Metabolism of diffuse intrinsic brainstem gliomas in children


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Progress in the development of effective therapies for diffuse intrinsic brainstem gliomas (DIBSGs) is compromised by the unavailability of tissue samples and the lack of noninvasive markers that can characterize disease status. The purpose of this study was to compare the metabolic profile of DIBSGs with that of astrocytomas elsewhere in the CNS and to determine whether the measurement of metabolic features can improve the assessment of disease status. Forty in vivo MR spectroscopy (MRS) studies of 16 patients with DIBSG at baseline and after radiation therapy were retrospectively reviewed. Control data for baseline studies of DIBSGs were obtained from 14 untreated regular and anaplastic astrocytomas. All spectra were acquired with single-voxel, short echo-time (35 ms), point-resolved spectroscopy. Absolute metabolite concentrations (mmol/kg) and lipid intensities (arbitrary units) were determined. At baseline, creatine and total choline (tCho) were significantly lower in DIBSGs than in astrocytomas elsewhere in the CNS (4.3 ± 1.1 vs. 7.5 ± 1.9 mmol/kg, p < 0.001; 1.9 ± 0.7 vs. 4.2 ± 2.6, p < 0.001). Serial MRS in individual subjects revealed increasing levels of tCho (p < 0.05) and lipids (p < 0.05) and reduced ratios of N-acetylaspartate, creatine, and myoinositol relative to tCho (all p < 0.01). Metabolic progression defined by increased tCho concentration in serial MRS preceded clinical deterioration by 2.4 ± 2.7 months (p < 0.04). Low tCho of DIBSG at baseline is consistent with low proliferative tumors.

Subsequent metabolic changes that have been associated with malignant degeneration preceded clinical deterioration. MRS provides early surrogate markers for disease progression. Neuro-Oncology 10, 32–44, 2008 (Posted to Neuro-Oncology [serial online], Doc. D06-00191, November 14, 2007. URL http://neuro-oncology.dukejournals.org; DOI: 10.1215/15228517-2007-042)

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Among pediatric brain tumors, diffuse intrinsic brainstem gliomas (DIBSGs) carry the worst prognosis. Because of their location, these lesions are considered inoperable. In addition, DIBSGs are highly resistant to chemo- and radiation therapy, and mean survival after diagnosis is less than 12 months. Because of the lack of new effective therapies, there has been no improvement in survival over the past several decades, so current management aims to preserve the quality of life for patients and to reduce family burden.1–7 It is believed that at diagnosis most DIBSGs present as low- or high-grade astrocytommas, but the frequency of each is unclear because biopsies are usually not obtained. At autopsy most lesions have progressed to anaplastic astrocytoma or glioblastoma, with extensive brainstem involvement.1,2,4–6,8 Although the prognosis is generally poor, clinical course differs considerably with respect to time from initial diagnosis to disease progression and to death. Whereas some patients deteriorate within a few months, atypical long-term survivors (>24 months) have been reported by several groups.1,3,5,9,10 This may indicate that lesions are initially wrongly classified, that there are less malignant DIBSGs with slower disease progression,
or that treatment is partially successful in slowing progression in some patients.

MR imaging is a powerful tool for initial diagnosis of DIBSG. However, beyond diagnosis, MRI frequently does not correlate with disease progression. Thus, in practice, because of the unavailability of tissue samples and the limited value of imaging, clinicians are effectively treating a “black box.” To better serve patients and to accelerate clinical research, novel noninvasive markers that can characterize disease status and assess response to therapy in individual patients are needed.

On most clinical MR scanners, proton MR spectroscopy (1H MRS) is available and can readily be integrated with MR imaging. With MRS, low-molecular-weight, mostly intracellular metabolites with concentrations above approximately 0.5 μmol/g, and abnormally elevated lactate (Lac) and free fatty acids and lipids can be observed in vivo. MRS studies of gliomas in adults have shown that progressing tumors are associated with metabolic profile alterations. Specifically, elevation of total choline (tCho), decreased metabolite ratios of N-acetylaspartate to total choline (NAA/tCho) and creativity to tCho (Cr/tCho), and increased levels of lipids have been consistently reported to be indicators for malignant degeneration. A metabolic progression consistent with a malignant transformation as described above has been observed. Though few patients have been studied, it has been suggested that MRS might be a useful early predictor of disease progression, preceding clinical and radiological deterioration.

The goals of this retrospective study were (1) to compare the metabolic features of DIBSGs at baseline, newly diagnosed astrocytomas elsewhere in the central nervous system (CNS), and control brainstem and to determine whether MRS of DIBSGs at baseline can provide surrogate markers predictive of clinical course; (2) to quantify metabolic changes that accompany progressive disease and to determine whether metabolic degeneration precedes clinical deterioration and radiological disease progression; and (3) to review MR spectra obtained from atypical long-term (>24 months) survivors for unusual features that would separate these subjects from other patients.

**Materials and Methods**

**Patients and Control Subjects**

1H MR spectra, MR images, and medical records of 16 pediatric patients with DIBSG studied between March 2001 and March 2006 were retrospectively evaluated (Table 1). These patients were diagnosed with DIBSG based on clinical symptoms at presentation, clinical course, and MR imaging. None of the patients underwent biopsy or autopsy. On MRI, lesions had the characteristics of diffuse intrinsic tumors as described previously. Specifically, on precontrast, T1-weighted MR images, the lesions were hypointense with indistinct margins, reflecting the infiltrative nature of this tumor. After contrast administration, rim-enhancing foci were detected within tumors of three patients. On T2-weighted MRI, these lesions were indiscernible hyperintense. Six patients had both MRS studies of the untreated lesion and at least one study after completion of therapy. A second group comprised six patients who underwent only one MRS study prior to therapy. The third group of patients contained four subjects who were studied with MRS only after therapy because they had had their initial scan elsewhere and had been referred to this institution after diagnoses were made. There were no outside MRS studies available for review.

MR spectra from DIBSGs were compared with data obtained from 14 children with anaplastic (n = 6) and regular (n = 8) astrocytomas elsewhere in the CNS. MRS results from a subgroup of these patients have been published previously. Control data for normal brainstems were obtained from 12 age-matched subjects (2–13 years; mean, 6.7 ± 4.1 years). These subjects were either enrolled in unrelated research studies or had clinical indications for MRS. Included were subjects with seizures, silt-cell disease, suspected encephalopathy, developmental delay, and suspected tumor. MR images of these subjects were all reported as normal.

**Clinical Information**

The medical records of all patients with DIBSG were reviewed for information about duration and dosage of radiation therapy (RT), chemotherapies, and drugs and their dosage such as steroids (Table 1). The time intervals from initial diagnosis to death (ΔT_survival), from initial diagnosis to clinical relapse (ΔT_relapse), and the time of event-free survival between the completion of radiation therapy and relapse (ΔT_tac) were obtained. Clinical course was considered “typical” for patients who survived less than 24 months. By this definition 12 patients had a typical clinical course, whereas there were three atypical long-term survivors. The family of patient 10 moved to a different country after the initial MR study, so we were unable to obtain information about this patient beyond that time. All other patients died with a mean survival time of 13.4 ± 9.8 months (median, 10.4 months). Patients were treated with standard dosage radiation therapy (approx. 5,900 cGy, given in 30 sessions over a 6-week period). All patients received steroids starting at the time of diagnosis, with duration and dosage individually adjusted according to clinical needs.

The Childrens Hospital Los Angeles institutional review board (IRB) approved the review of MRI, MRS, and clinical data of all subjects included in this study. Subgroups of patients were enrolled in various prospective studies, and parental consent was obtained. For the remaining subjects, the IRB approved the review of existing data and medical records for the purpose of generating control data. The requirement for parental consent was waived for these subjects.
MRS Acquisition and Quantitation of Metabolite Concentrations and Lipid Intensities

All MRS studies were integrated with clinically indicated MR imaging and were carried out on a 1.5-T MR system (Signa LX, GE Healthcare, Milwaukee, WI, USA). MR imaging studies were performed at diagnosis, after RT, and thereafter typically every 3 months. Patients were scheduled according to clinical priorities for one of two MR imaging systems that were available at this institution. Since only one MR system had MRS capabilities, the number of MR imaging studies exceeded the number of MRS studies. Patients aged 5 years and below were anesthetized with 100–200 mg/min/kg propofol throughout the MR study.

Single-voxel 1H spectra were acquired and processed as described in previous publications.26,27 Spectra were acquired prior to administration of contrast agent, using point-resolved spectroscopy with a short echo time of 35 ms, a repetition time of 1.5 s, and 128 signal averages. Total acquisition time including scanner adjustments for each spectrum was approximately 5 min. Sizes of the regions of interest (ROIs) typically varied between 4 and 8 cm³. Position and size of ROIs were documented on three MR images to ensure that spectra subsequently acquired in the same subject were obtained from the same region. Processing was performed using fully automated LCModel software (LCModel version 6.1 – 4 F, Stephen Provencher, Oakville, ON, Canada).

Absolute metabolite concentrations (mmol/kg tissue) of NAA, Cr, tCho, myoinositol (mI), Glx (glutamate [Glu] + glutamine [Gln]), and Lac (mmol/liter), as well as lipids (and possibly underlying macromolecules) at 0.9 ppm and 1.3 ppm (LipMM09 and LipMM13) were measured. Because the number of equivalent protons per lipid molecule or macromolecule is unknown, these entities cannot be quantified in absolute concentrations, so absolute intensities in arbitrary units (a.u.) are reported instead. Metabolite concentrations were corrected for the varying fractions of tumor and of necrotic or cystic fluid in the ROIs as described earlier.28 In previous studies of DIBSG, ratios of metabolites were reported.13,23,24

Table 1. Summary of demographics, therapies, clinical course, and MRS studies of patients with diffuse intrinsic brainstem gliomas

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>RT</th>
<th>Initial Post-RT Chemotherapy, Pretreatment</th>
<th>At Disease Progression</th>
<th>ĎT_survival (Months)</th>
<th>ĎT_relapse (Months)</th>
<th>ĎT_EFS (Months)</th>
<th>Time of MRS Studies (Months after Initial Diagnosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.5</td>
<td>M</td>
<td>RT only</td>
<td>—</td>
<td>—</td>
<td>6.4</td>
<td>5.5</td>
<td>4.3</td>
<td>Pre-Tx, 2.5, 4.3</td>
</tr>
<tr>
<td>2</td>
<td>12.7</td>
<td>F</td>
<td>RT + CP/C</td>
<td>—</td>
<td>TEM then TEM/IRI</td>
<td>10.4</td>
<td>3.9</td>
<td>1.9</td>
<td>Pre-Tx, 9.9</td>
</tr>
<tr>
<td>3</td>
<td>8.8</td>
<td>M</td>
<td>RT + TEM</td>
<td>TEM</td>
<td>Etoposide</td>
<td>11.1</td>
<td>6.7</td>
<td>5.1</td>
<td>Pre-Tx, 4.7, 6.5</td>
</tr>
<tr>
<td>4</td>
<td>7.0</td>
<td>F</td>
<td>RT + TEM</td>
<td>TEM</td>
<td>Cytoxan</td>
<td>8.9</td>
<td>6.3</td>
<td>4.6</td>
<td>Pre-Tx, 5.3, 8.2</td>
</tr>
<tr>
<td>5</td>
<td>7.4</td>
<td>F</td>
<td>RT + TEM/IRI</td>
<td>TEM/IRI</td>
<td>—</td>
<td>12.4</td>
<td>11.1</td>
<td>9.6</td>
<td>Pre-Tx, 2.7, 7.0, 11.1</td>
</tr>
<tr>
<td>6</td>
<td>3.6</td>
<td>F</td>
<td>RT + GT</td>
<td>TEM/IRI</td>
<td>—</td>
<td>10.8</td>
<td>7.1</td>
<td>4.9</td>
<td>Pre-Tx, 3.0, 5.7, 7.1</td>
</tr>
<tr>
<td>7</td>
<td>5.0</td>
<td>F</td>
<td>RT + GT</td>
<td>TEM/IRI</td>
<td>Taxotere (single dose)</td>
<td>27.6</td>
<td>25.4</td>
<td>23.5</td>
<td>Pre-Tx</td>
</tr>
<tr>
<td>8</td>
<td>4.3</td>
<td>F</td>
<td>No RT</td>
<td>because of poor clinical status</td>
<td></td>
<td>2.0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Pre-Tx</td>
</tr>
<tr>
<td>9</td>
<td>7.3</td>
<td>M</td>
<td>RT + TEM</td>
<td>TEM</td>
<td>Etoposide</td>
<td>8.4</td>
<td>5.0</td>
<td>3.1</td>
<td>Pre-Tx</td>
</tr>
<tr>
<td>10</td>
<td>4.0</td>
<td>M</td>
<td>No therapy as patient moved to different country after initial MR</td>
<td></td>
<td></td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Pre-Tx</td>
</tr>
<tr>
<td>11</td>
<td>8.2</td>
<td>M</td>
<td>RT + TEM</td>
<td>TEM</td>
<td>—</td>
<td>6.4</td>
<td>5.4</td>
<td>3.5</td>
<td>Pre-Tx</td>
</tr>
<tr>
<td>12</td>
<td>6.4</td>
<td>M</td>
<td>RT + GT</td>
<td>—</td>
<td>—</td>
<td>3.7</td>
<td>2.6</td>
<td>0.2</td>
<td>Pre-Tx</td>
</tr>
<tr>
<td>13</td>
<td>14.5</td>
<td>F</td>
<td>RT + TEM/IRI</td>
<td>TEM/IRI</td>
<td>—</td>
<td>13.3</td>
<td>12.5</td>
<td>10.8</td>
<td>3.0, 9.7, 12.3</td>
</tr>
<tr>
<td>14</td>
<td>11.1</td>
<td>F</td>
<td>RT + GT</td>
<td>—</td>
<td>TEM/Rocaltrol/Thalidomide</td>
<td>28.5</td>
<td>22.1</td>
<td>19.9</td>
<td>3.0, 4.7, 6.6, 8.9, 13.3, 18.2, 21.3, 23.1</td>
</tr>
<tr>
<td>15</td>
<td>5.5</td>
<td>F</td>
<td>RT + TEM/IRI</td>
<td>TEM/IRI</td>
<td>—</td>
<td>15.2</td>
<td>12.4</td>
<td>10.7</td>
<td>5.3, 10.7, 13.3</td>
</tr>
<tr>
<td>16</td>
<td>8.5</td>
<td>F</td>
<td>RT</td>
<td>—</td>
<td>Thalidomide/Thalidomide</td>
<td>36.1</td>
<td>31.5</td>
<td>29.2</td>
<td>31.1, 33.5</td>
</tr>
<tr>
<td>Total</td>
<td>7.0</td>
<td>M/</td>
<td>6 M/</td>
<td>2.6</td>
<td>10 F</td>
<td>13.4 ± 9.8</td>
<td>11.3 ± 8.9</td>
<td>9.4 ± 8.8</td>
<td>40 studies</td>
</tr>
</tbody>
</table>

Abbreviations: RT, standard radiation therapy; T_survival, time of survival after diagnosis; T_relapse, time until relapse after diagnosis; T_EFS, event-free survival after radiation therapy; Pre-Tx, pretreatment; CP/C, carboplatin + cereport; TEM, temozolomide (Temodar); GT, gadolinium-texaphyrin; IRI = irinotecan (Camptosar).

*Transient clinical improvement was observed for all patients undergoing RT with the exception of patient 12 who relapsed immediately after RT. Not all patients with DIBSG treated at this institution had MRS studies. Included in this retrospective review are only patients who had at some stage at least one MRS study.*
We have therefore also analyzed metabolite concentrations of NAA, Cr, mI, Glx, and Lac relative to tCho to allow qualitative comparison between data obtained in this study and findings previously published.

Assessment of Metabolic and Radiological Disease Progression of DIBSGs

The time of metabolic disease progression of DIBSGs was determined as follows. Increased tCho concentrations, increased lipids, and decreased metabolite ratios of Cr/tCho and NAA/tCho have been associated with high-grade and aggressive tumors. However, in gliomas that responded to RT, metabolites (including tCho) were reduced and lipids simultaneously increased. Thus, metabolic disease progression was defined as increased absolute concentrations of tCho alone. In its output, the LCModel-processing software provides concentrations and the Cramer-Rao lower bounds (concentrations $\pm$ CRLB) for each metabolite of a spectrum. A change of tCho was concluded when there was no overlap between two consecutively measured tCho concentrations $\pm$ CRLB. The time interval between clinical relapse, obtained from medical records, and the time of metabolic disease progression ($\Delta T_{\text{Clin-MRS}}$) was determined.

All but one MRI study (initial outside MRI study of patient 16 was not available) were digitized and loaded onto the Synapse PACS system (Fuji, Tokyo, Japan). T2-weighted, fluid-attenuated inversion recovery (FLAIR), precontrast and postcontrast, T1-weighted MR images were reviewed. The extension of the lesion was marked on axial T2-weighted MRI based on the hyperintensity of the lesions. Lesion volumes were calculated by adding up the areas marked on each slice and multiplying by the inferior-superior extension of the lesion. Each of the following criteria was considered to be indicative for radiological disease progression: (1) An increase in tumor volume of at least 25% following standard Children’s Oncology Group criteria. For these cases the timing of radiation therapy and any changes in the dosage of steroids were also considered when interpreting MR images. (2) The detection of new parenchymal lesions, enhancing or not enhancing, adjacent to or separate from the original lesion(s). (3) Leptomeningeal spread of the tumor. The time intervals between clinical relapse and radiological disease progression ($\Delta T_{\text{Clin-MRI}}$) and between metabolic progression and radiological disease progression ($\Delta T_{\text{MRI-MRS}}$) were determined.

Statistical Analyses

The two-sample Kruskall-Wallis rank-sum test, a non-parametric test, was used to compare the metabolic profiles at diagnosis of untreated DIBSGs with either control brainstem or untreated astrocytomas. Spearman rank correlation analysis was used to test the association of time to death with metabolite concentrations or lipid intensities. With the exception of one patient who was excluded because no follow-up was available, there were no censored observations, as all patients died of disease. Analyses of time to clinical deterioration or event-free survival gave similar results and are not reported. To determine significant changes in serial follow-up MRS exams, the slope of the linear regression of each metabolic measure was computed for each patient. If there were no relationship between clinical progression and serial changes in these measurements, one would expect the average slope for each measurement to be zero. The hypothesis of zero average slope was tested using one-sample, one-sided t-tests, which were justified because of previously described observations (e.g., increasing levels of tCho have been associated with progressing tumors). One-sample, two-sided t-tests were used to test whether mean $\Delta T_{\text{Clin-MRS}}$, $\Delta T_{\text{Clin-MRI}}$, and $\Delta T_{\text{MRI-MRS}}$ were significantly different from zero. Statistical computations were performed using Stata/SE 8.2 for Windows (Stata, College Station, TX, USA). No corrections for multiple comparisons were applied.

Results

Metabolic Profile of Diffuse Intrinsic Brainstem Glioma at Baseline

Twelve patients with DIBSG were studied at baseline with MRS (Table 1). In all cases, good quality spectra were obtained that could be compared with spectra from astrocytoma and normal brainstem (Fig. 1). In DIBSGs, lipid intensities were not prominent — with one exception (patient 8; survival, 2 months), wherein the LipMM13 and LipMM09 intensities were ten and four standard deviations, respectively, above that observed in the other 11 subjects (36.9 vs. 3.7 $\pm$ 3.0 and 13.1 vs. 4.8 $\pm$ 2.0 a.u.) (Fig. 1B; Fig. 2).

DIBSG vs. astrocytoma ($n = 14$). Although the mean tCho of anaplastic astrocytomas was higher than the mean tCho of regular astrocytomas (5.3 $\pm$ 3.8 mmol/kg vs. 3.4 $\pm$ 1.0 mmol/kg), the difference was not significant ($p = 0.3$). Because there were also no other statistically significant differences, data from anaplastic and regular astrocytomas elsewhere in the CNS were pooled. When comparing DIBSGs with all astrocytomas, Cr ($p < 0.001$), tCho ($p < 0.001$), and Glx ($p < 0.01$) concentrations were reduced, whereas NAA, mI, and Lac were not significantly different. Lac/tCho was higher in DIBSGs than in astrocytomas ($p < 0.01$). There were no other differences in metabolite concentration ratios relative to tCho between DIBSGs and astrocytomas. Neither LipMM09 nor LipMM13 was significantly different in DIBSGs compared with astrocytomas. Overall, a smaller scatter of metabolite concentrations was observed in the group of DIBSGs. For example, the standard deviation of tCho concentrations in DIBSG was approximately one fourth of that observed in astrocytomas outside the brainstem (Fig. 2; Table 2).

DIBSG vs. normal brainstem ($n = 12$). NAA ($p < 0.0001$), tCho ($p < 0.01$), and Cr concentrations ($p < 0.01$) of DIBSG were significantly reduced, whereas mI
(p < 0.01) and Lac (p < 0.0001) were increased. Glx concentrations were not significantly different in DIBSG and normal brainstem. The NAA/tCho concentration ratio was reduced (p < 0.0001), whereas ml/tCho (p < 0.001) and Lac/tCho (p < 0.0001) were elevated. LipMM13 was elevated in DIBSG compared with controls (p < 0.01), but LipMM09 was not (Table 2).

**Correlation of baseline MRS with survival time.** Eleven patients studied with MRS at baseline succumbed to their disease (no follow-up data available for patient 10). Spearman rank correlation analysis revealed that none of these metabolic measures was significantly associated with survival at the p < 0.01 level (Fig. 3). However, the very small sample size precludes discovering any but the most profound and probably unrealistic associations between MR spectra and survival.

**Metabolic Progression of DIBSG**

**Overall metabolic changes.** A significant decrease of NAA (p < 0.05) and an increase of tCho (p < 0.05) were observed in sequential studies. Also, the NAA/tCho, Cr/tCho, and ml/tCho ratios decreased significantly (all p < 0.01), whereas LipMM13 and LipMM09 (both p < 0.01) increased (Table 3; Fig. 4). The time courses of metabolites and lipids were notably different for patient 6. In this subject, all metabolite concentrations including tCho decreased after RT (at 3 months) and lipids were increased. Metabolite concentrations declined further over the next 3 months, but unlike in all other subjects, there was no further increase of lipids observed. Thereafter, metabolite concentrations increased again, whereas the lipid signal diminished (Fig. 5). The spectrum in this patient acquired at 7 months was comparable with the MRS before treatment.

Metabolic disease progression. The comparison of metabolic disease progression and clinical deterioration was limited to eight subjects (patients 1, 3, 4, 5, 6, 13, 14, and 15) in which at least two MR spectra taken before relapse could be evaluated. Metabolic disease progression preceded clinical deterioration in six of eight subjects, whereas for two subjects the progression coincided with clinical deterioration. Mean ΔTCho-MRS was $-2.4 \pm 2.7$ months, which was significantly different from zero (p < 0.04; one-sample, two-sided t-test). For patient 6 (see above) the initial pattern of MRS after radiation therapy was consistent with response. Spectroscopic changes thereafter were consistent with regrowing tumor. Also,
Fig. 2. Total choline (tCho) and lipids of diffuse intrinsic brainstem glioma (DIBSG) at baseline. In this study, mean tCho of DIBSG at baseline was reduced when compared with regular astrocytoma (A) and anaplastic astrocytoma (AA) elsewhere in the CNS or with normal control brainstem. Also, only one patient (Pt 8) showed prominent lipids at initial presentation. DIBSG clustered at low tCho concentrations and lipid intensities. Abbreviations: LipMM13, lipid signal at 1.3 ppm; a.u., arbitrary units.

Fig. 3. Correlation of metabolic features at baseline with survival. Increasing total choline (tCho) and lipids as well as decreasing creatine/tCho (Cr/tCho) and N-acetylaspartate/tCho (NAA/tCho) ratios have been associated with increasing malignancy. These features may thus identify already progressed lesions at presentation with possibly particularly poor prognosis; however, Spearman rank correlation analysis did not reveal that these or any other metabolic measures were significantly associated with survival when all data were pooled (A–D). Data points for patient 6 (Pt 6) are labeled as the time courses of metabolite concentrations and lipid intensities (LipMM13, lipid signal at 1.3 ppm) were notably different in this patient (compare to Fig. 5).

**Table 2.** Absolute concentrations (mmol/kg tissue) and metabolite concentration ratios, mean ± SD (median)

<table>
<thead>
<tr>
<th></th>
<th>DIBSG</th>
<th>Astrocytomas</th>
<th>Control Brainstem</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Age</td>
<td>7.0 ± 2.6</td>
<td>10.3 ± 5.6</td>
<td>6.7 ± 4.1</td>
</tr>
<tr>
<td>NAA</td>
<td>2.6 ± 0.9</td>
<td>2.4 ± 1.6</td>
<td>9.6 ± 1.6**</td>
</tr>
<tr>
<td>Cr</td>
<td>4.3 ± 1.1</td>
<td>7.5 ± 1.9**</td>
<td>5.2 ± 0.4 (5.2)**</td>
</tr>
<tr>
<td>tCho</td>
<td>1.9 ± 0.7</td>
<td>4.2 ± 2.6**</td>
<td>2.7 ± 0.3 (2.6)*</td>
</tr>
<tr>
<td>ml</td>
<td>9.7 ± 2.4</td>
<td>11.6 ± 3.9 (11.2)</td>
<td>7.5 ± 0.8 (7.5)*</td>
</tr>
<tr>
<td>Lac&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 1.9</td>
<td>2.6 ± 2.8 (2.5)</td>
<td>0.2 ± 0.5 (0.0)**</td>
</tr>
<tr>
<td>Glx</td>
<td>9.5 ± 3.0</td>
<td>15.8 ± 6.4 (14.7)*</td>
<td>11.0 ± 1.5 (10.8)</td>
</tr>
<tr>
<td>NAA/tCho</td>
<td>1.5 ± 0.6</td>
<td>0.8 ± 0.7 (0.9)</td>
<td>3.6 ± 0.7 (3.4)**</td>
</tr>
<tr>
<td>Cr/tCho</td>
<td>2.3 ± 0.6</td>
<td>2.2 ± 1.1 (1.9)</td>
<td>1.9 ± 0.3 (1.9)</td>
</tr>
<tr>
<td>ml/tCho</td>
<td>5.3 ± 1.2</td>
<td>3.6 ± 2.2 (2.7)</td>
<td>2.8 ± 0.4 (2.7)**</td>
</tr>
<tr>
<td>Lac/tCho</td>
<td>1.6 ± 1.0</td>
<td>0.7 ± 0.6 (0.5)*</td>
<td>0.1 ± 0.2 (0.0)**</td>
</tr>
<tr>
<td>Glx/tCho</td>
<td>5.3 ± 2.5</td>
<td>4.2 ± 1.3 (3.7)</td>
<td>4.1 ± 0.8 (4.0)</td>
</tr>
<tr>
<td>LipMM13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 10.0</td>
<td>8.6 ± 7.8 (6.1)</td>
<td>1.3 ± 0.8 (1.3)*</td>
</tr>
<tr>
<td>LipMM09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5 ± 3.0</td>
<td>8.9 ± 3.7 (8.9)</td>
<td>5.7 ± 2.0 (5.4)</td>
</tr>
</tbody>
</table>

Abbreviations: DIBSG, diffuse intrinsic brainstem glioma; NAA, N-acetylaspartate; Cr, creatine; tCho, total choline; Lac, lactate; Glx, glutamate (Glu) + glutamine (Gln); ml, myoinositol; LipMM13, lipid signal at 1.3 ppm; LipMM09, lipid signal at 0.9 ppm.

<sup>a</sup>Lac concentrations are reported in mmol/liter.

<sup>b</sup>Absolute intensity (arbitrary units).

*<i>p</i> < 0.01; **<i>p</i> < 0.001; ***<i>p</i> < 0.0001 vs. DIBSG (Kruskal-Wallis rank-sum test).
Table 3. Metabolic progression of diffuse intrinsic brainstem glioma with the mean slope of linear regressions fitted to each metabolite or lipid

<table>
<thead>
<tr>
<th>Measure</th>
<th>Change/Month (Mean ± SD)</th>
<th>p-Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>–0.15 ± 0.19</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cr</td>
<td>–0.09 ± 0.35</td>
<td>NS</td>
</tr>
<tr>
<td>tCho</td>
<td>0.09 ± 0.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ml</td>
<td>–0.14 ± 0.35</td>
<td>NS</td>
</tr>
<tr>
<td>Lac</td>
<td>0.15 ± 0.37</td>
<td>NS</td>
</tr>
<tr>
<td>Glx</td>
<td>0.31 ± 0.73</td>
<td>NS</td>
</tr>
<tr>
<td>NAA/tCho</td>
<td>–0.12 ± 0.11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cr/tCho</td>
<td>–0.13 ± 0.12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ml/tCho</td>
<td>–0.24 ± 0.21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lac/tCho</td>
<td>0.00 ± 0.16</td>
<td>NS</td>
</tr>
<tr>
<td>Glx/tCho</td>
<td>0.00 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>LipMM13</td>
<td>2.06 ± 2.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LipMM09</td>
<td>0.66 ± 0.51</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Abbreviations: NAA, N-acetylaspartate; Cr, creatine; NS, not significant; tCho, total choline; ml, myo-inositol; Lac, lactate; Glx, glutamate (Glu) + glutamine (Gln); LipMM13, lipid signal at 1.3 ppm; LipMM09, lipid signal at 0.9 ppm.

aOne-sample, one-sided t-test.

Also included in this retrospective review are data from three atypical long-term (>24 months) survivors. Most notable was a marked accumulation of lipids in one patient 14, reported in more detail below, showed a transient massive accumulation of lipids. Spectra acquired after RT from other patients did not indicate a response to therapy or stabilization but an apparently inevitable transition to high-grade gliomas.

**Radiological disease deterioration.** For six subjects, radiological disease progression preceded clinical deterioration, and for six subjects it coincided with or was noticed after relapse, whereas in two subjects radiological deterioration was not noted despite renewed clinical symptoms. Mean \( \Delta T_{\text{Clin-MRI}} \) was \(-0.6 \pm 1.9\) months and was not different from zero. Two patients did not have follow-up MRI studies (patients 8 and 10). Metabolic disease progression preceded radiological deterioration by \(1.4 \pm 3.6\) months but was not significantly different from zero.

**MRS/MRI of Atypical Long-Term Survivors**

Fig. 4. Serial MRI and MR spectroscopy (MRS) of a representative diffuse intrinsic brainstem glioma (DIBSG) patient. Transverse postcontrast T1-weighted fluid-attenuated inversion recovery MRI (repetition time/echo time/inversion time = 2,000/7/750, matrix size = 256 × 256, number of excitations = 1, echo train length = 6, acquisition time = 2 min 8 s), T2-weighted MRI, and MRS of patient 3 with typical clinical course. The metabolic progression observed in this patient was representative for most patients (not for patient 6; compare Fig. 5) with typical clinical course. Lesion volume on MRI decreased from initial 259 cm³ to 146 cm³ after therapy at 2.5 months and then further to 115 cm³ at 4.7 months. At that time it was noted that MRS, with increased total choline (tCho), lipids, and reduced creatine/tCho (Cr/tCho), was suggestive of disease progression. A ring-enhancing lesion detected at 2.5 months was not observed at 4.7 months. Clinical and radiological deterioration occurred at 6.5 months; this patient died 11 months after initial diagnosis. Spectra are scaled to measured concentrations to allow direct comparison of peak areas. Abbreviations: ml, myo-inositol; Glu, glutamate; Gln, glutamine; NAA, N-acetylaspartate; Lac, lactate; LipMM13, lipid signal at 1.3 ppm; LipMM09, lipid signal at 0.9 ppm.
atypical survivor (patient 14) after therapy (Fig. 6). For example, the LipMM13 signal (approx. 10,000 a.u.) in the spectrum shown in Fig. 6C was more than 800 standard deviations above the LipMM13 signal observed in other treated DIBSGs (27 ± 12 a.u.). Lipid intensity peaked between 5 and 12 months after therapy when lipids also became apparent on T1-weighted images. At 21 months after initial diagnosis, high choline relative to Cr was detected in a spectrum of a newly detected focus adjacent to the main lesion consistent with tumor. Baseline MRS was not performed in this subject. Patient 7, an atypical long-term survivor, had a baseline MRS comparable with what was observed in the majority of patients with DIBSG (Fig. 7A). An artifact, caused by a partially magnetic shunt, prevented the acquisition of interpretable MRS data from the lesion at follow-up. Spectra from the third atypical long-term survivor (patient 16) were obtained 31 and 34 months, respectively, after initial diagnoses at the final stage of the disease. At this late point the spectra resembled the pattern of progressed DIBSGs with prominent lipids and tCho (Fig. 7B).

Discussion

The overall goal of this study was to evaluate whether MRS provides novel information that can improve stratification and management of patients with DIBSGs. MRS is available on most clinical MR scanners. It is FDA approved for general use in the United States and can thus be ordered by clinicians for their patients. Because of recent advances in technology, the acquisition of MR spectra is now completely automated, and it provides robust measurements of metabolic profiles of tissue when used consistently. MRS studies of gliomas in the adult population showed that increased levels of tCho, decreased metabolite ratios of NAA to tCho (NAA/tCho) and Cr to tCho (Cr/tCho), and increased levels of lipids are indicators of malignant degeneration.15–22 DIBSGs are believed to progress to higher-grade gliomas (often glioblastomas at autopsy6,8), consistent with deterioration and death of 90% of patients within 2 years after initial diagnosis.3 Therefore, our general hypothesis was that metabolic progression may parallel disease progression and could be exploited (1) to assess disease...
progression at presentation and (2) to provide novel indicators for disease status during the latent phase.

**Metabolic Profile of DIBSG at Baseline**

The most notable finding was that the mean tCho concentration in DIBSGs was less than 50% of that measured in astrocytomas elsewhere in the CNS. Indeed, mean tCho concentration in DIBSGs was lower than mean tCho in normal-appearing brainstems in controls. Choline-containing compounds (mainly phosphocholine, glycerophosphocholine, and free choline) form a single peak in 1H MRS and were therefore referenced as tCho. Choline-containing compounds are involved in the synthesis and breakdown of phosphatidylcholine (lecithin), an important membrane phospholipid. In pathologically proven adult and pediatric brain tumors, higher tCho concentrations correlated with more malignant lesions. Significant positive correlations between Ki-67 staining and tCho concentrations have been found in gliomas. Also, in malignant gliomas, higher tCho levels (expressed relative to Cr or NAA) correlated with shorter survival time. Based on these and similar studies, the suggestion has been made that higher levels of choline metabolites are associated with increased rates of membrane synthesis and cell proliferation. Consequently, low tCho of DIBSG at baseline might be an indicator for tumors with low membrane turnover. This may partially explain the low sensitivity of DIBSGs to RT. tCho could serve as a surrogate marker to identify tumors that are less proliferative but are also less likely to respond to radiation therapy. Radiation treatment has considerable side effects in young children. Also, it requires daily visits with head immobilization over
a 6-week period. It has previously been shown that in gliomas responding to RT, all metabolites (including tCho) were reduced, whereas lipids increased. One explanation is the release of intracellular metabolites and the generation of fatty acids and lipids from membrane degeneration. We observed this pattern only in one patient (patient 6). tCho concentration at baseline in this patient’s tumor was four standard deviations above the mean tCho measured in all other subjects at baseline. Clearly, these observations are preliminary, and a prospective study with more subjects is needed to evaluate whether tCho concentrations could be surrogates for the sensitivity of brainstem tumors to radiation treatment.

We speculated that MRS may be useful to characterize disease progression at presentation, information that could be used for patient stratification. As discussed above, tCho is considered to be a marker for accelerated cell proliferation. But when all MR spectra acquired at baseline were evaluated, there was no correlation between tCho and survival. Lipid levels have been shown to correlate with necrosis, and they might be elevated under hypoxic stress prior to necrosis. This is more likely to occur in rapidly dividing tumors that outgrow their blood supply, such as glioblastomas. Only one patient presented with prominent lipids. This patient had the shortest survival time among the DIBSG patients. But the small sample size and the relative homogeneity of other spectra at baseline precluded the detection of a significant correlation. There were also no correlations between the NAA/tCho and Cr/tCho ratios and survival or other metabolic measures.

**Metabolic Progression of Typical DIBSG**

Laprie et al. and Thakur et al. reported reduced levels of NAA/tCho and Cr/tCho and the appearance of lipids in follow-up MRS studies. More specifically, in this study it was found that absolute concentrations of NAA decreased, whereas tCho concentrations increased. NAA is a marker for healthy neurons and axons. The NAA resonance was likely from residual axons. The NAA/tCho ratio decreased significantly. This is consistent with an earlier study in which increased levels of ml were reported in low- vs. high-grade gliomas. ml is believed to be a marker for astrocytes and is typically prominent in pediatric astrocytomas.

Thakur et al. reported that in two of two patients metabolic progression was observed, despite signs of clinical improvement. Both patients relapsed and died after a short time. Laprie et al. performed long-echo time (144 ms) two-dimensional and three-dimensional chemical shift imaging (CSI) in four patients at diagnosis and immediately after completion of therapy. They reported that in three patients, increased spectral abnormalities preceded clinical and radiological deterioration. In this study we observed that metabolic progression preceded clinical deterioration in six of eight subjects, whereas it coincided for two subjects. Thus, there is now evidence from three independent studies that MRS identifies subjects with progressing disease and impending relapse several months before clinical manifestation. This could be important for early assessment of efficacy of novel treatment strategies in individual patients.

The metabolic progression observed in most DIBSG patients suggests that radiation therapy had a limited impact on tumor cells. This is consistent with the observation that, although patients treated with RT have a median survival longer than would have been attained in the absence of RT, patients are not cured by RT; reports of complete radiographic responses, even transient ones, are exceedingly rare. Consequently, clinical symptoms were alleviated for only a limited time. At the present stage it is unclear whether a closer follow-up with MRS predicts imminent relapse in individual subjects. The rate by which tCho and lipids increased in typical patients was different for individual tumors. It is unlikely that there is a “threshold” for lipids or tCho or other metabolic markers above which relapse is imminent.

**Atypical Long-Term Survivors**

Although the prognosis is generally poor, patients with diffuse brainstem gliomas of similar appearance on diagnostic imaging sometimes differ considerably with respect to clinical progression and survival time after initial diagnosis. Atypical long-term (>24 months) survivors have been reported by several groups. It has been suggested that in those cases lesions were wrongly classified, but one has also to consider that there are subgroups of DIBSGs that are either less malignant or more responsive to therapy than others. After the review of medical records and MRI, we have found no evidence that any of the three atypical patients included in this study were incorrectly diagnosed. MRS at baseline was performed on only one of these subjects (patient 7) and was consistent with the MRS pattern of other typical DIBSGs prior to therapy. The pattern of MR spectra acquired at the time of relapse performed on two atypical survivors was consistent with what was observed in late disease in patients having a typical clinical course. From this we tentatively conclude that atypical long-term survival of a DIBSG patient is not necessarily associated with wrong diagnosis. A more likely explanation for longer survival is slower disease progression. But it was noted that MR spectra of one atypical long-term survivor (patient 14) acquired during the latent phase showed a striking accumulation of lipids. The tumor in this patient may have had a different response to treatment.

While reviewing patient records, however, we encountered one patient, not included in this retrospective review, who initially was wrongly diagnosed with DIBSG. This patient is still alive more than 4 years after initial diagnosis. Because of the clinical course and after extensive follow-up imaging, the diagnosis has been changed to a cystic or solid, demarcated brainstem lesion with better prognosis. MRS at diagnosis and several follow-up MRS studies of this lesion were highly consistent with the metabolic pattern observed for pilocytic astrocytoma, which is readily distinguishable from anaplastic and regular astrocytoma. It is beyond the scope
of this paper to evaluate the implications of MRS for brainstem tumors other than diffuse intrinsic gliomas. A comprehensive review of MRI/MRS of these lesions has been initiated in our institution, and results will be reported separately.

**Methodological Approach**

Single-voxel, acquisition-mode MRS was selected over CSI, wherein many spectra covering a larger volume of the lesion are acquired simultaneously. This ensures that the quality of individual tumor spectra is not adversely affected by unavoidable compromises accompanying CSI acquisitions from larger volumes. Particularly for infratentorial tumors, good magnetic field homogeneity and water suppression are not always achieved uniformly. Also, CSI requires the processing and review of many spectra and offers voxel shifting. While in principle this should be considered an advantage over single-voxel MRS, in practice the processing and quality control of CSI are more time consuming, and they require the expertise of a skilled MR spectroscopist. Thus, CSI is often not feasible in environments having sparse resources.

Employing a short echo time ensured a high signal-to-noise ratio of spectra and minimized signal loss of fast-decaying peaks of metabolites such as mI, Glu, and Gln. Good quality spectra (e.g., line width less than 6 Hz) were obtained in all attempted examinations. All MR spectra were processed with commercially available software that did not require user interaction. Using the above-described methods, we have acquired more than 3,500 spectra in more than 1,400 patients and generated control data of frequently studied brain regions such as occipital gray matter and parietal white matter. Absolute metabolite concentrations obtained in our institution in those regions are in excellent agreement with data reported by other groups.\(^{45–52}\) Absolute quantification was performed because it allows a more unambiguous interpretation of spectra by avoiding the often incorrect assumptions that reference metabolites such as creatine or choline are constant. Also, in recent studies it was shown that metabolite ratios exhibited higher coefficients of variation than absolute quantitation.\(^{53,54}\)

**Limitations**

Several limitations need to be acknowledged. Because of the small incidence of this disease, we have studied only a small number of subjects, and data were retrospectively evaluated. The number of MRS studies was smaller than the number of MRI studies because only one of two MR systems used at this institution had MRS capabilities. Therefore, the time interval by which MRS progression precedes clinical deterioration and radiological disease progression may have been underestimated. Findings reported for individual subjects such as atypical long-term survivors may be incidental and should not be considered representative of all atypical long-term survivors of DIBSG. Single-voxel MRS studies were limited in their capability to assess the heterogeneity of lesions and to distinguish areas of heterogeneous disease progression. Because of swelling, edema, lesion growth, shrinkage, or head positioning in the MR coil, the identification of identical ROIs in longitudinal studies was sometimes difficult. Lesions on T2-weighted MR images comprised unknown fractions of tumor and edema. Changes of lesion volumes thus reflect a change of tumor volume and a change of edema volume.

**Conclusions**

The mean metabolic fingerprint of DIBSGs at initial presentation was consistent with tumors having low proliferative rates. The metabolic progression during the latent disease phase was consistent with malignant transformation. This preceded clinical deterioration and was observed despite transient clinical improvement. MRS may be an important tool to characterize response to therapy and tumor progression in individual patients. This may compensate for the relatively small number of patients available for studies and allow a faster completion of clinical trials, thus enhancing development of effective therapy for this disease.

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