Towards μMRS using High-Resolution Magic-Angle Coil Spinning: application to brain metabolism

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INTRODUCTION:
High-Resolution Magic-Angle sample Spinning (HR-MAS) NMR spectroscopy of biopsies combined with chemometric statistical tools has now emerged as a powerful methodology for metabolomics NMR and has led to many important disease diagnosis, therapeutic target discovery and environmental assessment. This technique is also a method of choice when studying metabolism. However, due to the intrinsically poor detection sensitivity, NMR analysis often requires large tissue mass (5 to 10 mg). Such mass could compromise the metabolic evaluation due to the high degree of tissue heterogeneity (in tumor for example). Unfortunately, today there are no practical means for NMR analysis of small quantity of tissue (sub microgram), or any soft-matter, where sample magic-angle spinning is essential for high quality data acquisition. For this reason, we are developing NMR-based analytical tools with good sensitivity and with good metabolic spectral quality for nanogram tissue biopsies. Currently, one promising approach is the used of a simple micro-resonator (High-Resolution-Magic-Angle Coil Spinning (HRMACS)), in which it can wirelessly coupled to a MAS probe. Here, we present the use of this micro-resonator HRMACS to explore rat brain metabolism, and to see if it was possible to detect and discriminate any differential biomarkers between rest and activated brain.

MATERIALS and METHODS:
Animals: Wistar rats (200g, awake, n=4) underwent unilateral whisker stimulation during one hour. At the end of the stimulation, rats were euthanized by brain irradiation using focused microwaves (to avoid post-mortem evolution of the metabolism). About 200ng biopsies of activated and non-activated S1BF areas were analyzed ex vivo by 1H-NMR spectroscopy on a Bruker Advance 500 MHz equipped with a HRMAS probe with sample spinning at 300 Hz. The spectra were acquired with a 8-steps PASS sequence with a 90°-pulse of 6 μs and a total relaxation time of 2.36 s.

MACS Fabrication: The HRMACS resonator was constructed by manually hand winding a 10-turns solenoidal coil from a 30-μm copper (Cu) wire around a 840/600μm (outer/inner diameter) quartz capillary, forming a solenoidal length of 2.5 mm with a sample detection volume of about 200nL. The solenoid was soldered to a nonmagnetic 3.0 pF capacitor (American Technical Ceramics, US), to give a target frequency at 490 MHz for 1H detection at 11.75 T. The resultant coil quality factor is 43.

Sample Preparation: Sample preparation for HRMACS was preformed under a microscope with microtools. The brain biopsies were directly punched from the microwave-fixed brain using a 550/400 μm quartz capillary in the S1BF area (somatosensory cortex corresponding to the area that was activated by whiskers stimulation). The capillary was then sealed with hot paraffin wax to prevent leakage during the sample spinning, and inserted into the HRMACS resonator.

RESULTS and DISCUSSION: First, with this HRMACS, containing only 200ng tissue, we obtained high quality spectra in only 30 min with over 20 identifiable metabolites. Second, using a unbiased multivariate data analysis, the results do show that the micro-detection with HRMACS enable to discriminate the different groups: activated and rest brain. The OPLS loading plot clearly displays an increase in lactate and N-acetyl compounds (NAC) concentration in the activated groups. This increase in lactate content in the activated brain region agrees with previous results obtained with classical HRMAS biopsies and reflects an increase in lactate production during brain activation, a phenomenon also measured in the human brain. To our knowledge, it's the first time that an increase in NAC during brain activation is detected. Since the same amount of brain biopsy was analyzed for each sample, this result suggests that another metabolite such as choline rather than NAA should be taken as a reference when performing in vivo spectrum quantification.

CONCLUSION: Even with the small sampling (n=8), the results obtained with this new technique are consistent with the previous results measured from a classical HRMAS on a much larger panel. The ability of nanogram detection opens new possibilities to ease high-throughput screening, and also enables to analysis small biopsies, such as localize metabolic screening, where it is impossible for the conventional HRMAS approach (with 5-10mg). This work marks a significant leap towards needle NMR biopsy – a direct biopsy analysis in real-time clinical and surgical environments.

REFERENCES: