In vivo 1H MRS and ex vivo HR-MAS metabolic profile in diffuse liver pathologies

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Introduction: Nowadays, the only clinically available method for the evaluation of the degree of liver fibrosis is the histological inspection of liver biopsies which produces large variability inter and intra observer. In general, there is a direct correlation between the degree of progression of the fibrosis and the evolution of liver illness. The precise determination of biochemical and metabolic profiles in intact tissue promises to extend the possibilities of NMR as a medical diagnosis tool. The most powerful and least invasive technique for studying metabolic profiles 'in vivo' is ^{1}H MRS. ^{1}H-MRS in vivo is a non-invasive technique that provides information of microscopic biochemical changes that cannot be detected by conventional MRI. Combined with HR-MAS studies in intact liver tissue may constitute a powerful tool for diagnosis in clinical applications. In this work, we present a combined study of metabolic profiles on chronic diffuse liver diseases by MR, both 'in vivo' and 'ex vivo'.

Subjects and Methods:

In vivo data acquisition: in vivo liver MRS studies were performed in 69 patients with clinic diagnosis of chronic hepatitis, cirrhosis and steatosis, and in 3 healthy livers. All MRS spectra were recorded in a clinical MR unit operating at 1.5T. The protocol of MRS data acquisition included SV on liver (91.1cm^3, TE 136 ms). All spectra were transformed and analyzed with jMRUI program. The assignments of different resonances in vivo were checked with help of ex vivo HR-MAS spectra that make possible the identification of weak and overlapped signals. 35 patients have both in vivo and ex vivo measurements.

Ex vivo data acquisition: the whole HRMAS study was performed in 85 samples (76 of them were obtained by needle biopsy extraction, 3 of them during abdominal surgery and 5 during autopsies) at 4 C and 4 KHz spinning rate in a 500 MHz spectrometer. All samples were placed in 12 μL rotors. Approximately sample weight ranged from 0.8 to 10 mg. Three different types of spectral editing were obtained by recording 1D ^{1}H pre-saturation, 1D ^{1}H NOESY and 1D ^{1}H CPMG experiments. Transversal relaxation times (T_2) were measured in a 2D-CPMG experiment. 2D ^{1}H TOCSY and 2D 13C-HSQC experiments were also recorded on several sample for assignment and quantification purposes. Degradation of the sample was monitored through interleaved 1D spectrum.

Results: In vivo liver spectra show in general seven main groups of resonances originating from different types of lipids, compounds of Choline, TMAO, Glucose and Glycogen (Figure 1). The signals were overlapped in some cases but HR-MAS spectra helped in the assignation of resonances. The analysis of the spectra show significant differences between spectra of hepatic and cirrhotic patients in the relative intensity of water signal and in the ratio of intensities Lip I ($CH=CHCH_2$)/H_2O. Additionally, we observed some differences in other in vivo intensity ratios of metabolites with respect to water.

The quality, resolution and sensibility of the HR-MAS spectra were adequate in most of cases for quantification of different spectroscopic parameters (Figure 2). The assignments of 80 resonances lead to the identification of 22 metabolites on the spectra [1]. Spectroscopic parameters have been measured in metabolite peaks in the tissue spectra, with special emphasis in lipids signals and those metabolites important in hepatic metabolism. The parameters studied included T2 (figure 3), line shape, peak area and peak height, among others. In the statistical analysis, we detected some preliminary correlations between different spectroscopic parameters in the metabolite peaks and the different pathologies. In general, the most discriminative intensity ratios include lipids to differentiate between groups of patients. Our results may establish the basis for more robust, objective and reliable analysis of liver fibrosis based on needle biopsies. The method proposed here minimizes the operator dependence and may establish the basis to develop methodology in the in vivo human liver MRS studies.

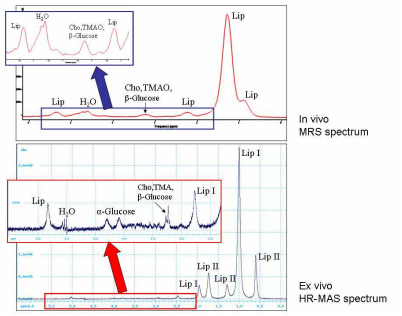

Figure 1: In vivo and ex vivo spectrum in a liver of patient with chronic hepatitis. HR-MAS spectrum helped in the assignation of in vivo resonances.

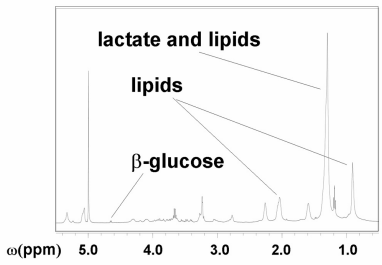

Figure 2. NMR spectra showed narrow line widths and adequate signal-to-noise ratios with well resolved spin-spin multiplicities

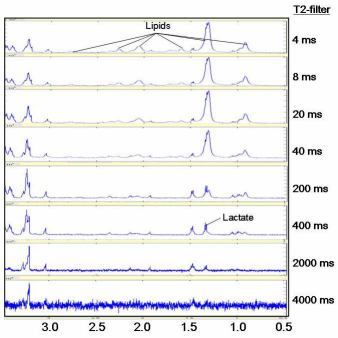

Figure 3: Example of T2 editing for T2 measurements on a liver needle biopsy. Different filtering delays have been indicated.

Reference: [1] B. Martínez-Granados, D. Monleón, M.C. Martínez-Bisbal, J.M. Rodrigo, J. del Olmo, P. Lluch, A. Ferrández, L. Martí-Bonmatí, B. Celda "Metabolite identification in human liver needle biopsies by high-resolution magic angle spinning 1H NMR spectroscopy" NMR in biomedicine 2006; 19:90-100

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