

Towards accurate quantification of metabolites and macromolecules in HR-MAS spectra of brain tumor biopsies using LCMoDel

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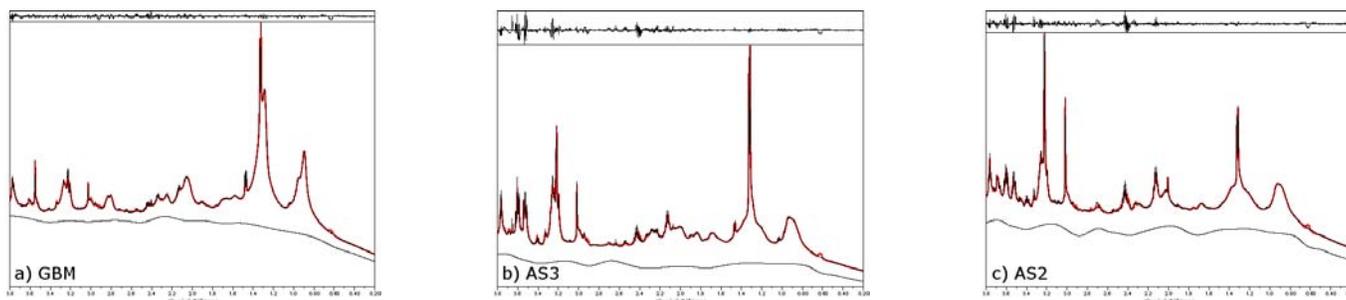
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Introduction

In vivo ¹H MRS is an attractive, non-invasive alternative to biopsy for the diagnosis of brain tumors. Lipid signals have been associated with malignant transformation [1], hypoxia and necrosis, but our understanding of the functional significance of the lipid and metabolite patterns *in vivo* is not entirely clear. High resolution magic angle spinning (HR-MAS) MRS provides well resolved spectra from intact biopsy tissue, resulting in more detailed biochemical profiles that can be correlated with the histological characteristics of the same tumor biopsy sample. Nevertheless, quantifying HR-MAS biopsy spectra faces the same problem as *in vivo* spectra, with many overlapping signals making accurate evaluation of the individual metabolite and lipid/macromolecule peaks difficult. LCMoDel [2] addresses the problem of peak overlap by fitting data to a linear combination of complete spectra from pure metabolite solutions, therefore utilising maximum prior information. In this study we describe creating an LCMoDel basis set that also incorporates the appropriate lipid and macromolecule peaks into the analysis of HR-MAS spectra of brain tumor biopsies.

Methods

All measurements were performed on a 600MHz Bruker Avance spectrometer using an HR-MAS probe spun at 5000Hz and 4°C. The low temperature was necessary to prevent enzymatic degradation when using biopsy material. Each metabolite solution (Alanine, Aspartate, Choline, Creatine, GABA, Glucose, Glutamine, Glutamate, Glutathione, Glycine, Lactate, Leucine, Lysine, *myo*-Inositol, NAA, Phosphoethanolamine, Taurine and Valine) was prepared in a phosphate-buffered solution (except Glycerophosphocholine and Phosphocholine), containing 50% D₂O, at pH7.2. Sodium formate and TSP were added for scaling and referencing. 35µl of each metabolite solution was pipetted into a 50µl insert and placed in a 4mm zirconium rotor. Spectra were acquired using a presat pulse sequence with a repetition time (TR) of ~30s, chosen to ensure complete relaxation of the *in vitro* basis spectra. For the biopsies [4 glioblastoma (GBM), 2 astrocytoma grade III (AS3) and 2 astrocytoma grade II (AS2)], 10-15mg of tissue were placed in 50µl inserts and the remaining space filled with D₂O. Presat and water spectra were collected from 6 of the biopsy samples (2 GBM, 2 AS3 and 2 AS2) using a TR of ~8s. The remaining 2 GBM samples were used to determine the contributions from lipids (Lip) and macromolecules (MM). Metabolite-nulled spectra were acquired by applying an inversion pulse (TI=650ms) prior to a Carr-Purcell-Meibom-Gill (CPMG) sequence over a range of TEs (5-90ms). The resulting spectra were analysed using MestReC's line fitting module (MESTRELAB Research, Spain) to determine the chemical shifts and linewidths of the Lip and MM peaks contributing to the tumor spectra. Multiple TEs were used to ensure consistency of the peaks being fitted. A total of 18 Lip/MM peaks were added to the basis set at δ3.2 (2), δ3.0, δ2.8 (2), δ2.25, δ2.05 (2), δ1.7, δ1.5, δ1.3 (5) and δ0.9 (3). The 6 biopsy samples were analysed using LCMoDel and quantified using the biopsy water signal as reference, assuming a water concentration of 44mM [3].



Results

The figure above shows the LCMoDel fits (red) to 3 different grades of glioma. Table 1 shows a comparison between the *in vivo* ¹H MRS and the biopsy analysis of the same grade 2 astrocytoma.

Table 1	Cr (mM)	mI (mM)	tCho (mM)	mI/tCho	tCho/Cr
<i>In vivo</i>	3.057	5.002	3.481	1.437	1.139
HR-MAS	2.703	2.937	2.578	1.139	0.954

Discussion

Incorporating the Lip/MM peaks into the analysis (determined from GBM biopsies) works well in the GBM and AS3 spectra as shown above; however, in the AS2 spectra the baseline was less smooth suggesting additional resonances are needed in the set of basis spectra that are most likely from MMs. We have aimed at including all biochemicals expected to contribute towards glioma spectra, but there are still unaccounted for residuals [top panels in the Figure above] from the LCMoDel analyses. These residuals consist of equal amounts of positive and negative-going sharp peaks in the region of multiplets such as Glx and mI and suggest there is a slight mismatch in the resonance patterns of the basis and *ex vivo* spectra. Most likely this is the result either of a difference in pH between solution and biopsy, or of ionic interactions of metabolites with proteins, which cause small relative peak shifts. For the tumor, for which we had both *in vivo* and *ex vivo* spectra, the correlation is very good, particularly for the peak ratios, which are not dependent on errors in water scaling.

Despite the slight mismatch for some multiplets, the overall fit provided by LCMoDel for HR-MAS data is very good. Further work is needed to provide model solutions of complex spectra to more accurately reflect their coupling patterns in the *ex vivo* environment where interactions with proteins is possible.

References

[1] Opstad KS *et al. Proc ESMRMB*, **22**, 327; 2005. [2] Provencher SW. *Magn Reson Med* **30**; 672-92; 1993. [3] Cheng LL *et al. Cancer Research* **58**; 1825-32; 1998

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