NMR Spectroscopy and Pediatric Brain Tumors

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Key Words. Brain · Imaging · Tumor · Spectroscopy · Pediatric · Metabolic activity

ABSTRACT

Proton nuclear magnetic resonance spectroscopy (1H-NMRS) is a noninvasive in vivo technique that utilizes conventional MR imaging hardware to obtain biochemical information from a discrete volume of tissue after suppression of the water signal. MR spectroscopy coupled with conventional MR imaging allows correlation of structural changes with biochemical processes in tissues by measuring specific metabolites present in brain tissue. NMRS is commonly used in the evaluation of patients with brain tumors. This article reviews the basic principles of spectroscopy and its use in evaluating pediatric patients with brain tumors. The Oncologist 2004;9:312-318

INTRODUCTION

Proton nuclear magnetic resonance spectroscopy (1H-NMRS) is becoming a common tool to noninvasively evaluate the brain for a variety of clinical conditions. It is frequently used in conjunction with standard magnetic resonance imaging (MRI) and positron emission tomography (PET) scans to evaluate tumors of the central nervous system (CNS). This article reviews the basic principles of spectroscopy and its role in evaluating pediatric brain tumors.

BASIC PRINCIPLES

MRI is the standard neuroimaging technique for brain tumors because it accurately delineates their anatomic location and extent. Magnetic resonance (MR) is the exchange of energy at a specific frequency as particles move between different energy states. MR images are constructed from the signals released from hydrogen nuclei of water and lipids in a strong magnetic field after radiofrequency excitation. The images obtained give information regarding the structure of the brain, but little or no information regarding its biochemistry or metabolism.

NMRS is a noninvasive technique that allows in vivo measurements of certain tissue metabolites. By suppressing the signals from water and lipids, the relative concentrations of nonwater, proton-containing metabolites from discrete tissue regions can be quantified. NMRS is based on...
the principle that small differences in the resonance frequencies of nuclei occur based on their chemical environment. Nuclei resonate at a frequency \( f \) defined by the Larmor equation:

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f = \gamma B_0
\]

where \( \gamma \) is the gyromagnetic ratio for the nucleus and \( B_0 \) is the strength of the external magnetic field. The actual \( B_0 \) for a given nucleus is determined by the shielding from the surrounding covalent electron structure. Therefore, the resonance frequency of a nucleus is influenced by its chemical environment, which can partially shield the nuclei from the external magnetic field [1]. Chemical shift is the small change in resonance frequency due to magnetic shielding at the nucleus by its neighboring atoms. It allows MRI to differentiate among chemical species; each metabolite is identified by the position of its peak (or chemical shift) on a frequency scale (Fig. 1) [2, 3]. The area under each peak is proportional to the concentration of the metabolite within the selected volume of interest, or voxel. This area can be expressed as an absolute number or as a ratio relative to a known internal or external standard [4].

Proton \(^1H\) spectroscopy is more advantageous than spectroscopy of other nuclei because \(^1H\) has the highest sensitivity, resulting in a higher signal-to-noise ratio and a lower acquisition time. \(^1H\)-NMRS can be obtained on a standard MRI scanner, with additional software to acquire spectra. Specific pulse sequences that suppress water and lipid signals are used [2]. \(^1H\)-NMRS can be performed using single-voxel or multivoxel imaging techniques (Fig. 2A) [5]. In single-voxel

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**Figure 1. \(^1H\)-NMR spectrum from normal brain tissue using a long TE (270 milliseconds).** The MR signal from the metabolites comes from the protons of hydrogen nuclei, but the frequency at which they resonate is influenced by the chemical environment, which can partially shield the nuclei from the external magnetic field. This shift in resonance frequency, called chemical shift, allows MRI to differentiate among chemical species. The \( x \) axis of the spectrum is the chemical shift in parts per million (ppm). The \( y \) axis is the relative signal intensity.

**Figure 2.** A) Single voxel spectroscopy. Note the large volume of interest and the heterogeneity of tissue included in the voxel. B) Multislice multivoxel spectroscopy. Each slice consists of a \( 32 \times 32 \) matrix (1,024 spectra). Each voxel volume is 0.8 cm\(^3\). The smaller voxel size allows measurement of spectra in more homogeneous areas of tissue. C) Output from the multivoxel technique. Data are displayed as low-resolution images. Axial MRI image is from a patient with a high-grade glioma. The spectrum on the right is from a voxel within the tumor as outlined by the box. The position coordinates represent the spatial position in the \( 32 \times 32 \) matrix of voxels, which can be related to the metabolite images on the bottom of the slide.
NMRS, data are acquired from a predefined single brain volume or region of interest at a time. The advantage is that the spectra are acquired in a short period of time; the disadvantage is that the volume of interest is usually large and contains heterogeneous tissue, for example, tumor, normal tissue, and cerebrospinal fluid. Certain tissue characteristics may alter the spectra, such as the presence of blood, calcifications, or cystic lesions. In multivoxel 1H-magnetic resonance spectroscopic imaging (1H-MRSI), spectra from a large number of discreet brain volumes are obtained simultaneously. The results are displayed in a topographic format in two dimensions, with a volume resolution of 1 cm³. Although more time is required to obtain the spectra, the voxel size is smaller and representative of more homogeneous tissue (Fig. 2B). Spectra can be acquired using short (e.g., 10 or 20 milliseconds) or long (e.g., 135 or 270 milliseconds) echo times (TEs) [6]. If long TEs are used, the acquired spectra have fewer metabolite signals, but there is less baseline noise and less signal overlap [7].

**Metabolites Measured by 1H-NMRS**

In the brain, the principal metabolite signals that can be measured by 1H-NMRS at a magnet strength of 1.5 tesla are N-acetyl aspartate (NAA), creatine (Cr), choline (Cho), and lactate (Lac). NAA is a neuronal marker present in normal, functioning neurons. It is thought to be present only in neuronal cell bodies, axons, and dendrites [1, 8, 9]. NAA is absent in areas of radiation necrosis and scar tissue [10]. Cr, including phosphocreatine, is important in energy metabolism and varies little in normal brain tissue. It is frequently used as an internal standard. However, if oxidative phosphorylation and anaerobic glycolysis cannot be maintained to supply ATP, for example, when a tumor outgrows its blood supply, Cr levels will be low [11]. Cr values are also low or absent in areas of radiation [11] or central necrosis. Cho and Cho-containing compounds are constituents of cell membranes and are, therefore, increased in the presence of increased cell density or increased cell turnover, which occurs in brain tumors [1, 12]. Cho levels and Cho:Cr ratios correlate with the degree of Ki-67 (cell proliferation) in gliomas [13, 14]. Lactate, which is a marker of anaerobic metabolism, is not usually detectable in the normal brain, but may be present in some brain tumors or areas of ischemic injury. A lactate peak may be more prominent in malignant than in low-grade gliomas [15]. Lipids also may be detected by NMRS in areas of destruction of myelinated white matter by tumor or necrosis [16]. A lipid peak is also visible in some glioblastomas and is associated with necrosis [17, 18]. Additional metabolites that can be measured by NMRS at short TEs include glutamate and glutamines, gamma amino butyrate (GABA), and myoinositol.

**Characteristics Affecting Spectra**

There are several important technical aspects that can affect 1H-NMRS. Patient motion must be minimized, particularly when using the multivoxel techniques and a voxel size of 1 cm³. Optimal magnetic field homogeneity should be obtained, which is generally accomplished by a process known as shimming. Since air and bone can interfere with spectra acquisition, their signals must be suppressed. Metal implants, such as shunts or orthodontia, will also interfere with obtaining spectra. Several techniques of metabolite quantification have been used in spectroscopy studies. Some investigators make use of an internal standard (creatinine or a reference voxel) and report metabolite values as ratios relative to the reference voxel. Others use an external standard, although this is not always practical as it increases scan time [3]. It is important to note that the technical aspects of NMRS are not standardized and may differ among studies.

There are age-related changes in brain metabolites, primarily in the first few years of life, during the time of rapid brain maturation and myelination. The newborn brain has a high water content and high Cho levels that decline as myelination progresses [19]. NAA, Cr, and glutamates increase in concentration with brain development, while Cho and myoinositol decrease [19, 20]. Adult norms for metabolite levels have been established, but these are not achieved until an age of 3-4 years [3, 4, 12]. Although the major changes in metabolite levels occur in the first few years of life, Kadota et al. [21] also found that, in patients aged 4-88 years, NAA:Cho ratios in gray matter gradually declined from childhood to old age. NAA:Cho ratios in white matter increased during the first decade of life, reached a maximum in the second or third decade, and then began to decline gradually. Since this change is gradual, its significance to an individual patient is not known. Only minor interindividual variations are seen in the spectra of adult cerebral cortices [20]. Differences in metabolite levels are found between gray and white matter, among different lobes of the brain, and between individuals of different genders [21, 22]. Some investigators use age-matched controls to overcome these obstacles. Others use the patient as their own control, and either use the contralateral normal-appearing brain as a control or measure the change in metabolites in a specific area over time.

**Current Uses of 1H-NMRS**

1H-NMRS has been used to study children with head trauma [23], metabolic disorders (e.g., adrenoleukodystrophy
1H-NMRS IN BRAIN TUMORS

1H-NMRS has been used to distinguish active tumors from radiation necrosis or scar tissue, document early treatment response, and distinguish different tumor types or grades. However, data obtained from NMRS cannot yet be used alone because of significant overlap and nonspecificity of the results. For example, it is difficult to distinguish non-neoplastic lesions (such as hamartomas, histiocytic lesions, or dysplastic lesions) from low-grade neoplasms by NMRS [5].

In general, 1H-NMRS of active brain tumors shows diminished or absent NAA, elevated Cho, and sometimes elevated Lac and lipid peaks (Fig. 3). In adult cerebral tumors, NAA:Cho and NAA:Cr ratios are often lower than those of normal brain tissue, while the Cho:Cr ratio is often higher [26]. Fulham et al. [15] performed 64 1H-MRSI scans on 50 adults with brain tumors and reported that NAA levels were lower in all tumors and in areas of radiation necrosis and that Cho levels were higher in most solid brain tumors. In a study using single-voxel NMRS on 75 children with brain neoplasms [16], all initial scans demonstrated lower NAA levels and elevated Cho levels, similar to findings in adults. Taylor et al. [27] found that the total Cho peak pretreatment was the most reliable indicator of malignancy. Others have also demonstrated that, like adult brain tumors, malignant pediatric brain tumors are characterized by an increase in the Cho:NAA ratio and a decrease in the NAA:Cr ratio [28], a general decrease in the NAA and Cr peaks, and an increase in Cho [29].

1H-NMRS has not only been used to identify tumors, but also to noninvasively grade and classify brain tumors. In a study of adult patients with CNS tumors, pattern analysis from 1H-MR was able to more accurately diagnose the tumor type than standard clinical methods [30]. A computer-based neural network that combined MRI, NMRS, and clinical data to predict tumor histology in pediatric patients with posterior fossa tumors were able to correctly identify 95% of tumors from 33 children [31]. However, NMRS results alone cannot be used to classify tumors, as some studies have shown considerable overlap in spectroscopy results among tumors of different histologies [32].

NMRS may be prognostic. In Byrd et al.’s study of 75 children with brain tumors [16], the more aggressive neoplasms had more elevated Cho levels and elevated Lac and lipids, which agrees with the adult data. In a study of 27 pediatric patients with recurrent or progressive brain tumors who were studied by 1H-MRSI [32], the maximum tumor Cho:NAA ratio was predictive of outcome. Patients with a maximum Cho:NAA ratio >4.5 had a median survival time of 22 weeks, and all 13 patients died by 63 weeks. Patients with a maximum Cho:NAA ratio ≤4.5 had a projected survival of more than 50% at 63 weeks (p = 0.0067, log rank test). NAA:Lac ratios <2.0 have also been associated with a poor prognosis [33]. NMRS may be predictive of

Figure 3. A) Spectrum from a patient with a high-grade glioma that progressed after radiation and chemotherapy. Note the elevated Cho and low NAA peaks. A small Lac peak is also present. B) Spectral pattern observed from a patient with presumed radiation necrosis. The scale on the y axis is reduced, exaggerating the baseline noise. Note the absence of any major metabolite signal.
tumor response. In one study, children who responded to radiation or chemotherapy had higher total creatine levels than those who did not [34]. In another study of 11 pediatric patients with low-grade gliomas, the tumors that progressed over a 2-year period had significantly higher baseline Cho:Cr ratios than those that remained stable [7].

NMRS can be used to follow tumors over time, since patients can serve as their own control after obtaining a baseline scan. One study demonstrated that the metabolite pattern of childhood low-grade gliomas with stable disease was consistent over time [27]. Another followed Cho levels of gliomas and reported a >45% increase in Cho in those tumors undergoing malignant degeneration [35]. NMRS may, therefore, be useful to follow patients with neurofibromatosis type 1 who have focal areas of signal intensity or low-grade gliomas over time in order to detect changes in the character of the lesions. NMRS can also detect changes associated with therapy. Several investigators have shown that a decrease in Cho correlates with tumor response [16, 35, 36] and may be seen prior to a change in tumor size by MRI [15]. Metabolites are low or absent, and lipids may be seen in areas of tumor or treatment-related necrosis [15, 16, 37, 38].

Although there have been similar NMRS findings in adult and pediatric brain tumors, there are several important differences noted. Increased Lac did not correlate with tumor grade in several pediatric studies [28, 39]. Juvenile pilocytic astrocytomas, which are histologically grade I lesions, were found to have high Cho, lipid, and Lac levels and low NAA and Cr levels, suggesting an aggressive or hypermetabolic neoplasm [5]. Interestingly, these lesions also showed significant activity on PET scans [40].

**Future Directions**

NMRS has been increasingly incorporated into the battery of tests used to study patients with brain tumors, but its ultimate role has not yet been defined. It can be used as a tool to help characterize CNS lesions noninvasively in conjunction with standard MRI and additional clinical information. Although contrast-enhanced areas on MRI scans are thought to represent breakdown of the blood-brain barrier in areas of tumor, these areas may include tumor, edema, and necrosis, while nonenhancing tissue may contain infiltrating tumor cells. 1H-MRSI can be used to identify areas with the highest Cho levels, providing an optimal target for biopsy and improving the diagnostic yield [41, 42]. NMRS may be useful to monitor low-grade gliomas, including those associated with neurofibromatosis type 1, in a longitudinal fashion for changes that may be associated with tumor activity. 1H-MRSI may also be used to track the effects of tumor and treatment on normal-appearing areas of the brain. We are currently using 1H-MRSI to study patients with neurotoxicities to explore the relationship between treatment and the development of neuropsychological or structural manifestations. Further development of NMRS and neural networks may make 1H-NMRS more specific, so tumor histology and grade can be determined noninvasively and response to therapy can be predicted and determined prior to seeing structural changes on MRI.

Several investigators are working on overcoming limitations of current clinical NMRS. One limitation is insufficient coverage of the brain by single-voxel or multivoxel techniques and the requirement for volume preselection [43]. Three-dimensional MRSI is one technique that is becoming more widely used for clinical studies of brain tumors. Using this technique, spectra are obtained throughout the volume of interest, including brain tumors and adjacent brain tissue. In addition, the spatial positions of the spectra can be selected retrospectively, allowing for retrospective comparisons [44]. Greater magnet strength may also improve 1H-NMRS. An initial study by *Gonen et al.* compared 1H-NMRS at 1.5 tesla with 3.0 tesla and found more reliable peak-area estimations and shorter acquisition times in four adult participants, which may allow for shorter scanning times and a better quantification of metabolites [45].

The ultimate role of NMRS in the evaluation and treatment of pediatric patients with brain tumors is still being defined but will nearly be significant. With higher resolutions, lower acquisition times, higher specificity, and more standardized methods of data acquisition and analysis, NMRS will be a useful parameter in noninvasively evaluating brain tumors in the pediatric population.

**References**


**ADDITIONAL READING**

