Proton magnetic resonance spectroscopy in pituitary macroadenomas: preliminary results

Laboratory investigation

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Object. The aim of this study was to correlate proton MR (1H-MR) spectroscopy data with histopathological and surgical findings of proliferation and hemorrhage in pituitary macroadenomas.

Methods. Quantitative 1H-MR spectroscopy was performed on a 1.5-T unit in 37 patients with pituitary macroadenomas. A point-resolved spectroscopy sequence (TR 2000 msec, TE 135 msec) with 128 averages and chemical shift selective pulses for water suppression was used. Voxel dimensions were adapted to ensure that the volume of interest was fully located within the lesion and to obtain optimal homogeneity of the magnetic field. In addition, water-unsuppressed spectra (16 averages) were acquired from the same volume of interest for eddy current correction, absolute quantification of metabolite signals, and determination of full width at half maximum of the unsuppressed water peak (FWHM<sub>water</sub>). Metabolite concentrations of choline-containing compounds (Cho) were computed using the LCModel program and correlated with MIB-1 as a proliferative cell index from a tissue specimen.

Results. In 16 patients harboring macroadenomas without hemorrhage, there was a strong positive linear correlation between metabolite concentrations of Cho and the MIB-1 proliferative cell index (R = 0.819, p < 0.001). The metabolite concentrations of Cho ranged from 1.8 to 5.2 mM, and the FWHM<sub>water</sub> was 4.4–11.7 Hz. Eleven patients had a hemorrhagic adenoma and showed no assignable metabolite concentration of Cho, and the FWHM<sub>water</sub> was 13.4–24.4 Hz. In 10 patients the size of the lesion was too small (< 20 mm in 2 directions) for the acquisition of MR spectroscopy data.

Conclusions. Quantitative 1H-MR spectroscopy provided important information on the proliferative potential and hemorrhaging of pituitary macroadenomas. These data may be useful for noninvasive structural monitoring of pituitary macroadenomas. Differences in the FWHM<sub>water</sub> could be explained by iron ions of hemosiderin, which lead to worsened homogeneity of the magnetic field. (DOI: 10.3171/JNS/2008/109/8/0306)

Key Words • hemorrhage • magnetic resonance spectroscopy • pituitary macroadenoma • preoperative diagnosis • proliferation

Pituitary adenomas are the most common sellar space-occupying lesions and are generally regarded as benign proliferations of cells of the anterior lobe of the pituitary gland. However, some pituitary adenomas demonstrate more aggressive behavior such as recurrences, rapid progression in size, or even intracranial metastasis in rare cases. This capacity for invasive or aggressive growth adversely affects the success of treatment and disease prognosis. The ability to predict tumor behavior has led to an interest in estimating the proliferative potential of pituitary adenomas.22,29,30 It is believed that invasive or recurrent pituitary tumors generally have high growth fractions,1,33 whereas other lesion subgroups do not.1,17 Disease prognosis in a particular individual cannot be absolutely predicted based on a proliferation index such as immunostaining for the proliferation-associated antigen Ki 67 (MIB-1), but the preoperative assessment of the proliferative potential of these tumors may provide helpful information for treatment planning and prognosis.8,15

To date, MR imaging is the method of choice for the evaluation of pituitary tumors. It allows imaging of soft
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tissue without interference from the osseous surroundings of the sella and produces images in any plane. The pituitary gland, cavernous sinus, and infundibulum are outside the blood–brain barrier and normally enhance immediately on contrast-enhanced T1-weighted MR images, which usually provide information on the malignancy of brain tumors.\(^2\) Signal intensities and enhancement patterns of larger adenomas are variable on MR images. Areas of increased signal on T1-weighted MR images may be due to calcifications, high concentrations of protein, or hemorrhage.\(^3\) A T2-weighted MR sequence is helpful in confirming hemorrhage, but unfortunately does not provide unambiguous findings.\(^2,4\)

Proton MR spectroscopy is a method that renders information on tumor metabolism, which is unavailable with MR imaging. Although no detectable metabolite in vivo \(^1\)H-MR spectroscopy is specific for the infiltration of tumor cells, it is possible to detect changes in metabolite concentrations in tumor tissue compared with those in normal brain tissue.\(^2\)\(^3\)\(^4\) It is common knowledge that brain tumors show increased levels of Cho.\(^5\)\(^6\)\(^7\) In \(^1\)H-MR spectroscopy the Cho signal is composed of choline, phosphocholine, and glycerophosphocholine. It is thought to be a marker for increased cell membrane proliferation or higher cellular density.\(^2\)\(^3\)\(^4\)

In this study we evaluated the feasibility of \(^1\)H-MR spectroscopy in patients harboring pituitary macroadenomas and its utility in the preoperative, noninvasive assessment of the tumor proliferative potential and the presence of intratumoral hemorrhage. Absolute metabolic values of Cho were calculated from single-voxel \(^1\)H-MR spectroscopy data and correlated with the MIB-1 PCI from intraoperative tissue samples.

**Methods**

**Patient Population**

Conventional MR imaging and \(^1\)H-MR spectroscopy examinations with subsequent tumor resection and a detailed histopathological evaluation were performed in 37 patients harboring pituitary macroadenomas. The mean patient age for the entire population was 55 ± 16 years (mean ± SD), and the sample included 15 women. There were 26 nonfunctioning adenomas (11 null cell adenomas and 15 adenomas showed immunohistochemical staining for FSH and/or LH, respectively) and 11 hormonally active adenomas (1 ACTH- and 10 GH- and/or PRL-secret ing adenomas). The presence or absence of hemorrhage within an adenoma was observed intraoperatively.

Informed consent was obtained from all patients before inclusion in the study, as required by the local ethics committee.

**Magnetic Resonance Imaging and \(^1\)H-MR Spectroscopy**

The MR imaging examinations were performed on a 1.5-T clinical whole-body unit (MAGNETOM Sonata, Siemens) equipped with a standard head coil. For diagnosis of a pituitary adenoma the conventional MR imaging protocol consisted of an axial T2-weighted half-Fourier acquisition single-shot turbo spin echo sequence (5-mm-thick sections, TR 1000 msec, TE 89 msec, matrix 240 × 256, FOV 234 × 250 mm), coronal and sagittal T2-weighted turbo spin echo sequences (3-mm-thick sections, TR 3850 msec, TE 111 msec, matrix 358 × 512, FOV 230 × 230 mm), and coronal and sagittal T1-weighted turbo spin echo sequences (3-mm-thick sections, TR 450 msec, TE 12 msec, matrix 307 × 512, FOV 270 × 270 mm). Lesion volumes were estimated by measuring the height (head–feet direction), width (left–right direction), and length (anterior–posterior direction) of the lesions on T2-weighted MR images. With the assumption that an ellipsoid is an acceptable approximation of the shape of a lesion, we used the following formula to calculate the lesion volume:

\[
V_{\text{lesion}} = (\pi/6) \times \text{height} \times \text{width} \times \text{length}.
\]

For \(^1\)H-MR spectroscopy of each pituitary macroadenoma the VOI (voxel) was situated to avoid both susceptibility effects by covering most of the macroadenoma and partial volume effects resulting from neighboring structures such as the cavernous sinus, paranasal sinuses, internal carotid arteries, sella turcica, and optic chiasm. Voxel dimensions were adapted to obtain optimal homogeneity of the magnetic field (shim). Magnetic resonance spectroscopy data from the voxel were obtained using a point-resolved spectroscopy sequence (TR 2000 msec, TE 135 msec) with 128 averages. Water suppression was achieved using 3 chemical shift selective pulses before the 90° excitation pulse.

Absolute metabolite concentrations were determined by water scaling, a method in which the resonance area of the unsuppressed water signal is used as an internal reference. Appropriate water-unsuppressed MR spectroscopy data were obtained immediately after the water-suppressed measurement from a voxel identical in localization and size. Sequence parameters for the water-unsuppressed data acquisition were also identical, differing only in the number of acquisitions, which was reduced to 16. The water-unsuppressed spectra were also used for eddy current correction and the determination of FWHM\(_{\text{water}}\). Absolute metabolite concentrations of Cho were computed using LCModel (linear combination of model spectra),\(^8\) which is a user-independent frequency domain spectral fitting program (Stephen Provencher, Inc.). Water scaling and fitting were performed automatically. Estimated uncertainties (Cramér–Rao lower bounds) served as main guidelines for judging the spectra of absolute metabolite concentrations. Only metabolite spectra with LCModel-estimated uncertainty of < 15% SD of the evaluated concentrations were included in this study. Concentrations were corrected for relaxation times effects using T1 and T2 times from the literature\(^9\)\(^10\)\(^11\) and were expressed in mM (mmol/L).

The FWHM\(_{\text{water}}\) was determined using the freely available program csx (version for Linux, Kennedy Krieger Institute) for postprocessing of MR spectroscopy data measured without water suppression. The FWHM\(_{\text{water}}\) was calculated after reconstruction via a Fourier transformation and automatic phasing of the data. No time-domain or frequency-domain filter was applied.
Immunohistochemical Analysis

The surgical specimens were fixed in buffered formalin for embedding in paraffin as well as Epon 812. For classification of the adenomas H & E- and PAS-stained paraffin sections and toluidine blue-stained Epon sections were used for analyzing the structure. To determine hormone expression antibodies against the pituitary hormones GH, PRL, ACTH, thyroid-stimulating hormone, FSH, LH, and α-subunit were used on paraffin sections in an ABC technique. For proliferation, Ki 67 (MIB-1) produced by Zytomed (Berlin, Germany) as a monoclonal antibody against recombinant Ki 67-protein (clone K2, dilution 1:750, retrieval with microwave and citrate buffer) was used. The nuclear stainings of Ki 67 were counted in the high-power fields. The PCI documents the positive nuclei as a percentage of all adenoma cells.

Statistical Analysis

Data were analyzed using statistical software (SPSS, version 14.0, SPSS Inc.). Unpaired Student t-tests were used for comparisons of the FWHM\textsubscript{water} values between patients with nonhemorrhagic and hemorrhagic macroadenomas, and for comparisons of lesion volumes, [Cho], and MIB-1 PCIs between endocrinological types. Linear regression analyses were calculated for correlations between [Cho] and the MIB-1 PCI. The correlation coefficient was interpreted while considering the specifications for interpretation as outlined by Zou et al. For all tests the level of significance was set at a probability level < 0.05.

Results

Lesion diameters in all 37 patients ranged from 11 to 53 mm (mean diameter 26 ± 9 mm), and the lesion volumes (V\textsubscript{lesion}) from 1.28 to 52.50 cm\textsuperscript{3} (mean volume 11.49 ± 10.80 cm\textsuperscript{3}). In 10 patients the voxel dimensions were limited to the minimal possible value of 10 mm in each direction because of the number of small-volume lesions that were found in the range of 1.28–3.87 cm\textsuperscript{3} (mean ± SD, 2.63 ± 1.08 cm\textsuperscript{3}). For these patients the spectra showed no quantifiable metabolite peak, which was caused by an insufficient signal-to-noise ratio rather than the presence of hemorrhagic changes. For the remaining 27 patients the lesion volume ranged from 4.35 to 52.50 cm\textsuperscript{3} (mean 14.78 ± 10.94 cm\textsuperscript{3}), and the volumes of the voxel were defined as 1.30 to 6.27 cm\textsuperscript{3}. For the acquisition of reliable MR spectroscopy data with sufficient signal-to-noise ratio, the diameter of the lesion must be > 20 mm in at least 2 directions in space. Information about the lesion volume regarding the endocrinological type of pituitary adenoma is presented in Table 1. There were no significant differences in lesion volume among null cell adenomas, FSH- and/or LH-secreting adenomas, and hormonally active adenomas.

In the group of 27 patients with lesion volume > 4 cm\textsuperscript{3}, 16 (13 with a nonfunctioning adenoma and 3 with a GH- and/or PRL-secreting adenoma) showed a quantifiable Cho signal in the spectra preoperatively and no hemorrhagic changes in the macroadenoma intraoperatively. A representative case of a 76-year-old man with a nonfunctioning adenoma is presented in Fig. 1. The position and size of the voxel is depicted as a white rectangle overlaid on sagittal T2-weighted (Fig. 1A) and coronal T1-weighted MR images (Fig. 1B). The water-suppressed spectra fitted using the LCModel and the water peak of the water-unsuppressed experiment were both obtained from the same voxel and are shown in Fig. 1C and D, respectively.

For 11 patients (7 with a nonfunctioning adenoma and 4 with a GH- and/or PRL-secreting adenoma) harboring pituitary macroadenomas with intratumoral hemorrhage, the quality of the spectra was not adequate for quantification of a Cho signal. Preoperative standard MR imaging provided no indication of hemorrhagic changes in any of the lesions. Figure 2 features a representative case of a 66-year-old man with a hemorrhagic prolactinoma. The water-suppressed spectra showed no quantifiable Cho peak and a distorted baseline because of residual unsuppressed water. The water peak of the water-unsuppressed experiment was significantly broader (FWHM\textsubscript{water} = 19.53 Hz) compared with that in the nonhemorrhagic case (FWHM\textsubscript{water} = 5.86 Hz) in Fig. 1D.

Among these 27 patients with sufficient lesion volumes we found a clear difference in the FWHM\textsubscript{water} between the 2 subgroups without and with hemorrhage (Fig. 3). The subgroup of patients with nonhemorrhagic macroadenomas showed FWHM\textsubscript{water} values ranging from 4.39 to 11.72 Hz (mean 8.74 ± 2.49 Hz), and the subgroup with hemorrhagic macroadenomas a range from 13.35 to 24.41 Hz (mean 17.97 ± 4.01 Hz). This difference was significant (p < 0.001, unpaired t-test).

Quantification of spectra from the 16 patients with nonhemorrhagic pituitary macroadenomas revealed a Cho concentration in the range of 1.8–5.2 mM (mean 3.6 ± 1.0 mM). Immunohistochemical analysis of the samples demonstrated an MIB-1 PCI ranging from 0.5 to 3%. Figure 4A features images obtained in a 51-year-old man with a nonfunctioning adenoma. A Cho concentration of 3.7 mM was calculated using the LCModel for the spectra (Fig. 4C) from a voxel situated in the lesion. The histological sample showed 1% MIB-1–positive cells. For all 16 patients a linear regression model for the correlation of [Cho] and MIB-1 PCI revealed a strong positive correla-
Discussion

The application of MR spectroscopy methods is common and well accepted in the diagnosis of brain tumors, even in a clinical routine. The purpose of our study was to estimate the utility of single-voxel MR spectroscopy of pituitary macroadenomas. In detail we evaluated the value of $^1$H-MR spectroscopy in the assessment of the proliferative potential and hemorrhage of these lesions. We found that a prerequisite for the application of $^1$H-MR spectroscopy in patients with pituitary macroadenomas is a lesion diameter $> 20$ mm in at least 2 directions in space to obtain reliable MR spectroscopy data. For lesions that fulfill this condition the concentration of Cho correlated well with the MIB-1 PCI, whereas hemorrhagic adenomas showed no Cho signal, which we assumed to be caused by the interference of various products in the hemoglobin breakdown. This assumption is supported by the FWHM$_{\text{water}}$ findings.

To our knowledge in vivo $^1$H-MR spectroscopy of pituitary macroadenomas has not been reported in a larger series. A report by Usenius et al.\textsuperscript{31} featured 2 cases in the application of in vivo $^1$H-MR spectroscopy and 6 cases in in vitro $^1$H-MR spectroscopy. Arnold and colleagues\textsuperscript{3} reported their experience with $^{31}$P-MR spectroscopy in evaluating 3 patients with pituitary adenoma. In 2 papers Kinoshita et al.\textsuperscript{12,13} described spectra and molar concentrations in vitro studies. The in vivo metabolite concentration of Cho in 2 patients with pituitary adenomas ($2.7$ mM), as measured by Usenius and coworkers,\textsuperscript{31} is in the range of values in our study. The in vitro Cho concentration in adenoma biopsy samples, as determined by Usenius et al. ($3.5$ mM for 6 specimens) as well as Kinoshita et al. (1.3

Fig. 1. Sagittal T2-weighted (A) and coronal T1-weighted (B) MR images obtained in a patient with a nonhemorrhagic null cell adenoma, showing the position and size of the VOI (white rectangles) in the $^1$H-MR spectroscopy experiment. Graphs demonstrating the water-suppressed spectra fitted using the LCModel (C, solid line) and the unsuppressed water peak obtained from this voxel (D).
mM for 2 specimens), are also in this range but not fully comparable to our in vivo findings. These authors did not correlate MR spectroscopy data with immunohistochemical parameters, as it was not actually within the scope of their studies. Several studies have revealed a correlation between the Cho concentration and the MIB-1 proliferation index in gliomas, meningiomas, and in meningiomas. Although we detected a clear correlation between aggressive growth and the proliferation of pituitary adenomas in the study by Buchfelder et al., more recently Honegger et al. found a significant correlation between the MIB-1 proliferation index and the growth velocity of nonfunctioning pituitary adenomas. However, there was no correlation between the MIB-1 proliferation index and invasiveness. Hence, the correlation between the MIB-1 proliferation index and \(^1\text{H}-\text{MR spectroscopy may influence decision making especially when a surgical versus a conservative treatment is considered. The major drawback is the inhomogeneity of proliferation within an individual adenoma and thus the intratumoral variation in the PCI. Proton MR spectroscopy has the advantage of being able to assess an averaged signal from the whole lesion.}

The increased FWHM\(_{\text{water}}\) in patients with hemorrhagic pituitary macroadenomas can be explained by the effect of various products in the breakdown of hemoglobin on the homogeneity of the magnetic field and increased relaxation rates of the magnetic moments. The different derivatives of hemoglobin that follow a hemorrhage have paramagnetic effects on both T1- and T2-weighted MR images. This action is caused by the deposition of iron ions in the tissue and has 3 main effects: a shift in the local resonance frequency because of magnetic susceptibility alterations; a decrease in the T2 relaxation time due to diffusion through local gradients; and local line broadening, that is, increased FWHM\(_{\text{water}}\) due to field inhomogeneity within a voxel.

The limitations of this feasibility study are a lack of comparative data. Additional studies in which authors apply higher field strengths and/or short echo time MR spectroscopy are needed to create correlative data and
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Fig. 3. Boxplot revealing the FWHM_{water} values for the subgroups of patients with and without hemorrhagic macroadenomas. Horizontal lines are the medians, and the ends of the boxes represent the lower and upper quartiles (25th and 75th percentiles, respectively). N = the number of patients in the subgroups.

to detect other metabolites (for example, lactates or lipids) that can be used as prognostic indicators. However, 1H-MR spectroscopy can be used as an additional tool to monitor intratumoral changes following drug or radiation therapy, which are not detectable by conventional MR imaging.

Conclusions

Proton MR spectroscopy of large pituitary macroadenomas can be used as an adjunct in the therapy planning stages. In cases of hormonally active tumors (for example, prolactinomas), knowledge of the tumor’s inclination to hemorrhage may result in either early surgery or close observation.

Our findings demonstrate that MR spectroscopy provides additional information on proliferation and hemorrhage, which may be helpful in caring for patients with pituitary macroadenomas.

References


Fig. 4. Sagittal T2-weighted MR image (A) obtained in a patient with a nonhemorrhagic nonfunctioning adenoma (FSH secreting), showing the position and size of the VOI (white rectangle) in the 1H-MRS experiment. Photomicrograph (B) showing the results of the immunohistochemical analysis for the proliferation of a surgical tumor specimen. Graph (C) revealing the water-suppressed spectra fitted using the LCModel (red line). Graph (D) demonstrating the correlation between [Cho] and the MIB-1 PCI in the 16 patients with nonhemorrhagic pituitary macroadenomas. The solid black line and the correlation coefficient (R) represent the result of a linear regression model. Original magnification × 440 (B).


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