Invited Review

In Vivo Molecular Imaging for Planning Radiation Therapy of Gliomas: an Application of 1H MRSI

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Gliomas are infiltrative lesions that typically have poorly defined margins on conventional magnetic resonance (MR) and computed tomography (CT) images. This presents a considerable challenge for planning radiation and other forms of focal therapy, and introduces the possibility of both under-treating macroscopic tumor, and over-treating regions of normal brain tissue. New therapy systems are able to deliver radiation more precisely and accurately to irregular three-dimensional target volumes, and have placed a premium on definition of the spatial extent of the lesion. Proton MR spectroscopic imaging (H-MRSI) has been proposed as an in vivo molecular imaging technique that assists in targeting and predicts response to radiation therapy for patients with gliomas. The evidence that supports the use of H-MRSI for planning radiation treatment is reviewed, together with the technical requirements for implementing data acquisition and analysis procedures in a clinical setting. Although there is room for improvement in the spatial resolution and chemical specificity obtained at the conventional field strength of 1.5 T, there are clear benefits to integrating H-MRSI into treatment planning and follow-up examinations. Further work is required to integrate the results of the H-MRSI examination into the treatment planning workstation, and to improve the quality of the data using more sensitive phased array coils and higher field strength magnets.

Key Words: brain tumors; radiation therapy; magnetic resonance spectroscopy; improved targeting; molecular imaging


RECENT IMPROVEMENTS in the hardware and software available for planning and delivering focal radiation therapy have made it possible to target irregular three-dimensional volumes with a high degree of accuracy and precision. Intensity modulated radiation therapy (IMRT) and radiosurgery are two examples of such technology. These techniques have the potential for utilizing a higher, and presumably more effective, dose in treating the most malignant region of the tumor, as well as reducing the dose that is deposited in surrounding normal structures. It is also possible to simultaneously prescribe different doses to varying parts of the tumor using IMRT. To use this technology most effectively, it is critical to have access to non-invasive imaging modalities that are able to visualize differences between normal and abnormal tissue morphology and to distinguish regions of tumor from edema, inflammation, or necrosis. It would also be valuable to identify lesions, or certain parts within a lesion, that are likely to be radioresistant, and to predict which components of the tumor are the most malignant. Although conventional T2-weighted and contrast enhanced T1-weighted magnetic resonance (MR) images are widely used for planning focal therapy, they reflect the morphologic, rather than molecular or functional, properties of the tumor.

One of the most promising techniques for monitoring the molecular properties of a tumor is MR spectroscopy (MRS). This method has been used to obtain information about the chemical composition of solutions, tissue extracts, ex vivo tissue samples, and in vitro cell systems in animals and humans. For in vivo systems, it is possible to combine the chemical specificity of MRS with the spatial localization techniques that have been developed for MR imaging to obtain signals from multiple different regions of tissue. Limitations of this approach have been the relatively low signal-to-noise ratio (SNR) of many metabolites at the field strengths used for in vivo applications, and the broadening of metabolite peaks caused by variations in susceptibility in living systems. Recent improvements in hardware and software for acquiring in vivo MRS data have made it possible to obtain spectra with more accurate localization, better SNR, and higher spectral resolution. These developments are particularly exciting for the translation of MRS approaches from in vitro to in vivo model systems, and for relating information about events occurring at the molecular level to clinical outcome.

This paper examines the possibility of using multi-voxel proton MRS imaging (H-MRSI) as an in vivo molecular imaging technique for planning focal radiation therapy in patients with gliomas. These are the most
common primary brain tumors in adults, and represent a major challenge to the oncologist because of their spatial heterogeneity and complex patterns of infiltration into normal tissue. The clinical procedures associated with treatment planning are considered, followed by a discussion of the data acquisition and analysis techniques necessary for this application. Evidence that supports the relevance of MRS for focal treatment planning of gliomas has come from a number of studies that compared the differences in the spatial extent of the anatomic and metabolic lesions. These results are reviewed and placed in context by considering how they relate to the prognosis and patterns of response to radiation in patients with gliomas. Advances in technology that would contribute to the practical application of this methodology are identified and their potential impact is discussed.

RADIATION THERAPY OF GLIOMAS
Malignant gliomas have relatively poor prognosis with conventional therapies. Although surgical resection is an important therapeutic tool, it is rare that the tumor is completely removed, and adjuvant treatments, such as radiation or chemotherapy, are usually required to impede the growth of residual tumor (1–4). Survival times are 20–36 months for grade 3 gliomas and 9–12 months for grade 4 gliomas following a combination of surgery and standard external beam radiation therapy (5,6). Novel treatment regimens, such as brachytherapy or radiosurgery, have been used to deliver a boost to specific portions of the tumor, either at the time of initial treatment or for focal recurrence. Although it is not yet clear whether there is a strong dose-response beyond 60 Gy, there continues to be interest in using higher doses to target the most malignant region of the tumor. Early experience suggests an increase in local dose may improve time to progression and survival in high-grade gliomas (7). This approach carries an increased incidence of side effects, especially if the radiation is not delivered accurately to the target.

The goal of dose escalation by means of a local boost is to target all of the active disease while sparing normal tissue as much as possible. Accurate target definition thus becomes of prime importance. Examples of the dose distributions for gamma knife radiosurgery and fractionated radiation therapy are shown in Figure 1. The standard approach for defining target volumes for fractionated radiation therapy in patients with high grade gliomas is to deliver the highest dose to the lesion determined from a contrast enhanced T1-weighted MR imaging or a computed tomography (CT) scan, plus a margin of 1–4 cm (1,5,8). The size of this margin is designed to compensate for a lack of appropriate target discrimination in areas beyond obvious contrast enhancement. Earlier studies have shown that serial biopsies of patients undergoing craniotomy for malignant glioma demonstrate tumor cells more than 3 cm distant from the contrast-enhancing margin (5), and that about 80% of relapses occur within a 2-cm margin from the original tumor location (1,9). In order to treat microscopic spread within the edematous regions, some pro-
tocols deliver a moderate dose to the T2-weighted region of hyperintensity with an additional, high-dose boost to the region that is expected to correspond to macroscopic tumor (7,10). Target volumes for boosts that are delivered by IMRT or radiosurgery are typically restricted to the enhancing volume. Even in cases where a high-dose boost is delivered, it is usually necessary to supplement radiation therapy with one or more forms of chemotherapy.

Improved definition of the radiation target requires the identification of macroscopic areas of active tumor that are suitable for treatment with high-dose radiation. This is termed the gross tumor volume (GTV). Surrounding areas that are suspicious for tumor extension are considered suitable for a moderate dose of radiation and are termed the clinical target volume (CTV). Based on these definitions, an integrated boost irradiation and dose escalation protocol for high-grade gliomas can be defined that will deliver a higher, and presumably more biologically effective, daily dose to the GTV (2.5–3.0 Gy), with a simultaneous lower dose (2.0 Gy) to the CTV (11). The goal is to improve local control while sparing normal tissue. While the technology for delivering this type of dose regimen is available, it is clear that conventional imaging methods are unable to provide an adequate definition of the GTV and CTV.

The use of fractionated radiation therapy in patients with low-grade gliomas is controversial. These lesions tend to occur in younger, otherwise healthy subjects, and survival times are much longer. Distinguishing which lesions are likely to progress to higher grade is critical for deciding whether the patient should receive immediate therapy. The benefits of treatment must be weighed against the long term damage to normal tissue. For patients with bulky disease, the primary treatment is surgical debulking with immediate radiation for residual disease. Younger patients with limited residual disease may have delayed radiation at the time of suspected progression. When neurocognitive function was evaluated in patients after cranial irradiation, there was a correlation of toxicity with delivered dose and volume of brain irradiated (12). This suggests that if radiation therapy is to be used, it should take advantage of conformal delivery techniques. The dose is typically 54 Gy delivered in 1.8 Gy/day fractions to a target that is based upon the T2 lesion plus a 2-cm margin. It would also be valuable if a sub-region of the lesion could be identified as being appropriate for delivery of a focal high-dose boost.

**MRS OF BRAIN TUMORS**

Both P-31 and H-MRSI have shown differences in the metabolic properties of brain tumors relative to normal parenchyma and necrosis (13–31). Because of its higher sensitivity, and hence improved spatial localization, H-MRSI has proved to be the technique of choice for in vivo studies of brain tumors at 1.5 T. The most common pulse sequences use point resolved spectroscopy (PRESS) or Stimulated echo acquisition mode spectroscopy (STEAM) excitation to localize to a small region (four to eight cc) of tissue inside the brain. Suppression of the large water resonance uses chemical shift selective radiofrequency (rf) pulse (CHESS), and suppression of lipid is achieved either by excluding it from the voxel of interest or by using an echo time of 144 or 288 msec to take advantage of its relatively short T2 relaxation time. At 1.5 T and with an echo time of 144 msec (see Fig. 2a), normal brain shows peaks corresponding to N-acetylaspartate (NAA), choline containing compounds (Cho), and creatine (Cr). NAA has been shown by labeled antibody studies to be confined to neurons (24). As seen in the ex vivo spectrum in Fig. 2e, the Cho peak includes contributions from free Cho.
phosphocholine, and glycerophosphocholine. These resonances reflect membrane synthesis and turnover. The level of Cr indicates the energetic status of the tissue, and is typically used as a reference for estimating changes in other metabolites. At shorter echo times, it is possible to observe peaks corresponding to myo-inositol (mI), glutamine (Glu), and glutamate (Gln). While the ability to detect such resonances adds to the chemical specificity of the measurements (25,26), there may be a trade-off in terms of the accuracy of the quantitative information that can be obtained because of the presence of overlapping resonances and the increased potential for lipid contamination.

In brain tumors, there is a reduction in NAA (see Fig. 2b and 2c) that may be attributed to a low density of neuronal cells. This may be completely absent in high-grade gliomas or brain metastases (27–31). The majority of studies have also reported increased Cho in tumors as compared with normal brain tissue (32–34). Whether the differences in Cho peak areas were reflective of changes in concentration is uncertain because there has also been evidence that the T2 relaxation time of Cho may be prolonged. Cr resonances were extremely variable in brain tumors, but had an overall tendency to be decreased relative to normal tissue (see Fig. 2b). This variability resulted in a greater overlap between tumor and normal Cho/Cr ratios than Cho/NAA ratios. Although lactate (Lac) and lipid resonances (Lip) have also been observed in spectra from tumor and necrosis (25), there are large variations in intensity for individual lesions (19,25). Alanine (Ahn) has been shown to be present in meningiomas (33). Many of the studies that employed H-MRSI of brain tumors have focused on the diagnosis and definition of tumor grade (25,33,34). The most promising of these have applied multivariate statistical analysis of levels of Cho, Cr, NAA, Lac, Lip, and Ahn (33,34). Some more recent studies have also applied short echo-time MRS, and observed changes in ml with tumor grade (35).

METHODS FOR ACQUIRING H-MRSI DATA OF BRAIN TUMORS

Single voxel STEAM and PRESS H-MRSI techniques are relatively easy to implement and have been automated for routine clinical use. Although they do give valuable data for analysis of tumors, they suffer from the inaccuracy of conventional MR imaging in defining the extent of solid neoplasm and in evaluating heterogeneity within the tumor mass. For defining the spatial extent of the tumor and examining variations in tissue properties within the anatomic lesion, it is critical to acquire information from multiple voxels. To obtain such data in a time efficient and reliable fashion requires the application of MRSI techniques. The majority of studies of brain tumors have used two-dimensional MRSI (14,17,28,29). Although this has advantages over single voxel MRS, it does not address the presence or spatial extent of tumor outside the chosen plane. Follow-up examinations are also complicated by the need for reproducible patient positioning and slice selection. Techniques that provide volumetric coverage of the lesion are two-dimensional multi-slice MRSI (19), and three-dimensional MRSI with oscillating gradients or spiral k-space sampling strategies (36,37). Posse (36) incorporated two- and three-dimensional MRSI rectilinear scanning techniques into STEAM for brain imaging with both head and surface coils to achieve resolutions ranging from 0.4–2 cc. Adalsteinsson (37) used similar methods, but without STEAM localization, to achieve 1.4 cc resolution over a field of view (FOV) of 24 cm × 24 cm × 10 cm.

While three-dimensional MRSI with oscillating gradient or spiral k-space sampling provides good data for other types of neurological diseases, we have found it difficult to obtain robust water and lipid suppression from brain tumor patients who have undergone surgical resection. Several groups have implemented acquisition techniques that combine PRESS volume selection with three-dimensional phase encoding (PRESS-MRSI) to obtain arrays of spectra restricted to the morphologic lesion and surrounding tissue (38). The size of the selected volume can be as large as 100 to 200 cc, with a spatial resolution of one to two cc when using a head coil, and as fine as 0.2 cc using surface or phased array coils. The acquisition time for a phase encode matrix of 8 × 8 × 8, 16 × 8 × 8, or 12 × 12 × 8 with a one-second repetition time is eight to 19 minutes. The use of volume selection has allowed the elimination of subcutaneous lipids, and permits shimming to be optimized over a limited region of the brain. The three-dimensional nature of the acquisition facilitates the analysis of tissue heterogeneity and registration of coordinate systems between successive examinations (39). This has meant that spatial and temporal changes in metabolic parameters can be directly correlated with anatomic lesions, CT treatment planning images, and radiation dose distribution (40).

Even with PRESS MRI, there are circumstances where CHESS water suppression and lipid suppression are too sensitive to variations in magnetic susceptibility and relaxation times to provide good data quality. When this occurs, the residual water and lipid resonances produce baseline distortions and inaccurate measurements of spectral intensities. The bandwidth of typical PRESS selection pulses may also cause errors in the estimation of metabolite ratios on the edge of the selected volume due to the induced chemical shift artifact, and may deleteriously affect the phase and intensity of weakly coupled spins, such as Lac (38). With the small FOVs used for three-dimensional phase encode matrices, the aliasing of residual water or lipid resonances from outside the selected volume may prove to be a significant problem. One solution is to apply spectral/spatial pulses (41,42) that are simultaneously selective in the chemical shift domain and one spatial dimension. These provide more robust water suppression but are relatively long and are unable to accommodate short echo times.

To improve the definition of the selected volume and further eliminate signal from unwanted lipids, it is possible to add spatially selective saturation bands. The precision of the box selection depends upon the bandwidth of the rf pulses, and is substantially improved by using very selective saturation (VSS) pulses. These pulses were developed by Tran et al and are short.
enough (two to three msec) that several of them may be combined (43). They may be used to both sharpen the edges of the selected volume and to eliminate portions of the volume that include subcutaneous lipid. We have found that VSS pulses are likely to be extremely important for treatment planning because they can assist in increasing coverage of the lesion. Although the diagnostic significance of the Lac and Lip peaks is not yet clear, it is possible to make use of J-difference Lac editing schemes to distinguish between them (44). Lac and Lip are relatively high in grade 4 tumors and may help in evaluating the extent of necrosis, and hence the sensitivity to radiation therapy.

CORRELATION OF MRSI DATA WITH TREATMENT PLANNING IMAGES

Although the software tools for acquiring research MRSI data are available on many clinical scanners, the capabilities for reconstruction and post-processing of the data are much more restrictive. This has necessitated a major effort in developing off-line analysis packages, and has limited the number of clinical applications of the technology. A large number of different methods have been proposed for analysis of in vivo MRSI spectra (45–47). Key issues for reliable and reproducible quantification of metabolite parameters are the use of as much prior information concerning peak positions and relative intensities as possible, and the removal of artifacts, such as residual water or broad baseline components. For MRSI data, further complications can be introduced by spatially dependent variations in frequency and phase corrections (47). Phase mapping and water referencing are two methods that have been developed for treating these problems for in vivo data (48).

Correlation of spectral parameters with the anatomy is made possible by plotting arrays of spectra with grids marked on the MR images, displaying metabolic maps of peak areas as gray level images, and superimposing contours or color overlays on MR images (47). To define the spatial extent of the lesion for patients with brain tumors, we have focused on characterizing spectra based upon their Cho/NAA ratios. The assumption used for this analysis was that normal tissue has relatively small variations in Cho/NAA when compared with the increased Cho and reduced NAA observed in histologically confirmed tumor. It was then possible to classify voxels into normal and abnormal based upon an iterative procedure that selectively removed outliers from a linear regression of Cho to NAA. The estimate of Cho/NAA obtained from the slope of the regression line for normal voxels was robust as long as the selected region included sufficient normal-appearing brain tissue surrounding the lesion. A quantitative index was determined for each voxel by calculating the distance of its Cho and NAA values from the regression line (49). Division of this index by the standard deviation (SD) of the distances for the normal voxels provided a parameter, which we have termed the Cho-to-NAA Index (CNI), that was comparable between different studies and could be used to highlight regions with a high probability of being tumor. An example is seen in Figure 3 where the CNI map is displayed at low resolution as an interpolated map that matches the resolution of the corresponding anatomic image, and as contours overlaid on the T1-weighted MR image. Other indices, such as Cho-to-Cr (CCrI), Cr-to-NAA (CrNI), and Lac/Lip levels (LLI) may be derived, and are of interest for defining radiation sensitivity (50).

In order to be useful for treatment planning, the MRSI data must be directly correlated with the images used to define the radiation target. This is achieved by registering the treatment planning CT or MR images with the anatomic images acquired during the MRSI examination. A number of different approaches to image registration have been implemented (51–55), including the use of internal anatomic markers, and volume and surface matching. Studies in the brain have indicated that the accuracy of registration for automated or semi-automated techniques is to within one to two pixels (55). The application of this methodology is demonstrated in Figure 4. The treatment planning CT data were registered with the MR images from the MRSI examination to determine translations and rotation angles to relate the two coordinate systems (39). The MR imaging data were reformatted and the MRSI data reconstructed to correspond to the CT images. The CNI images were calculated and interpolated to the resolution of the CT images. Contours of the CNI images were then superimposed on the anatomic images as shown in color and in black and white after being transferred using a DICOM protocol to a clinical picture archiving communications system (PACS) workstation. From there, the images were directly accessible to the treatment planning workstation, and were used to determine how the radiation treatment plan would have been modified. These data provide evidence for the feasibility of including the CNI or other metabolic indices in defining the treatment plan.

PLANNING FRACTIONATED RADIATION THERAPY USING MRSI

To define the role of MRSI in planning focal therapy, it is important to determine whether it provides information that is different from gadolinium (Gd)-enhanced T1-weighted and T2-weighted MR imaging. A major emphasis of our recent studies has therefore been in comparing the spatial extent of metabolic and anatomic lesions. Grade 4 gliomas are the most prevalent and the most heterogeneous lesions. The majority show enhancement on T1-weighted images after injection of Gd, and they frequently have hypointense central regions that represent necrosis. They are known to be highly
Figure 3. Steps in calculating CNI contours for a patient with a grade 4 glioma. The image on the upper left is a T1-weighted post-Gd MR image, with the PRESS box and phase encode grid superimposed on it. The array of spectra on the upper right is from the corresponding location; the lower left image is of the calculated CNI at the same resolution of the spectra; the lower middle image is the interpolated CNI image with CNI 2, 3, and 4 contours superimposed on it; and the lower right image is the anatomic image with the same contours superimposed on it.
vascular with an increased number of enlarged vessels. In the region of central necrosis, the spectra typically have characteristic peaks of Lac and Lip. The level of these peaks is highly variable. The Gd-enhancing volume ranges from a thin rim to a complex nodular structure. The NAA in the enhancement is typically very low.

The highest Cho is generally outside or on the edge of the enhancement, with levels that are highly variable. The Cr tends to be reduced in the enhancing lesion but may be both increased and decreased in the T2 hyper-intensity.

In a preliminary study of 12 newly-diagnosed grade 4 gliomas, we compared the regions with CNI greater than 2, 3, and 4 with the enhancing volume for the purposes of investigating how the use of the metabolic data might modify the radiation target (56). This analysis showed that adding the CNI lesion to the enhancing volume would increase the size of the target by 150% for the region with CNI greater than 2, by 60% for the region with CNI greater than 3, and 50% for the region with CNI greater than 4. The median distances that the CNI regions extended outside the enhancing volume were one to two cm. The region with CNI greater than 2 was, on average, only about 50% of the T2 lesion. While it did extend beyond the T2 lesion in some cases, it would only have extended the T2 lesion by about 10% if it was used for targeting purposes. Figure 5 shows the T2 lesion, the Gd-enhancing lesion, and the metabolic lesion for a patient with a grade 4 glioma. The metabolic lesion, as defined by the region where the CNI was greater than or equal to 3, was virtually disjoint from the enhancing lesion. Closer inspection of the spectral data indicated that there was Lac/Lip in the enhancing volume, elevated Cr in the posterior portion of the T2 lesion, and elevated Cho both anterior and posterior to the enhancing lesion. This underlines the heterogeneity within the lesion.

Grade 3 gliomas also vary in their anatomic characteristics. In a recent study, we found that approximately 40% are non-enhancing, 20% are weakly enhancing, and 40% have obvious enhancing components (Catalaa et al, unpublished results). When they were present, the enhancing volumes were smaller than for grade 4 lesions, and did not have obvious regions of central necrosis. Levels of Lac and Lip were much lower and smaller in spatial extent. The voxels with maximum Cho and maximum CNI were frequently in non-enhancing regions directly beyond the enhancing portion of the lesion, and the levels of Cr tended to be higher than for grade 4 lesions. An analysis of metabolic lesions for 22 grade 3 lesions showed even more dramatic results than for the grade 4 gliomas (56). Adding the CNI lesion to the enhancing volume would increase the size of the target by 500% for the region with CNI greater than 2, by 300% for the region with CNI greater than 3, and 150% for the region with CNI greater than 4. The median distances that the CNI regions extended outside the enhancing volume were two to three cm. The region with CNI greater than 2 was, on average, about 70% of the T2 lesion. While it did extend beyond the T2 lesion, it would only have extended the target by about 15%.

The majority of grade 2 gliomas do not enhance, and appear as relatively uniform regions of hyperintensity on T2-weighted images. The levels of Cho were variable, but tended to be higher for oligodendrogliomas than astrocytomas (57). Some lesions had residual NAA, and there was more likely to be Cr than in higher grade gliomas. There were some lesions that had small peaks corresponding to Lac or Lip, but the levels were much lower than for grade 4 gliomas. Analysis of the CNI contours for 20 grade 2 gliomas showed that the metabolic abnormality was usually within the T2 lesion, but that when it existed, the extension was relatively small and usually directed along white matter tracks (Catalaa et al, unpublished results). In the two grade 2 patients who had Gd enhancement, the maximum CNI corresponded with the small volume of patchy enhancement. If the treatment volume was modified from the usual T2 hyperintensity plus the two to three cm margin to include the T2 lesion plus the region with CNI greater than 2, there would have been a substantial reduction in the target volume, and hence, presumably in the radiation damage to normal brain tissue.

EVALUATION OF RESPONSE TO FRACTIONATED RADIATION THERAPY

Response to therapy is currently evaluated by a combination of radiological and clinical criteria. The Gd-enhanced lesion on T1-weighted MR images is widely used as an assessment of lesion burden, and the T2 lesion is considered to be a combination of tumor, edema, and post radiation changes. Quantitative analysis of such lesions may be performed using image segmentation techniques (58,59). There is considerable ambiguity in interpretation of the anatomic image because the enhancing volume typically includes some combination of residual/recurrent tumor and necrosis. As one of the first effects of radiation is upon the vasculature, interpretation of changes in the leakiness of the blood–brain barrier is problematic. There are also changes in image intensity and T1 relaxation times in previously normal appearing white and gray matter regions that receive moderate doses of radiation (60,61). Several authors have reported a reduction in Cho for patients who responded to therapy, and a retention or increase in Cho for patients who progressed (62–67). This is interpreted as a decrease in the number of viable cells, and is often accompanied by an increase in the peak corresponding to Lac/Lip. Other changes that have been reported are transient reductions in NAA and relative increases in Cho in normal appearing white matter at around four months after radiation (68). In cases where patients have been followed to determine the late effects of radiation, there were reductions in NAA, Cho, and Cr in regions showing elevated T2 intensity, and reductions in Cho in normal appearing white matter (69).

Figure 6 shows examples of such changes from three-dimensional MRSI datasets for a patient with a grade 4 glioma being treated with fractionated radiation therapy. The spectra in the upper panel correspond to a sum of voxels from normal appearing white matter adjacent to the CTV that received approximately 30–40 Gy. The levels of Cho, Cr, and NAA before therapy show the same characteristic levels as the spectrum in Figure 2. At the end of treatment and for the next four months,
the metabolite levels decreased, with a preferential loss of NAA and Cr. The largest effect would have been seen at two months after treatment, where the Cho/NAA ratio was close to 1.0. By six months after radiation, the metabolite levels had recovered and were close to normal. The middle panel shows serial changes in a voxel from the center of the enhancing lesion. Before radiation, there was elevated Cho and decreased Cr and NAA, with a small peak corresponding to Lac/Lip. This is consistent with the presence of tumor and some necrosis. The intensity of the Cho, Cr, and NAA decreases with time, and the Lac/Lip increases to a maximum four months after the end of radiation. This is interpreted as representing a reduction in tumor and formation of treatment-induced necrosis. The lower panel in Figure 6 is the sum of voxels from the borders of the enhancing and non-enhancing volume. The metabolite levels are initially very low, presumably corresponding to a mixture of edema and micro-necrosis. With time, the metabolite levels increase, and at the six-month follow-up, there are high Cho levels, lower levels of NAA and Cr, and a large Lac/Lip peak. This is interpreted as a mixture of recurrent tumor and necrosis. It is important to note that there are differences in both the spatial and temporal patterns of metabolite levels in the lesion and surrounding tissue. Care must be taken in inter-

**Figure 5.** Comparison of T2-weighted lesion (red), post-Gd T1-weighted lesion (green), and metabolic lesion (orange, CNI = 3) for a patient with a grade 4 glioma.

**Figure 6.** Patterns of changes in spectra following radiation therapy for three different spatial locations corresponding to radiation effect in normal appearing white matter (red), tumor response (blue), and tumor progression (mauve) at time points corresponding to immediately before radiation therapy, at the end of radiation therapy, and at two, four, and six months later.
pret ing changes in normal-appearing white matter during this time frame as corresponding to tumor recurrence as opposed to transient radiation effects.

Figure 7 demonstrates the ability of three-dimensional MRSI to predict radiological recurrence for a grade 4 glioma. The Gd-enhanced T1-weighted image that was obtained after surgery and before conventional radiation therapy showed no enhancement. The MRSI data that were obtained at this time had CNI values that were abnormal in regions medial and posterior to the cavity, with suggestion of involvement of the corpus callosum. Four and one-half months after fractionated radiation therapy to a total dose of 60 Gy, the Gd-enhanced T1-weighted images showed two nodules of hyperintensity that exactly corresponded to the region with abnormal CNI. These findings suggest that the MRSI data are superior to the anatomic images in indicating the existence of residual tumor, and that the spatial extent of the metabolic lesion should be taken into account in planning focal therapy. Whether treating the region with elevated CNI to a higher dose would have been effective in halting disease progression is not clear, but it would certainly have defined a target region under circumstances where the anatomic data did not.

**METABOLIC VS. ANATOMIC LESIONS FOR RECURRENT GLIOMA**

To investigate differences in the extent of morphologic and metabolic abnormalities for recurrent lesions, Gd-enhanced MR imaging and three-dimensional MRSI data from 100 patients with primary brain tumors were analyzed: 50 with grade 4 glioma, 17 with grade 3 glioma, and the remainder with low-grade tumors (70). Patients presented with suspected recurrent tumor and had all received prior radiation therapy. Abnormal spectra were identified as being suggestive of tumor if they had levels of Cho, Cr, and NAA below the random noise in the spectrum. If there was decreased NAA with Cho less than normal, they were defined as mixed in character, and if Cho was higher than normal with decreased NAA, the spectra were considered to be suggestive of tumor.

Although all the patients had regions of abnormal metabolism within the vicinity of the lesion, only 88 had clearly defined Gd-enhancing lesions. There were metabolic abnormalities outside the enhancing lesion in 76 patients, including 46 with spectra that were suggestive of tumor. The grade 4 gliomas were characterized by a very high percentage of contrast-enhancing lesions, high percentage of metabolic lesions with necrotic voxels, and a lower percentage (36%) of tumor-suggestive voxels outside the contrast-enhancing lesion. Mid- and lower-grade tumors were more likely to have non-enhancing lesions, had necrotic voxels, and more tumor voxels (56%) outside the enhancing lesion. These findings are consistent with observations from histological analysis of surgical samples that have shown recurrent high-grade lesions have more extensive regions of necrosis. Recurrent low-grade gliomas were also seen to be more likely to have regions of enhancement than newly diagnosed lesions. This may have been due to parts of the lesion progressing to higher grade, or to the effects of radiation. Further studies are required in order to determine whether there is a clear metabolic signature for transformation from low to high grade. Such dramatic differences between the extent of abnormalities in contrast-enhanced MR images and metabolic parameters are likely to be critical for assessment and treatment of patients with primary brain tumors.

**EVALUATION OF GAMMA KNIFE RADIOSURGERY FOR GLIOMAS**

Gamma knife radiosurgery is able to deliver a single radiation dose to irregular shaped lesions less than 4 cm in linear dimension. It is commonly used for gliomas to avoid open surgery for small regions of recurrent tumor. The target is typically defined as the enhancing volume plus one to two mm. Because the dose distribution is well confined, it provides a good model system for understanding the changes in metabolism caused by radiation. It is for this reason that it was selected for our initial studies of response to therapy using three-dimensional MRSI. Patients with recurrent gliomas were divided into two groups depending on whether the metabolic lesion was confined to the target or whether it extended well outside the target. Strong evidence for the predictive value of MRSI came from following these patients to determine time to further treatment, increase in enhancing volume at six months after therapy, and survival (71). For populations of grade 4 gliomas, there was a significant difference in each of these parameters between the two groups. Median survival for patients whose metabolic lesion was outside the target was 36 weeks, as compared with 96 weeks for those with the lesion confined to the target. In some cases, the metabolic lesion was small enough to be included within the gamma knife target, but in others, the lesion was too large to be treated adequately. This suggested that the MRSI was valuable for both defining the target and for eliminating patients with large metabolic lesions from consideration of using this type of focal therapy. Alternatives would be surgical resection or chemotherapy.

Figure 8 gives an example of where extending the radiation target to treat the metabolic lesion would have been feasible. For this small grade 4 glioma that was close to the cortex, it was possible to obtain an MRSI dataset with a spatial resolution of 0.3 cc using a surface coil. The MRSI data showed two tumor-like voxels, but only one of them lay within the target. The second voxel received a relatively low radiation dose and became enhancing just one month after therapy. The enhancement grew larger with each follow-up, but its spatial extent was about two months behind that of the metabolic lesion. By eight months after gamma knife radiosurgery, the lesion had grown so large that a resection was performed. Histology confirmed a mixture of active tumor and necrosis. Similar results were observed for other patients who had follow-up MRSI examinations, and there was clear evidence of recurrence in regions predicted by the MRSI data (72). This suggested that the MRSI would be valuable for treatment selection, planning the radiation target, and following response to gamma knife radiosurgery for recurrent gliomas.
CONCLUSIONS AND FUTURE DIRECTIONS

Studies in patients with gliomas have provided compelling evidence that the spatial extent of the lesion defined by MRSI is different from lesions observed using traditional MR imaging techniques. Modifying the target for focal therapy to include the MRSI lesion is therefore expected to have a major impact upon treatment effectiveness and patient outcome. Developing MRSI as a tool for routine use in radiation therapy planning and follow-up requires improvements in coverage of the lesion and in the SNR of the spectral data. Optimization of methods for lipid suppression are critical for achieving adequate coverage of the anatomic lesion and surrounding normal appearing tissue without compromising

Figure 7. Prediction of the location of tumor recurrence based on CNI contours. The image on the left is the post-operative Gd-enhanced T1-weighted image, the image in the middle is the same image with the CNI contours superimposed, and on the right is an aligned image obtained 4.5 months later. The new regions of Gd enhancement correspond closely to the previous CNI = 3 contours.

Figure 8. Response to gamma knife radiosurgery for a patient with a grade 4 glioma. The small Gd-enhancing lesion was chosen as a target for the radiation therapy. As seen in the spectra obtained at the time of treatment, there was a voxel with elevated Cho and decreased NAA just outside the target volume. Just one month after treatment, the enhancing lesion had already started to expand into this region, and the Cho remained elevated. As seen in the aligned serial images on the right side, the Gd-enhancing lesion continued to enlarge over the next six months until it got so large that a further surgery was performed. The histological analysis of tissue samples obtained during this surgery confirmed recurrent/residual tumor.
spectral quality. Higher spatial resolution is achieved using surface or phased array rf coils. A number of coils have been developed for high-resolution MR imaging and MRSI (73–75), and have provided up to eight-fold higher SNR near the surface as compared to a conventional head coil. Resolutions as small as 0.2 cc have been achieved for brain tumors and other focal lesions near the cortex (75, 76) at 1.5 T. The increase in the size of the matrix required to cover the lesion at such fine resolution may require the implementation of oscillating or spiral k-space encoding techniques.

Further limitations on the data acquisition are the magnetic field strength and echo times used for clinical applications of H-MRSI. Improvements in spectral dispersion and spatial resolution can be achieved using newly released 3-T clinical scanners. Reduction of the echo time would provide further improvements in SNR, and allow estimation of levels of ml, Glu, and Gln. While there is limited evidence about the prognostic significance of these metabolites, there is some indication from ex vivo and single voxel in vivo MRS studies that changes in levels of ml may be indicative of a higher degree of malignancy (32–34, 77). In addition to studying new markers, it is important to perform a more thorough analysis of the prognostic significance of the spatial distribution of all the metabolites observed in long TE spectra. Examples of the variations in Cr intensity for patients with grade 3 and 4 lesions are seen in Figure 9. Some lesions have relatively high Cr throughout, whereas others have relatively small regions with elevated Cr. These differences are expected to reflect changes in the oxygenation of different tumors, and may therefore have significance for predicting radiosensitivity. Since Lac is a marker of anaerobic metabolism, and Lip increases following cellular necrosis, these compounds may also be important as early markers of therapeutic success. This type of in vivo molecular imaging may have a major impact upon our understanding of the mechanism of action of new treatments, and has applications to many other diseases.

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Figure 9. Heterogeneity in Cr levels for patients with grade 3 and grade 4 gliomas. The Gd-enhanced images have superimposed the PRESS box (white) and CNI = 2 contour (yellow). The green area corresponds to the region where the CCrl is greater than 2, and the red area corresponds to the region where the CrNl is greater than 2. This indicates that the majority of the lesion on the left has elevated Cr, the middle lesion had a relatively small region with increased Cr, and the lesion on the right had elevated Cr in the non-enhancing portion of the lesion.
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