All patients also underwent the same studies 3 weeks after initiation of therapy and follow-up standard MRI was accomplished to determine radiological response after completion of therapy.

Radiographic treatment outcomes based on the “crossed diameter product” were classified according to the World Health Organization criteria as follows: CR, complete resolution of tumor contrast enhancement and cessation of all steroids; partial response (PR), >50% decrease in tumor volume observed at least 4 weeks after the conclusion of therapy and on stable or decreased dosage of steroids; stable disease (SD), <50% decrease or a <25% increase in tumor volume and stable or decreased dose of steroids; and progressive disease (PD), >25% increase in tumor volume and on stable or increased dose of steroids. Written informed consent was obtained from all subjects, and all images and medical records were obtained according to protocols approved by the University of Michigan Medical School Institutional Review Board.

MR1. Water diffusion-sensitive images of the brain were acquired on a 1.5 T human MRI system (General Electric Medical Systems, Milwaukee, WI); capable of single-shot echo-planar imaging (EPI) (33). The diffusion spin-echo EPI sequence (TR/TE = 10,000/100 msec) was set to acquire at least 14, 6-mm-thick, contiguous axial-oblique sections through the brain at a given diffusion sensitivities (i.e., “b factors”) along all three orthogonal directions. A set of diffusion-weighted images at high diffusion sensitivity ($b_2 = 1,000 \text{ sec/mm}^2$) and low sensitivity $b_1 \approx 0$ (i.e., T2-weighted) were collected in 80 sec. Mean ADC maps were then calculated from the three orthogonal directions because this quantity represents a rotationally invariant scalar of the diffusion tensor (34). That is, ADC maps were then calculated according to

$$ADC_i = \frac{1}{(b_2 - b_1)} \log_e \left[\frac{S_{b_1}}{S_{b_2}} \right], \quad [1]$$

where S_{b1} and S_{b2} are signal intensities at low- and high-diffusion weighting, respectively, and i is the direction along which the diffusion sensitization gradients were placed (x , y , and z). These ADC maps were then averaged to calculate the scalar invariant mean ADC:

$$ADC_o = \frac{[ADC_x + ADC_y + ADC_z]}{3}. \quad [2]$$

The quantity, ADC_o , is a scalar invariant of the diffusion tensor, thus, it avoids complexities introduced by anisotropy in brain tissue (34–36).

Image Analysis. All MR images were spatially coregistered by using the pretreatment T2 weighted images as the reference dataset. This step allowed all images of a given patient to be viewed and analyzed from a fixed frame of reference. The coregistration was performed by using a “mutual information for automatic multimodality image fusion” (MIAMI FUSE) algorithm (37). After this coregistration, brain tumors were manually segmented on the images by neuroradiologists. For this study, tumor tissue was defined as tissue that was either contrast-enhanced on T1-weighted images or was contained within a “ring” enhancement. Tumor cross-sectional diameters were measured by the radiologists for calculation of the crossed diameter product.

For each patient, diffusion changes were quantified by using a multiparametric analysis. ADC values of each voxel within the tumor fDM at 3 weeks were plotted as a function of their pretherapy ADC (Fig. 1C, scatter plot). Because shrinkage or growth of the tumor during the time between scans may have occurred, only voxels that were present in both the pretherapy and posttherapy tumor volumes as segmented by radiologists were included. All tumor voxels were objectively segmented into three different categories: red voxels (V_R) for which the ADC increased significantly, blue voxels (V_B) for which the ADC decreased significantly, and green voxels (V_G) for which the ADC did not change significantly. The three-color fDM was overlaid on the anatomical image. The mean of 15 independent observation prediction intervals (see statistical analysis below) was used as the significance threshold. The total volume of the voxels within each of the three categories (red, blue, and green) was calculated for each patient at week 3 (Fig. 1C) and normalized against the total tumor volume to give three normalized tumor volume segments for each patient V_R , V_B , and V_G , respectively.

Statistical Analysis. The thresholds for determining whether there was a significant change in diffusion within a voxel were determined empirically from 15 coregistered data sets from five different patients. For each coregistered data set, a volume of interest >50 ml within the contralateral brain containing a range

Table 1. Patient summary

Response	Tumor type	Age	Gender	Therapy
PR	AA	41	M	Radiation
	AO	37	M	Chemotherapy
	AO	56	F	Radiation
	Germ cell	10	F	Chemotherapy
	Germ cell	15	M	Chemotherapy
	PNET	8	F	Chemotherapy
	SD	AA	36	M
AO		47	F	Radiation
GBM		40	F	Radiation
GBM		67	F	Radiation
GBM		28	M	Radiation
PNET		13	F	Combination
PD		A	44	F
	A	45	F	Combination
	A	63	F	Radiation
	AO	64	M	Radiation
	GBM	46	M	Radiation
	GBM	20	M	Radiation
	GBM	42	F	Radiation
	GBM	45	F	Chemotherapy

AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; PNET, primitive neuroectodermal tumor; GBM, glioblastoma multiforme; A, astrocytoma.

Fig. 2. MRI of three patients with oligodendrogliomas. MR image datasets obtained from three different patients diagnosed with anaplastic oligodendrogliomas. Images shown are at 3 weeks into a seven-week fractionated ionizing radiation regimen. Regions of interest were drawn for each tumor image by using anatomical images. (A, C, and E) Shown are the regional spatial distribution of ADC changes (fDMs) of a single slice through each tumor as color overlays for the PD, SD, and PR patients, respectively. The red pixels indicate areas of increased diffusion, whereas the blue and green pixels indicate regions of decreased and unchanged ADC, respectively. The scatter plots (B, D, and F) show quantitatively the distribution of ADC changes for the entire three-dimensional tumor volume for each corresponding patient (A, C, and E), respectively.

of ADC values from normal gray and white matter was correlated with the reference dataset by using linear least squares analysis. The 95% confidence intervals and standardized residuals were then determined from the results of the linear least squares analysis.

To test whether changes in diffusion images of brain tumors were correlated with patient radiographic outcome (PR, SD, and PD), the volumes V_R , V_B , and V_T ($V_T = V_R + V_B$) were then compared by using a one-way analysis of variance test (ANOVA). Wilson's scoring method (38) was then used to determine the sensitivity, specificity, and predictive values and accuracy of fDM for predicting outcomes.

Results

Patient Diagnosis, Therapy, and Outcome. A total of 20 patients with primary brain tumors were enrolled in the study as detailed in Table 1. Prescribed therapeutic interventions included chemotherapy, ionizing radiation, or combined therapy. Imaging was initiated just before the start of treatment and again during therapy at 3.2 ± 0.4 weeks (mean $\pm$ SD). Standard radiographic followup of tumor response beginning at 4 weeks after treatment classified 6 patients as PR, 6 patients as SD, and 8 patients as PD (Table 1).

Clinical Studies. Fig. 2 shows representative examples of fDMs overlayed on T2-weighted images from three patients with anaplastic oligodendrogliomas. Each of these patients received fractionated radiation therapy (total dose of 70 Gy in 2-Gy daily fractions 5 days a week over 7 weeks) and were subsequently classified as PD (Figs. 2A and B), SD (Figs. 2C and D), and PR (Figs. 2E and F). The three colors used in these overlay images represent three different categories of diffusion values determined at 3 weeks after initiation of treatment. The red voxels indicate the

regions of the tumor that had a significant increase in ADC (standardized residual > 1.96), the blue voxels indicate regions of significant reductions in ADC values (standardized residual < -1.96), and the green voxels represent tumor regions with no significant change ($-1.96 < \text{standardized residual} < 1.96$). The total volume of the voxels within these three categories was then calculated for each patient at 3 weeks (PD patient, Fig. 2B; SD patient, Fig. 2D; and PR patient, Fig. 2F) and normalized against the total tumor volume to give three normalized tumor volume segments for each patient V_R , V_B , and V_G . Values for the PD patient ($V_R = 0.9\%$, $V_B = 1.1\%$ and $V_G = 98.0\%$), SD patient ($V_R = 2.7\%$, $V_B = 17.8\%$, and $V_G = 79.5\%$), and the PR patient ($V_R = 41.1\%$, $V_B = 15.2\%$, and $V_G = 43.7\%$) reveal significant differences between these patients that could be quantified at 3 weeks after initiation of therapy when less than half of the total radiation dose has been delivered and almost 2 months earlier than the radiographic response was defined in these patients.

Sensitivity and Specificity of fDMs. The box plots (Fig. 3) summarize the volumes of diffusion change obtained from fDM for each of the patient-response groups for the entire cohort of patients. The PR group had a $V_R = 33.2 \pm 5.9\%$ (mean $\pm$ SEM) (Fig. 3A) at 3 weeks posttreatment initiation (Fig. 3A), which was significantly larger ($P < 0.001$, power = 95.7%) than the SD ($V_R = 5.6 \pm 1.4\%$) and PD ($V_R = 1.1 \pm 0.4\%$) groups, which were also significantly different from each other ($P = 0.005$, power = 98.9%). As shown in Fig. 3B, the PR group had a $V_B = 4.0 \pm 2.8\%$ at 3 weeks posttreatment initiation, which was not significantly different from the V_B values of the SD and PD groups ($V_B = 10.6 \pm 3.2\%$, $P = 0.4$ and $V_B = 1.9 \pm 0.6\%$, $P = 0.2$, respectively). However, the V_B values were significantly different between the SD and PD patients ($P = 0.01$). The sum of the total

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