Assignment of glial brain tumors in humans by *in vivo* 1H-magnetic resonance spectroscopy and multidimensional metabolic classification

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This study presents a simple approach for the noninvasive assignment of glial brain tumors according to malignancy by single-voxel proton magnetic resonance spectroscopy at short echo times (TE $\leq$ 50 milliseconds). Based on peak area ratios, a five-dimensional data set was obtained for each investigated subject. This vector was then projected along metabolic coordinates in a two-dimensional metabolic space. These coordinates had been determined in a previous study (Hagberg G et al., 1995, *Magn Reson Med* **34**: 242–252). Tumor assignment was done without any knowledge of histology by comparing the location of the new cases to the features of the previous study. All 11 investigated glioblastomas multiforme, as well as 4 of 5 astrocytomas grade II, could easily be assigned to the groups of high- and low-grade tumors, respectively. Classification was more difficult in the case of a cystic astrocytoma grade II and one astrocytoma grade III. Two spectra measured in normal-appearing matter of glioblastoma patients were not classified as healthy. Using single-voxel proton magnetic resonance spectroscopy at short echo times with the knowledge of a base study, a straightforward, fast, and noninvasive differential diagnosis of glial brain tumors is possible.

Keywords: 1H-MRS, human, glioma, malignancy, classification, ODV.

INTRODUCTION

The histological assignment of human brain tumors using different localized 1H-magnetic resonance spectroscopy (1H-MRS) techniques is a long-standing problem. Several investigators have tried to find or describe tumor-specific spectra [1]. However, most of these studies have been performed using longer echo times (e.g., TE = 135–272 milliseconds). In those measurements the definition of the baseline is improved at the expense of the visibility of resonances having shorter relaxation times or being strongly coupled, such as *myo*-inositol, glutamine, glutamate, lipids, or proteins. Important additional metabolic information can thus be obtained using shorter TE. However, a diagnosis based on single peaks in short echo time MRS is still very difficult because the spectral patterns of different tumor types show some trends but overlap in a wide range [1–3].

Howells et al. [4,5] presented a method to improve the spectral analysis of rat tumors using automated procedures. According, the information content of all metabolites together can be extracted using cluster analysis both with [4–6] or without [6,7] feature extraction techniques [8]. First applications of this

method to *in vivo* proton spectra of the human brain were presented by Confort-Gouny et al. [9].

The purpose of the present study was to apply the metabolic features found in a previous study of 21 healthy controls and humans with gliomas [6] to a new cohort of patients with a suspected glial brain tumor and other healthy volunteers. The new cases, investigated on a different magnetic resonance (MR) scanner, were classified by observing their location within the previous features as low-grade or high-grade gliomas or as healthy.

MATERIALS AND METHODS

Data acquisition and postprocessing

Thirty-five spectra of 17 patients with a suspected glial brain tumor and 9 healthy volunteers (HV) were investigated at 1.5 T with a Siemens Magnetom SP 4000 MR system (Siemens Medical Systems, Erlangen, Germany) using the standard circularly polarized head coil. After image-guided localization, the position of the cubic volume of interest of 8 mL was chosen to be as close as possible to the center of the tumor, so that the volume of interest was filled with at least 75% tumor tissue. A STEAM sequence with an echo time of 50 milliseconds was used, the mixing and repetition times were 30 milliseconds and 1500 milliseconds, respectively; 256 acquisitions were performed. Water suppression was achieved by three consecutive CHESS pulses followed by dephasing gradients [10]. None of the patients had ever received stereotaxic biopsy, open surgery, or radiation therapy before MRS. Between MR imaging and MRS was a delay of at least 1 day to minimize possible spectral disturbances caused by the application of the paramagnetic contrast agent. In eight patients contralateral normal-appearing matter was also investigated. Stereotaxic biopsy or open surgery was performed within a few days after MRS. One patient returned to MRS 3 months after radiation therapy.

Spectra were postprocessed with a correction for residual eddy currents [11], zero-filling to 4096 data points, Gaussian filtering (time constant: 256 milliseconds), and, if necessary, phase corrected in zero and first order after Fourier transformation. Peak areas were calculated using the standard equipment software. The resonances of lipids, proteins, and lactate (Lip) between 0.7 and 1.7 ppm; the N-acetylated compounds (NAA) at 2.0 ppm; macromolecules, glutamine, and glutamate (MGG) between 2.1 and 2.55 ppm; total creatine (Cr) at 3.0 ppm; the choline-containing compounds (Cho) at 3.2 ppm; and the resonance of both glycine and *myo*-inositol (GI) at 3.55

ppm were integrated. For quantification of the NAA peak, the upfield half of the resonance was integrated and the result multiplied by a factor of 2. Severe lipid contamination effects could be excluded by positioning the volume of interest sufficiently apart from the skull and by applying slice selection gradients of 3 mT/m. This was necessary because the lipid resonances played an important role in the metabolic classification (see below).

The study protocol was approved by the Ethical Committee of the Basel University Hospital. All subjects were informed of the purpose of the study and gave their consent.

Metabolic classification

For each measured subject the ratio of the area of each metabolite over the area of Cr was calculated. Thus, five ratios were obtained for each patient. The ratios were then projected along metabolic coordinates (ODV_1, ODV_2) into a two-dimensional space. These coordinates had been determined using the orthonormal discriminant vector method (ODV) [12] in a previous study of 13 patients with the same inclusion criteria (suspected glial brain tumor, no previous therapy, biopsy, or surgery before MRS) and 8 healthy volunteers, all measured with identical measurement parameters on a different MR system, a Siemens Magnetom Helicon GBS II at 2.0 T [6]. In this study, the coordinates shown in equation 1 permitted a separation of all investigated cases into three distinct groups: healthy volunteers, low-grade gliomas (i.e., astrocytoma and oligodendroglioma World Health Organization grade I and II), and high-grade gliomas (astrocytoma grade III and glioblastoma multiforme):

$$ODV_1 = 0.4232 \, NAA/Cr - 0.3116 \, MGG/Cr$$
$$- 0.6605 \, Cho/Cr - 0.5348 \, GI/Cr$$
$$- 0.0372 \, Lip/Cr, \tag{1}$$

$$ODV_2 = 0.4422 \, NAA/Cr - 0.3909 \, MGG/Cr$$
$$+ 0.7321 \, Cho/Cr - 0.3317 \, GI/Cr$$
$$+ 0.0748 \, Lip/Cr.$$

Each measured subject was thus represented by one single point in the two-dimensional metabolite space. Figure 1 displays the distribution of the previous cases (solid symbols) within this space. High-grade and low-grade tumors, as well as healthy volunteers, could clearly be separated by ODV.

In the present study the same coordinates were used for a histological assignment of 37 new spectra. The position of each point of the new subject group (with unknown histology, when classification was done)
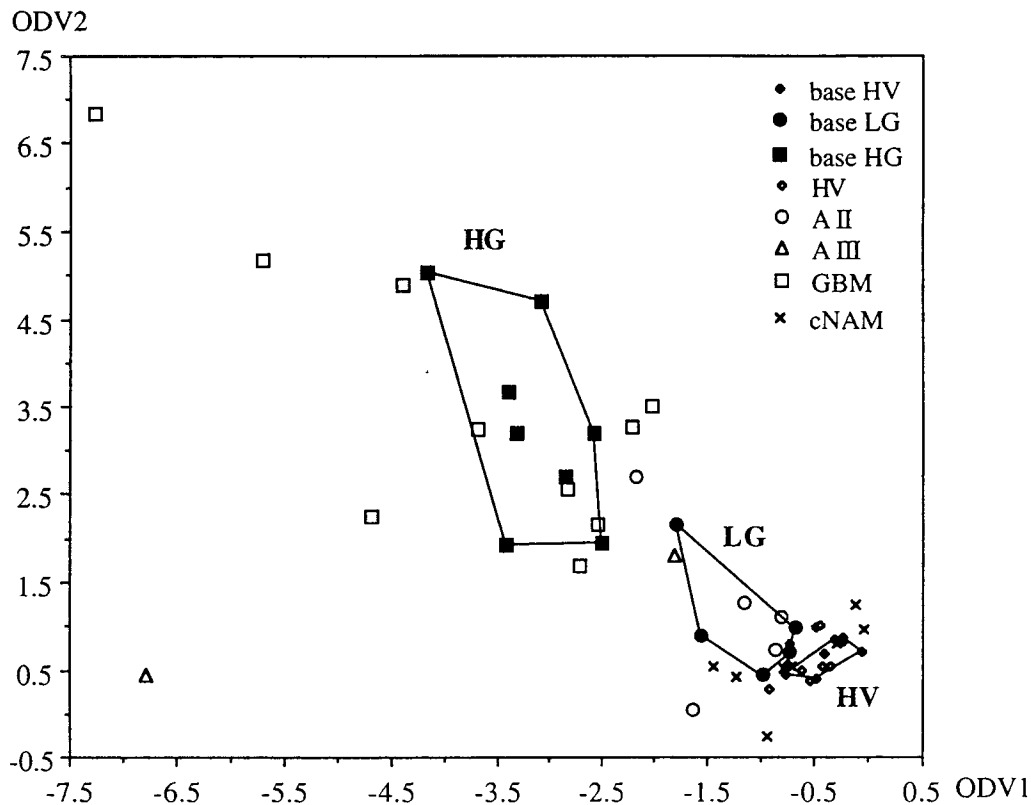
ODV2

Fig. 1. Distribution of all investigated cases within the metabolic space as defined by equation 1. The outlined areas form the basic features as regions of different histology found in the base study (solid symbols). The new cases of the present study are indicated by open symbols and crosses. The cases were classified as HG or LG or as HV depending on distance from previously defined regions. The data point of one glioblastoma multiforme is located beyond the left edge of the figure at (−11.2, 22.4). A II, astrocytoma grade II; A III, astrocytoma grade III; GBM, glioblastoma multiforme; cNAM, contralateral normal-appearing matter.

was compared with the metabolic features of the previous study (with known histology). The new subjects were then assigned to the group to which the distance was smallest: healthy (HV), low-grade glioma (LG), or high-grade glioma (HG). This histological assignment by MRS and ODV was then compared with the histology obtained afterwards by stereotaxic biopsy.

RESULTS

Stereotaxic biopsy revealed 10 glioblastomas multiforme (GBM), two astrocytomas grade III, and five astrocytomas grade II. Metabolic coordinates were calculated from the spectral results, as described above. The new cases are shown in Fig. 1 (open symbols and crosses), together with the results of the base study. All 10 GBM were nearest to the high-grade gliomas of the base study. Four of five astrocytomas grade II were assigned to the LG gliomas, one to the HG. The two astrocytomas grade III were assigned as one LG and one HG. All HV were nearest to the HV group of the base study, and contralateral normal-appearing matter of tumor patients was assigned as normal in six cases and as low grade in two cases.

Figure 2 shows the tumor spectra of two patients

with glioblastoma multiforme. In metabolic space, the spectra shown in Fig. 2 are located at the coordinates (−11.2, 22.4) and (−3.69, 3.25), respectively (Fig. 1). Both spectra were clearly assigned to the HG gliomas, although the spectra look very different.

The patient returning for a second MRS after stereotaxic biopsy and radiation therapy had a GBM. The spectra were located at (−2.55, 2.17) and (−2.83, 2.57) (before and after biopsy/therapy, respectively). They could be correctly assigned in both measurements to the HG group (Fig. 1).

DISCUSSION

The spectra from the glioblastoma multiforme show the largest spread within the metabolic space, indicating their heterogeneous and dedifferentiated tissue. Nevertheless, all 11 investigations of GBM were assigned correctly. Four of the five astrocytomas grade II were also assigned correctly, and the fifth was located between the features of low- and high-grade gliomas. This measurement had been performed within the cysts of a large cystic astrocytoma. Here, the integral of the choline signal was approximately as large as all of the remaining resonances together, and lipid signals
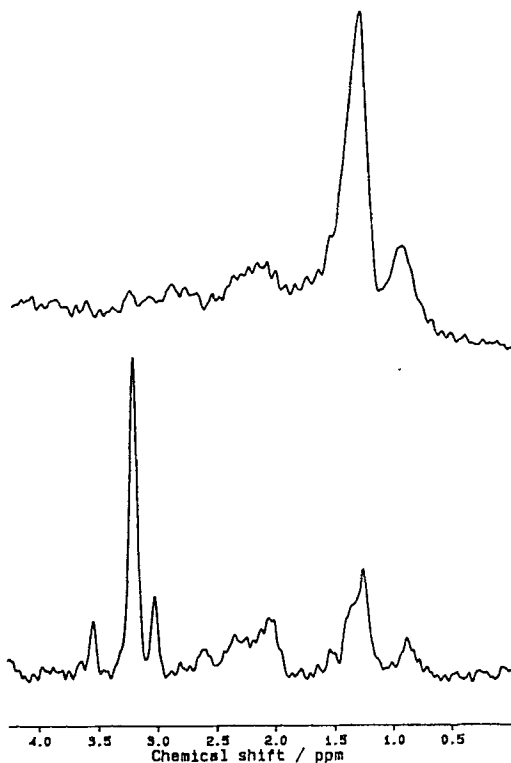
Fig. 2. MR spectra of two different glioblastomas multiforme. Both spectra were correctly assigned as high-grade glioma.

were also small. Thus, this case was located slightly closer to the HG group in metabolic space, inasmuch as choline has, according to equation 1, the strongest effect on the metabolic coordinates. On the other hand, the general state of the patient was not very healthy, and morphological histology, as determined by both computed tomography and MR imaging, resulted also in a high-grade glioma. Especially this case might demonstrate the problems encountered with the very limited size of stereotaxic material and thus the possible usefulness of the technique presented as an additional diagnostic tool for an improved characterization of human glioma.

The assignment of the two astrocytomas grade III was most difficult. One of the astrocytomas grade III was located close to the LG group, and the other was nearer to the high-grade gliomas. At the moment we can only speculate whether this different outcome in assignment reflects the difficulties in classifying tumor histology by brain biopsy analysis or perhaps a different outcome of the patient's course.

The points of all nine HV were assigned correctly, but some were located close to the area of low-grade gliomas. This finding can be explained by the fact that the humps of lipids, proteins, and lactate were not integrated in the HV spectra of the base study. Be-

cause these resonances may also appear in short echo time ¹H-MRS of healthy subjects [13–15], the indicated area of HV in Fig. 1 might have to be slightly extended to the LG area. The use of better localization sequences with shorter TE and stronger slice selection gradients to prevent lipid contamination could improve the separability of the pathology groups; these were, however, not available at the time of the base study.

The use of identical measurement parameters in all investigations is crucial for the success of the presented approach. The change from a main magnetic field strength of 2.0 T to 1.5 T could not be avoided in the present study, but it should have only a minor influence on the evaluated peak ratios [16]. More important is the optimum choice of the volume of interest within the center of the tumor. The present study also clearly demonstrates that the choice of contra- or ipsilateral normal-appearing tissue as an internal reference in tumor patients may lead to false spectral interpretations. The approach operates very well, although singular metabolic ratios should in principle not be used to quantitate brain metabolites [6,17].

Depending on the quantification method, absolutely quantified proton spectra of tumor tissue might be seriously influenced by altered relaxation times of the metabolites of interest. Normally, these relaxation time changes will be in the same direction for different metabolites. In this case we can partly reduce this addition source of quantification error just by the use of metabolite peak ratios, as has been done in the approach presented. Hence, the linear combination of metabolite peak ratios remains probably the fastest and most model-independent way to classify brain tumor spectra noninvasively.

CONCLUSION

Single-voxel ¹H-MRS at short echo times (TE ≤ 50 milliseconds) allows a differential diagnosis of glial brain tumors by the use of linear combinations of metabolic ratios plotted in a two-dimensional space. The method fails only in a few cases. A careful observation of the MR images may be necessary in the cases where the assignment is ambiguous. The approach does not require a method for an absolute quantification of the metabolites if the same or very similar measurement parameters are used as in the base study. The change from 2.0 to 1.5 T between base and actual study had no measurable deteriorating effect on the results of the present study. The method is simple and noninvasive and can easily be performed as part of routine MR imaging within about 15 minutes. Contralateral normal-appearing brain matter may show

pathological spectra and should not be taken as a reference in tumor patients.

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