MR Spectroscopy of very small volumes (<0.4 µl) of 13C-labelled metabolites using microcoil detection: application to online measurements of cerebral microdialysate

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Introduction: *In vivo* ^{13}C MR spectroscopy in small animals remains challenging due to the low sensitivity of the ^{13}C nuclei (γ_{13C} = 1/4 γ_{1H}), long longitudinal relaxation time (tens of second) and low natural abundance (1.1%) of ^{13}C. The SNR (signal-to-noise ratio) of ^{13}C studies are usually increased with the use of high magnetic field (7T and above) pre-clinical MR scanners and the administration of enriched ^{13}C-labeled substrates to the animal. However, when very small volumes (microliter and below) are investigated, the sensitivity of ^{13}C MRS becomes a major issue and alternative solutions are required. Although hyperpolarization techniques (DNP, PHIP) are very effective approaches for enhancing the sensitivity of ^{13}C MRS, these sophisticated techniques are currently only available in a limited number of imaging centers. Alternatively, for very small volumes of biological tissue/solutions, the NMR detection can be improved with the use of microcoils optimizing the filling factor and the sensitivity per unit volumes [1-3]. In this study, we report the use of a microsolenoid for the online detection of ^{13}C-labelled metabolites at 4.7T on nanolitres (< 400 nl) of cerebral microdialysate in rats.

Material and methods: A microdialysis catheter (CMA 7 metal free 2mm, Kista, Sweden) with a cutoff of 6kDa was implanted stereotaxically in the cortex region of female Wistar rats. MRI acquisitions were performed with a 4.7 T Biospec spectrometer (Bruker, Ettlingen, D), using a dual-tuned 8 mm diameter proton/carbon surface coil (Doty Scientific, Columbia, USA) and a custom-made microsolenoidal coil (wire diameter: 100 µm, coil ID: 600 µm, number of turns: 14, length: 1.5 mm, inner volume: 424 nl). The coil was centered above the surface coil and the outlet tubing of the microdialysis probe was passed through it as shown in Fig. 1. Animals were located in the magnet in decubitus position with the brain positioned below the surface coil. The microdialysis probe was perfused at a rate of 8 µl/min with a solution of artificial cerebrospinal fluid (ACSF, pH 7.3), containing either (i) 1 M [3-^{13}C]-lactate and 3mM Gd-DOTA, or (ii) 3M ^{13}C-urea and 3mM Gd-DOTA. MRI gradient echo (GE) acquisitions (TE=2.6 ms, TR=113ms, FOV 35*35 mm, matrix 256*256, slice thickness 1mm, averages=6, flip angle=60°) were obtained with surface coil in three orthogonal planes to control the positioning of the microdialysis probe and the release of the perfusate into the brain. ^{13}C spectra were obtained using a non-selective RF excitation (TR=400 ms, averages= 200, flip angle=40°, scan time 1.20 minutes). Proton decoupling was obtained with the surface coil.

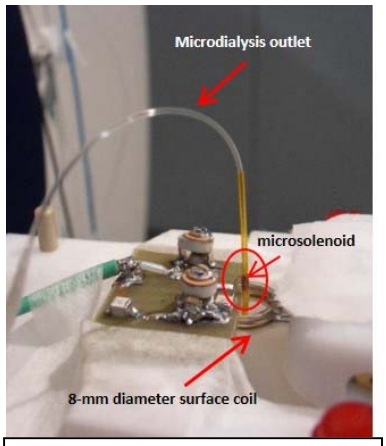

Fig 1. Photograph of the surface proton coil, the microsolenoid coil and the microdialysis outlet. Animal's head is positioned below the surface coil.

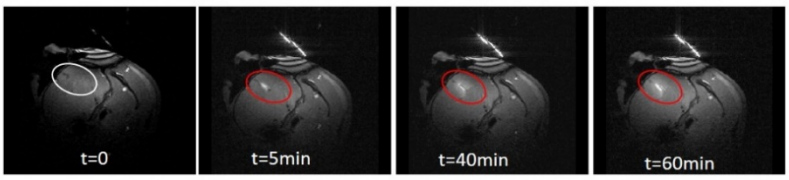

Fig 2. T_1-weighted MR brain images at different time points following the start of perfusate infusion. Brain tissues in the vicinity of the microdialysis probe are progressively enhanced by Gd-DOTA.

Results: The microdialysis probe and its positioning in the brain were accurately visualized using the GE images (in plane pixel resolution of 130 µm). Signal enhancement of the brain tissue due to the presence of the perfusate and Gd-DOTA was observed up to a distance of 2 mm from the microdialysis probe (Fig. 2). Representative ^{13}C spectra of the dialysate obtained in 80 seconds are displayed in Fig. 3. Typical SNR and linewidth of the lactate peak are equal to 10 and 15 Hz (0.3 ppm) respectively. In this particular dataset, the increasing intensity to a plateau of the [3-^{13}C]-lactate peak was attributed to the in vivo steady state equilibrium of lactate release and recovery.

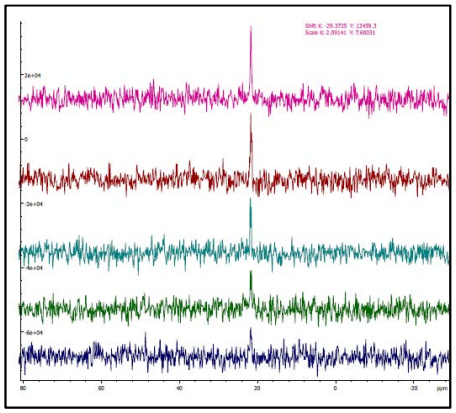

Fig 3. Selected stacked spectra from 400 nanoliters solution obtained at 10 minutes interval. Peaks at 21.5 ppm correspond to 3-^{13}C-lactate.

Discussion: The Gd-based contrast agent associated with T_1-weighted MRI sequences was very effective for visualizing the release of infusing perfusate solution into the brain tissue. The presence of Gd-DOTA proved also beneficial for shortening the longitudinal relaxation times of ^{13}C-labeled metabolites. As a result, 400 milliseconds repetition times were used and metabolites could be detected in less than one minute from a very small volume (< 400 nanoliters) of dialysate. The detection of such small quantities (< 36 µg) was made possible with the use of a micro NMR probe. As long as the length-to-diameter ratio is unchanged, the sensitivity per unit volume of solenoidal coil is known to increase inversely with its diameter [4]. In this study, the diameter of the microsolenoid was designed in tightly accommodate the outlet tubing of the microdialysis probe. Hence from a simple rule of thumb, one can estimate a ten-fold NMR sensitivity gain as compared to a conventional 8-mm diameter surface coil.

Conclusion: Intracerebral microdialysis probe and MRI/MRS techniques are complementary tools and their combined use offer important synergetic potential. For instance, the visualization of tissue integrity, the monitoring of appropriate positioning (despite the use of stereotaxic device for microdialysis implantation) and the imaging of the perfusate distribution are of prime interest. Similarly, the possibility of quantifying on-line and in real time the concentration of ^{13}C-labelled metabolites in dialysate represents an additional step towards *in vivo* NMR investigation of metabolism at submillimetric scale.

References: [1] *Olson et al. Science, 270, 1967 (1995)* [2] *Wong et al., Anal. Chem., 84, 3843 (2012)* [3] *Radecki et al., PNAS, 111, 23, 8667 (2014).* [4] *Hoult et al., JMR, 24, 1, 71 (1976).*