Diagnosing Brain Tumors by High Field ^{1}H NMR

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Abstract: *Conventional NMR imaging has increased our ability to detect brain tumors. However, it has not enhanced at the same degree the ability to diagnose tumor type. Hydrogen nuclear magnetic resonance spectroscopy (^{1}H NMR) performed in low magnetic fields provides a non-invasive method of examining a wide variety of metabolites in the human brain in vivo. The aim of this study is to determine the characteristic in vitro NMR spectra, using high magnetic field, of various brain tumor extracts and to provide a better interpretation of spectra obtained by in vivo NMR. Tissues samples were obtained from 47 patients with CNS (Central Nervous System) tumors. From the PCA (Principal Components Analysis) analysis, we observed a trend toward association between neuroepithelial and non-neuroepithelial tumors with creatine levels. Moreover, considering only astrocytomas, the aggressive tumor increases directly with the glycine/inositol ratio and inversely with creatine levels. These results reflect the high catabolism and osmotic imbalance in tumor tissue. Thus, in vitro NMR spectra contributes to understanding tumor metabolism and can be used as a tool in the development of in vivo NMR methods by the adjustment of parameters to improve non-invasive brain tumor diagnoses.*

Conventional NMR imaging has increased our ability to detect brain tumors. However, it has not enhanced at the same degree the ability to diagnose tumor type. Hydrogen nuclear magnetic resonance spectroscopy (^{1}H NMR) performed in low magnetic fields provides a non-invasive method of examining a wide variety of metabolites in the human brain *in vivo*. Interpretation of *in vivo* acquired spectra can contribute to tumor diagnosis. This requires good correlation among tumor types, chemical compositions (spectral characterization and abundance of metabolites), and ^{1}H NMR spectral features. To better establish the chemical compositions of specific tumors from biopsy samples, tissue extracts should be first evaluated by high field ^{1}H NMR.Therefore, this research focuses on the spectral characterization of brain tumor metabolites by high resolution ^{1}H NMR to build a data set for *in vivo* NMR spectra and imaging diagnosis.

We used 50 brain tissue samples, of which 47 were from brain tumors and 3 from patients with intractable temporal lobe epilepsy (control samples). The biopsy samples were frozen in liquid nitrogen right after surgical removal and then stored at -80 °C. Frozen tissues were weighed, crushed under liquid nitrogen and then extracted with aqueous perchloric acid, followed by standard neutralization procedures.[1] The solutions were lyophilized and dissolved in D_2O containing 0.625 mMol.L^{-1} sodium d_4-trimethylsilylpropionate. The latter had a twofold function: internal reference and concentration standard. The ^{1}H NMR spectral acquisition was performed in a Varian INOVA-500 spectrometer (B_0 = 11,7 T)

operating at 499,885 MHz for ^{1}H. The intense residual water (HDO) signal was suppressed using selective pulse excitation followed by pulsed field gradient (wet1d). The data matrix with the signals amplitudes and frequencies shifts was submitted to PCA analysis.

Some spectral features were identified as diagnostic and allowed the classification of some tumor groups by comparison with their previous diagnoses and clinical evolution. Creatine methyl signal (δ 3.05, s) was responsible for a differenciation between neurepithelial and non-neuroepithelial tumors. The latter group of tumors was characterized by low levels of creatine when compared to those of the neuroepithelial group. Furthermore, among the neurepithelial class, we observed that the relationship between tumor aggressiveness and thecreatine levels were inversely proportional.

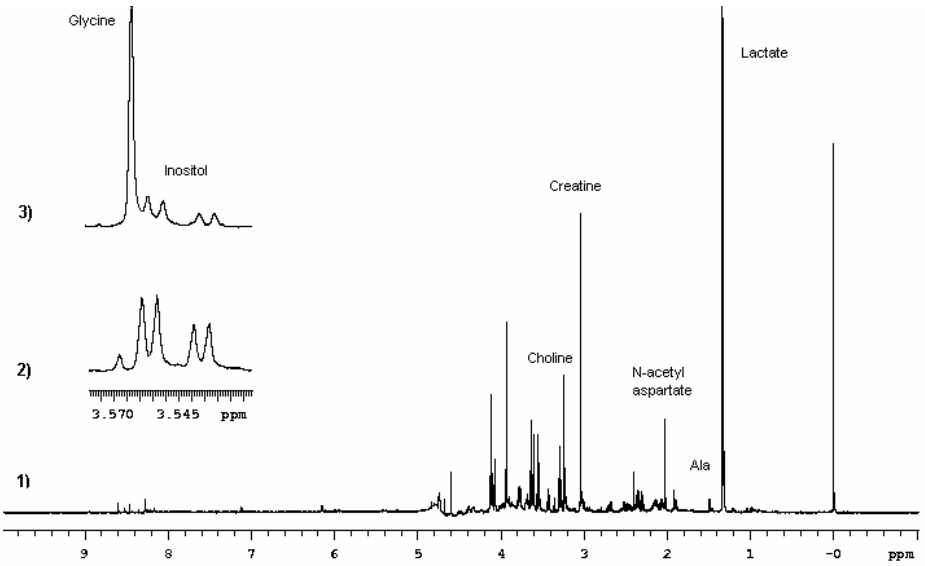

Figure 1. Full spectrum of a typical sample (499.886MHz; D_2O) (1); amplification of the 3.50 – 3.60 region of a low grade astrocytoma sample (2); amplification of the same region of a high-grade astrocytoma sample (3).

The spectral region ranging from δ 3.50 to δ 3.65 made possible the aggressiveness grading of astrocytomas tumor type. Thus, high glycine/inositol ratios were characteristic of high aggressiveness while low ratios were associated to non-aggressive tumors.

Creatine is indicative of the celular metabolic degree. The reduction in creatine may correspond to the exhaustion of energy reserves from rapid cell proliferation and ischemia, both related to tumor aggressiveness. Furthermore, it is common knowledge that non-neuroepithelial cells show low creatine levels.[2]

An increase in glycine, an inhibitory neurotransmitter, has been associated with increasing malignancy in extracts of astrocytomas.[3] It is possible that this amino

acid is involved in the osmotic stress to which the tumor cells are submitted.[4] Inositol represents an osmolyte of plasma membranes that is elevated as a result of neuron destruction. It is missing in areas of necrosis, common in aggressive tumors and could explain the low inositol/glycine ratios.[5] Finally, the results so far obtained indicate that high resolution ^{1}H NMR are consistent with previous evidence found in the literature for *in vivo* NMR[6]. Our work also provides detailed spectral information about tumor cells composition, which can be used to optimize *in vivo* NMR experiments by parameters adjustment.

References

1. A. C. Petroff, D. D. Spencer, J. R. Alger, J. W. Prichard, *Neurology* **39** (1989) 1197.
2. A. A. Tzika, D. Zurakowski, C. Young, T.Poussaint, *Neuroradioogy* **43** (2001) 169.
3. J. Peeling, G. Sutherland, *Magnetic Resonance in Medicine* **24** (1992) 123.
4. K. Tuz, B. Ordaz, L. Vaca, O. Quesada, H. Pasantes-Morales, J. *Neurochemistry* **79** (2001) 143.
5. J. F. Norfray, T. Tomita, S.E. Byrd, B.D. Ross, P.A. Berger, R.S. Miller, *American Journal of Roentgentology* **173** (1999) 119.
6. I. Mader, W. Roser, G. Hagberg, M. Schneider, R. Sauter, J. Seelig, E. W. Radue, W. Steinbrich, *MAGMA* **4** (1996) 139.