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A Review of the Detection of Amino Acids in Tumor Tissue with ^{1}H Magnetic Resonance Spectroscopy *in vivo*

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Abstract

The biochemical components of tumor tissue are generally different from normal tissue. A variety of techniques, including of magnetic resonance (MR) are used to study metabolic compounds and their associated biochemical pathways in tumor tissue *in vivo*. This review provides an overview of applications of MR spectroscopy (MRS) to the identification of biochemical characteristics, e.g. amino acids present during tumor growth. MRS is a technology that allows spatial localization and structural studies of amino acids and is gaining in use as a diagnostic tool. This review is intended to provide information about potential applications of MRS to non-invasive probing of the underlying amino acid biochemistry in tumors. Articles discussed in this review are classified into the following categories: hardware for ^{1}H MRS, acquisition methods, software, and ^{1}H MRS of amino acids in tumor tissue *in vivo*.

Keywords: Amino acids; Metabolites; Cancer; Magnetic resonance spectroscopy

Abbreviations: MR: Magnetic Resonance; MRS: Magnetic Resonance Spectroscopy; NMR: Nuclear Magnetic Resonance; MRI: Magnetic Resonance Imaging

Introduction

Methods allowing quantification of cancer metabolites *in vivo* based on Magnetic Resonance (MR) spectral profiles have been developed and are currently being considered for clinical routines. MR Spectroscopy (MRS) is noninvasive, nondestructive and enables serial measurements of tissue. MRS spectra can be obtained from the entire sample or from specific regions of interest (localized MRS). MRS is the *in vivo* application of (NMR) spectroscopy, and is implemented on (MRI) clinical or experimental scanners. Identification and quantification of amino acids in cells or organs in a totally non-invasive manner are the unique advantages of MRS. The spectra are a plot of signal intensity (roughly proportional to metabolite concentration) verses chemical shift. MRS can be applied to the study of cancerous tissue *in vivo* and *in vitro* and allows identification and quantification of amino acids in tumor tissue. The spectroscopic profile of tumor tissue is currently used for diagnosis [1], assessment of therapy response [2], and therapeutic monitoring [3,4]. Potentially, MRS can identify key biochemical changes in tumor tissue. In metabolic profiles of tumors, amino acids are participating in various metabolic pathways and their presence and concentrations are often dependent upon pathological conditions. Amino acids play a crucial role in tissue metabolism [5], therefore this review focuses on applications of 1H MRS to study amino acids in tumor *in vivo*.

Tumor cell metabolism has been use in clinical diagnosis for many years. Over the last three decades, X-ray computed tomography (CT) [6], positron emission tomography (PET) [7], PET combined with magnetic resonance imaging (MRI) [8], a combination of PET/CT [9] PET/CT/MRI [10] and fluorescence-mediated molecular tomography (FMT) [11] have been commonly utilized for visualization of the distribution and therapeutic effects of drugs in cancer tissue *in vivo*. While imaging techniques provide information about the integrated function of multiple transporters and enzymes involved in a metabolic processes, spectroscopic techniques can detect metabolic biomarkers and track the expression of one molecule. ^{1}H MRS has been developed to enable identification and quantification of metabolites *in vivo*. An advantage of ^{1}H MRS is that no imaging probe needs to be injected, since endogenous levels of metabolites are measured [12]. Currently, ^{1}H MRS is used mostly in clinical practice for detailed analysis of brain tumors [13,14]. In contrast to imaging techniques, the fundamental goal in ^{1}H MRS *in vivo* technique is to compare levels of metabolites in pathologic tissue with levels in normal tissue and detect increased activity of these metabolic processes.

Hardware for ^{1}H MRS

In vivo MRS can be used complementary to clinical MRI, providing quantitative analysis of the tumor tissue without the use of radioactive tracers. MRS spectra of amino acids in the human body can be obtained using the ^{1}H nucleus [15]. The advantages of using ^{1}H MRS *in vivo* are the high sensitivity of the ^{1}H nucleus, the near 100% abundance of this isotope, and the presence of this nucleus in amino acids and most metabolites. ^{1}H MRS can be performed after acquisition of diagnostic MR images and most clinical MRS measurements are performed using clinical MR imaging systems (with field strengths of 1.5T-3.0T or higher) with a suitable radiofrequency (RF) coil. Signal intensity depends upon field strength. When using 3.0T MR a scanner, the detected signal is nearly doubled from that obtained in 1.5T MR. Theoretically, the signal increases proportionally to the square of the static field strength whereas the noise increases linearly. This implies that the signal-to-noise ratio (SNR) of a 3.0T system is higher than at

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1.5T. In practice, the actual improvement is only in the 30-60% range due to susceptibility effects [16,17]. Using ^{1}H MRS, smaller metabolite concentrations can be detected in samples of small volume with higher field strengths and improved SNR. Field strengths as high as 7, 9.4 and 11.7 are now in use for clinical MR [18].

In vivo ^{1}H MRS requires selection of a volume of interest (VOI). The magnet creates magnetic field gradients in three directions (G_x, G_y, and G_z) across the selected volume. For the improvement of SNR, a volume coil is used close to the measured object for an increase in signal [19]. A surface coil yields a higher SNR than a volume coil, but the sensitive region is much smaller. ^{1}H MRS cannot be used to image all tumors. For example, breathing and peristalsis can lead to severe magnetic susceptibility difficulties and affect the local magnetic field homogeneity which results in line broadening and loss of signal. Tumors located in the lungs, stomach, and gastrointestinal tract are difficult to analyze with MRS [20].

^{1}H MRS Acquisition

In order to acquire a MRS spectrum, it is necessary to localize the MR signal acquired to a particular region in the body, either by exciting signals only within a specific volume (or voxel), known as single voxel MRS or by performing a hybrid MRS and imaging experiment, known as magnetic resonance spectroscopic imaging (MRSI, also referred to as chemical shift imaging, CSI). The first method is called single voxel spectroscopy (SVS) and is based on volume selection in tissue [21]. Magnetic resonance spectroscopic imaging (MRSI), originally introduced as chemical shift imaging (CSI) [22] is also used. In MRSI, to collect spectra, ^{1}H MRS is followed by ^{1}H MRI. ^{1}H MRI images provide important information about geometry (size and volume). The area of samples from which spectra will be collected is selected from MR images. In ^{1}H MRS, techniques such as chemical shift-selective water suppression are used [23]. The acquisition of ^{1}H MRSI faces two major challenges: removal of the water signal and avoiding contributions from fat which contribute to susceptibility effects that decrease the signal of the target. Thus, the size of the excited region and its proximity to structures which produce variations in susceptibility are important factors for MRSI [24]. The reported diagnostic accuracy of MRSI is higher than that of choline PET/CT, with a sensitivity of 70%-90% and a specificity of about 80% [25,26]. Diagnostic accuracy can be further increased by combining MRSI with dynamic contrast-enhanced MRI [25].

The predominant SVS techniques used for acquisition of ^{1}H MRS, mostly applied to the brain, are known as Point Resolved Spectroscopy (PRESS) [27]. In the PRESS sequence, the spectrum is acquired using one 90° pulse followed by two 180° pulses. The first 180° pulse is applied after a time TE1/2 from the first pulse (90°) and the second 180° pulse is applied after a time TE1/2+TE. The signal occurs after a time 2TE. Spoiler gradients dephase the nuclei outside the VOI and reduce their signal [28].

Stimulated Echo Acquisition Mode (STEAM) is also used as a SVS technique. In this sequence all three pulses applied are 90°. After a time TE1/2 from the first pulse, a second 90° pulse is applied. The time elapsed between the second and the third is conventionally called the "mixing time" (MT) and is shorter than TE 1/2. Both PRESS and STEAM use three frequencies selective RF-pulses combined with linear magnetic field gradients to select three perpendicular slices, their intersection defining the VOI. Thus, the total time for the STEAM technique is shorter than PRESS. Spoiler gradients are also needed to reduce signal from regions outside the VOI [29]. The main difference between STEAM and PRESS is the acquisition of a stimulated echo obtained by three 90° pulses in STEAM and a second echo obtained by a 90° excitation pulse followed by two 180° refocusing pulses in PRESS. PRESS consists of one 90° excitation and two 180° refocusing RF-pulses. STEAM consists of three 90° excitation pulses and the signal from VOI is acquired as a stimulated echo [30,31]. If we can assume that equal volumes of tissue are observed using the same acquisition parameters, then PRESS has approximately a factor of 2 times larger SNR than STEAM because the stimulated echo in STEAM is formed from only half the available equilibrium magnetization. STEAM is somewhat more convenient because it is usually easier to produce a 90° pulse with a sharp slice profile than a 180°. High bandwidth 90° excitation pulses are also more available than high bandwidth 180° pulses, and require less power. The better slice profiles usually obtained with the STEAM sequence, the SNR improvement with PRESS may be less than a factor of 2 [16].

The quantitation of metabolite peaks in individual spectra depends upon the magnitude of residual water or lipid peaks, as well as the existence of any broad resonances which distort absolute peak intensities. Typical echo times for STEAM MRSI are 18-30 ms, although it is possible to obtain data with TE of less than 10 ms [32]. The complexity of the observable peaks and residual protein or lipid signals are significantly reduced in spectra acquired at longer echo times [32]. PRESS MRSI with its intrinsically higher sensitivity is more commonly used at echo times of 135, 144, 270 or 280 ms, where the relatively short T2 of lipids allows for the detection of lactate. The estimation of peak intensities from both STEAM and PRESS MRSI data requires the removal of residual baseline components, correction for spatially dependent variations in chemical shift and correction for both constant and spatially dependent phase shifts. In SVS, typical voxel sizes used in clinical studies range from 4 to 8 cm3 [32]. The quality of single-voxel spectra is highly dependent on voxel positioning, since the inclusion of surrounding normal tissues will contaminate the tumor spectra. For similar reasons, single-voxel spectroscopy is limited for assessment of heterogeneous tumors. In MRSI, a matrix of spectra is acquired either over a plane (2D MRSI) or a volume (3D MRSI). The voxel size in clinical 3D MRSI is 1 cm^3 or more when using a standard 1.5 T clinical MRI system and typical matrix sizes are 8×8×8 voxels [32]. However, the quality of the spectra can be poorer than in single-voxel spectroscopy because of greater magnetic field in homogeneities in the sampled volume, particularly with regard to achieving adequate water suppression.

Software

Using ^{1}H MRS, it is possible to study the structure, localization, and quantity of amino acids in tumors if software is employed to isolate signals. In ^{1}H MRS the splitting due to coupling result in signals that have lower peak intensity and a broader footprint along the chemical shift axis. Also, due to many compounds present in an *in vivo* sample, many signals can overlap. One common feature of the MR spectrum is the appearance of multiplets, signals associated with a single hydrogen environment that are split into a number of sub-peaks. This phenomenon arises because of spin–spin coupling, or J-coupling, whereby the field experienced by a spin is affected by adjacent spins within the molecule [33]. Software packages such as LC Model [34] or jMURI [35], are used to provide the concentration of metabolites. The jMRUI software is based on a Mat lab/Java programming. In the LC Model, deconvolution of spectra is employed using a basis set of reference spectra [36].

^{1}H MRS of Amino Acids

The metabolic pathways of amino acids are known as a useful source of information in diagnostic medicine [37-42]. Amino acids are simple, monomeric subunits that make up the structure of thousands of different proteins. They are composed of a carboxyl group and an amino group bound to an alpha, beta or gamma carbon. The standard 21 biological amino acids are classified into two groups: essential (the body cannot make these amino acids, they must come from dietary sources) and non-essential (the body can synthesize these amino acids). The first group (essential) consists of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryphtophan and valine. The non-essential amino acids are alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, proline, serine and tyrosine. Amino acid profiles are obtained in medical practice from blood plasma in the diagnosis of prostate cancer [37], breast cancer [38,39], and pancreatic cancer [40,41]. Blood amino acid profiles *in vitro* have been accessed with high resolution micro SPECT/MRI. Hydroxylation of phenylalanine to tyrosine is a physiologically important example where amino acids in brain tissue in vivo were studied by ^{1}H MRS [42]. The range of the observed brain phenylalanine concentrations were 0.20-0.76 mmol/L when compared to brain plasma concentrations of 0.47-2.24 mmol/L. These studies confirmed that metabolites or amino acid concentration measured in tissue can be lower than blood due to consumption of amino acids in varied metabolic pathways [42]. Below, Table 1 lists the amino acid content for 13 amino acids detected in normal and malignant tissue using gas chromatography [43]. Amino acids concentrations were examined in the malignant tissue of patients diagnosed with squamous cell carcinoma of the head and neck who were undergoing tumor resection surgery (Table 1).

The tumor tissue possessed higher concentrations of threonine, valine, serine, aspartate, glutamate and glycine compared with the surrounding normal tissues. Higher concentrations of amino acids may be indicators of dynamic processes of biosynthesis rate, or that tumor cells utilize more amino acids as sources of energy [43-45]. Cells take up amino acids by a set of transport molecules. Amino acid transport systems have been functionally characterized by affinity for specific amino acids, sodium dependency, and sensitivity to inhibitors before the actual transporter proteins had been identified. Based on functional characteristics of tissue, 13 major amino acid transport systems can be differentiated [45]. The concentrations of amino acids in normal tissue and cancer tissue bring the low mM detection limits into context. The ^{1}H MRS limit of detection is around 1 mM, however, many of the endogenously occurring ^{1}H metabolites are present in cellular concentrations in the mM range. ^{1}H MRS detects a number of metabolites present in relatively low concentrations (<10 mM), when water and fat suppression techniques are used. Spatial resolution can be improved at longer acquisition times and with increasing magnetic field strength. Changes in viscosity, in homogeneity of the magnetic field and many exchange processes can also affect the line shape.

Nb.	Amino Acid	Malignant Tissue	Normal Tissue
1	Glutamate	6.20 ± 2.69	1.93 ± 0.71
2	Glycine	5.55 ± 1.59	2.55 ± 0.68
3	Alanine	4.19 ± 0.52	3.20 ± 0.94
4	Aspartate	2.27 ± 1.41	0.55 ± 0.28
5	Serine	1.43 ± 0.48	0.87 ± 0.32
6	Proline	1.43 ± 0.48	0.92 ± 0.46
7	Leucine	1.34 ± 0.29	0.92 ± 0.46
8	Lysine	1.23 ± 0.53	0.85 ± 0.36
9	Threonine	0.90 ± 0.24	0.50 ± 0.29
10	Isoleucine	0.57 ± 0.10	0.36 ± 0.15
11	Phenylalanine	0.50 ± 0.15	0.39 ± 0.22
12	Tyrosine	0.32 ± 0.09	0.21 ± 0.09

Table 1: The amino acids content (μmol/g) in malignant and normal tissues from patients with squamous cell carcinoma of the larynx.

Currently there are few published examples of the use of ^{1}H MRS for the detection and identification of amino acids in tumor tissue *in vivo*. In particular, studies of ^{1}H MRS detection of amino acids in tumor tissue have focused on glutamine, glycine, and alanine. Glutamine is used by the cell for both bioenergetics and biosynthetic needs. Glutamine can be used as an amino acid for protein synthesis, as a carbon source, or as the primary nitrogen donor for multiple essential biosynthetic reactions in the cell. Once taken up by the cell, much of the glutamine is converted to glutamate by mitochondrial glutaminase, an enzyme whose levels are often unregulated in tumors and tumor lines [46]. Glutamine metabolism is also unregulated in tumor tissue glutaminase is required for the proliferation of both the human B lymphoid tumor line and PC3 prostate cancer cells, suggesting that targeting this pathway is a viable therapeutic option. Both glutamine and glutamate contribute to anabolic metabolism; glutamine supplies nitrogen for nucleotide and hexosamine synthesis while glutamate is the nitrogen donor for the synthesis of many non-essential amino acids. It is now well appreciated that many of the signaling pathways that promote oncogenesis also reprogram glutamine metabolism, and in many cells the dependence on glutamine is absolute, a condition termed glutamine addiction [47]. Glutamine is a precursor of glutamate. Glutamate is involved in neurotransmission. Gamma-aminobutyricacid (GABA), also present but in lower concentrations during normal physiological conditions may overlap with the glutamin and glutamate resonance at 1.5T field strength. The main advantage of the higher field strength is in the increased spectral resolution which allows for improved delineation of resonances such as glutamine and glutamate [48-50]. Numerous peaks are observed, the principal peaks seen in brain tumor ^{1}H MRS at 1.5T include branch chain amino acids (0.9-1.0 ppm), lipid (0.9-1.5 ppm), lactate (1.3 ppm), alanine (1.5 ppm), n-acetyl aspartate (NAA; 2.0 ppm), choline (3.2 ppm), creatine (3.0 ppm and 3.9 ppm), and myoinositol (3.6 ppm). For this reason, amino acids, lactate, and lipid peaks overlap [51]. N-acetyl-aspartate (NAA) produces a large resonance in a H_2O suppressed 1H spectrum. The peak may contain up to 20% of contributions from aspartyl-glutamate (NAAG). NAA is generally associated with neurons and axons in the adult brain. A decrease in levels of NAA indicates loss or damage to neuronal tissue, which results from many types of brain pathology. Its presence under normal conditions indicates neuronal and axonal integrity [52]. NAA is found in high concentration (~10 μmol/g) exclusively in the nervous system [53]. Interestingly, a ^{1}H MRS study at 11.7 T show that the NAA synthesis rate in isoflurane-anesthetized rats was 0.19 ± 0.02 mol/g/h (mean ± standard deviation, n=12) [54]. ^{1}H MRS alone or in conjunction with ^{1}H MRI, or some other imaging technique, can be used to detect significantly abnormal concentrations of tumor tissue metabolites. ^{1}H MRS may also be helpful in the differentiation of high grade from low grade brain tumors, and perhaps in separating recurrent brain neoplasm from radiation injury [55-58]. Biochemical monitoring of neuroblastoma with ^{1}HMRS could enable individual therapy as well as provide early pharmacodynamic evaluation of novel anticancer therapeutics [59].

Alanine and glycine have been detected using localized single voxel 1H MRS in the intracranial hemangiopericytomas tumor *in vivo* [60]. To confirm the *in vivo* findings researchers conducted a molecular

study using *ex vivo* high resolution magic angle spinning MRS. Recently, glycine has been detected by optimized PRESS sequence in brain tumors at 3T. The glycine concentration was estimated at 0.6 ± 0.1 mM and the study revealed that a subset of human gliomas contains elevated glycine levels 1.5-8 fold relative to normal tissue [61]. In addition to commonly measured metabolites such as total creatine, NAA, choline, glutamine, glutamate, lactate, concentrations of alanine, aspartate, glutathione, γ-aminobutric acid, phosphorylethanolamine, and taurine were quantified using *in vivo* ^{1}H NMR spectra from the human brain at 7T [62]. In the brain of patient with amyloidosis, the intensities of amino acids were relatively increased in white matter, whereas the concentration of choline, creatine, and NAA were reduced when measured at 1.5T [63]. An increase of the amino acids resonances in the spectral area between 2.1 and 2.5 ppm might be directly related to the increased amyloid content caused by the disease. In the brain spectrum peaks between 3.7 and 3.8 ppm overlap with resonances of myo-inositol, glucose, and creatine [63]. It has been demonstrated that ^{1}HMRS has great potential to aid cancer management, e.g. in the detection and localization or the assessment of its aggressiveness.

Conclusion

^{1}H MRS is becoming an increasingly important diagnostic tool for detection of abnormal levels of amino acids in tumor tissue. To date, ^{1}H MRS has been used to determine abnormal concentrations of glutamine, glycine, and alanine. Incorporation of ^{1}H MRS in clinical practice can enhance identification of normal and cancer tissue components including amino acids. ^{1}H MRS is a unique technique that can be correlated with imaging and other clinical techniques for the interpretation and data correlation. ^{1}H MRS offers non-invasive monitoring of normal and cancerous tissue as well as response to treatment.

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