In vitro spectroscopic method under clinical conditions for in vivo Mannitol detection

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Introduction

Under clinical conditions, at 1.5 Tesla, spectral overlap and resolution may disturb component identification measurement, biochemical and medical interpretation. For example, at short echo time, identification of components which contribute to the large spectral overlap in the 1.1-1.5 and 3.6-3.9 ppm regions is difficult. Therefore, it is important to be accurate in frequency assignment in order to detect differences between lactate 1.27-1.33 ppm doublets, neural progenitor cells (NPC) with a prominent peak at 1.28 ppm (1) and the CH_2 lipid peak at 1.25-1.35 ppm in normal hippocampus and in pathological human hippocampus, where this detection plays an even more important role. Among myo-inositol, glutamate, glutamine and glucose resonances, Maioriello *et al.* (2) and Sankar *et al.* (3) focus on an unusually large peak at 3.8 ppm that fits none of the standard metabolites. In (2), assumptions of Mannitol for the 3.8 ppm large peak were confirmed by *in vitro* tests. Identification, in human brain, of lesser-known metabolites may be improved by employing accurate prior knowledge. Nevertheless, variability in MR proton spectroscopy results from many parameters such as pH, temperature, internal reference, chemical environment or field inhomogeneity. We have chosen to study in detail the reference parameters, temperature and pH effect on frequency assignment for the drug Mannitol® (an anti-edema drug) in order to clearly identify, extract and measure it in patients' brain.

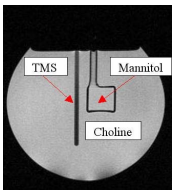
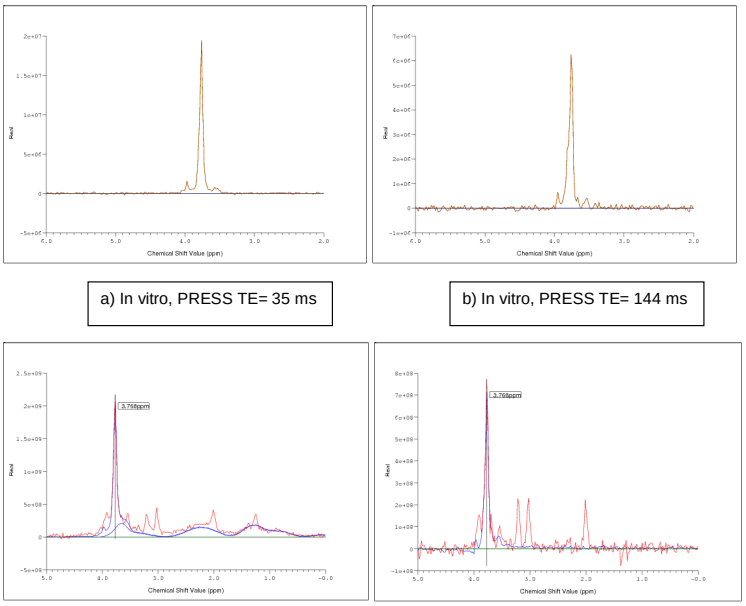


Fig 1: Phantom

Materials and Methods

^{1}H-MRS experiments were performed on a clinical 1.5 Tesla scanner using a phased array coil (8HR brain by General Electric Medical System). A spherical phantom (Fig. 1) made of glass with about a 2 l volume (of Choline -Cho- at 10mMol.L^{-1}) as well as an NMR glass tube (with Tetramethylsilane -TMS- at 1% in deutered-chloroform -$CDCl_3$-) and a spherical phantom of glass with about a 12 ml volume of Mannitol were used for experiments. The localized ^{1}H MR spectra (volume size of 8 cc) were acquired using GE probe PRESS and probe STEAM sequences with several TEs and a TR of 1500 ms. The post-processing analysis was provided by a home-written program (in Scilab©INRIA-ENPC code, open source), named SCI-MRS LAB which suppresses the water residual peak by using a linear exponential combination (centered on water region) and a Hankel combination (the rest of the spectrum). Measurements were repeated at least four times in order to check long term reproducibility and twice during each session (short term reproducibility). A first choice based on damping factor, signal to noise ratio and spectral resolution criteria for the reference spectra was then confirmed by further analysis using statistical tools such as hierarchical clustering analysis and normality test. All measurements were modelized from the reference.



a) In vitro, PRESS TE= 35 ms

b) In vitro, PRESS TE= 144 ms

c) In vivo, PRESS TE= 35 ms

d) In vivo, PRESS TE= 35 ms

Fig. 2 In vitro and In vivo Mannitol spectra

Results

Hierarchical clustering emphasizes the temperature effect for all experiments. The Shapiro-Wilk test was performed and all p-values were higher than the significance level choice of 5%, so our assumption of normality of residuals is consistent with the null hypothesis.

At 1.5 Tesla and without mixture of products, the results correspond to what we expected: the temperature effect is about 0.01 ppm per degree Celsius. No pH dependency was found for Mannitol resonances. Mannitol chemical shift, reported with reference to Cho or 1% TMS in $CDCl_3$ resonances, is temperature stable.

At 37° C referring to a 3.185 ppm Cho peak (4), Mannitol resonates at 3.767 ppm ± 0.001 (Fig. 2a 2b) and the confidence interval (at 95%) is [3.762; 3.771] ppm.

In vitro results were applied to Mannitol detection in the brain of one young patient: a 13-year-old boy (50 kg) with renal disease and cardiac arrest who received intravenous Mannitol (20%) for three days. Spectroscopic examinations made in the left internal occipital cortex, one day after Mannitol was stopped, showed a Mannitol peak four times higher than the NAA (Fig. 2c 2d).

Discussion and Conclusion

This spectroscopic study was done using a special test object which is composed of three separate compartments. Such a phantom allows us to acquire spectroscopic data referred to 1% TMS in $CDCl_3$ and Choline for any metabolite desired. Usually, the method proposed by Provencher (5) is used to acquire spectroscopic signals but the mixture of products (reference, buffers, conservatives and metabolite) and the DSS use (mainly due to D_2O manipulation) is not suitable under clinical conditions and for long term reproducibility studies. In the case of several spectroscopic measurements, statistical tools were introduced in order to choose a spectral pattern with a confidence interval for frequency assignments and to validate reproducibility of patterns. To our knowledge, no such methodology has been proposed. *In vitro* measurements of Mannitol obtained under the same conditions as *in vivo* examinations confirm and clarify obvious results (2,3). Acquisition and post-processing methods under clinical instrumental conditions presented in this study may be applied to acquiring other spectroscopic signals such as metabolites or anti-epileptic drugs. Studying the dependence of spectroscopic patterns on the chemical environment in repeated measurements improves prior knowledge use and may decrease false detections.

Acknowledgments : This work was supported by French Normandy region (FNADT) subsidies.

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