Solid-state NMR adiabatic TOBSY provides enhanced sensitivity for multidimensional high-resolution magic-angle-spinning H1 MR spectroscopy in burn trauma

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Introduction– Burns are lesions often due to direct transfer of energy from any source of heat to the body. The thermal injury may determine severe metabolic alterations due to the liberation of inflammatory mediators and hormonal disturbances induced by stress. Burn trauma in skeletal muscle has both local and systematic effects, as functionally debilitating changes are seen to occur at local and distant site, especially when burn size exceeds 30% of total body surface area (1,2). Nuclear magnetic resonance Spectroscopy HRMAS has been used to explore lipidic accumulation after burn trauma (3). On these bases we perform a solid-state NMR method that maximizes the advantages of high-resolution magic-angle-spinning (HRMAS) ^{1}H MRS applied to intact burn tissue biopsies when compared to more conventional liquid-state NMR approaches. Numerical simulations and experimental results of an optimized adiabatic TOBSY (Total through Bond correlation SpectroscopY) solid-state NMR pulse sequence for two-dimensional ^{1}H-^{1}H homonuclear scalar-coupling mixing indicate that a significant SNR gain (>100% theoretically and 20-50% experimentally) relative to its liquid-state analogue TOCSY (TOtal Correlation SpectroscopY) sequence is attainable (4). Multidimensional ^{1}H-MRS is crucial for unambiguous assignment and quantification of overlapping ^{1}H spectra of tissues. Hence, ensuring the best sensitivity is highly desirable. Here we present experiments using our novel 2D TOBSY HRMAS ^{1}H MRS, which aim to suggest its use as a sensitive MR sequence to investigate burn metabolic injury.

Materials and Methods– Six mice, subjected to 5% total burn surface area (TBSA), were analyzed using *ex vivo* HRMAS ^{1}H NMR spectroscopy. Burn injury was inflicted by immersing the left leg of mice in 90° C water for 4 seconds; three days after burn mice were sacrificed and the skeletal muscle tissue underlying the burned (and contralateral non-burned) leg was harvested, immediately frozen in liquid nitrogen and stored at -80°C. All HRMAS ^{1}H MRS experiments were performed on a Bruker Bio-Spin Avance NMR spectrometer (600.13 MHz) using a 4mm triple resonance (^{1}H, ^{13}C, ^{2}H) HRMAS probe (Bruker). The tissue samples were transferred to a ZrO2 rotor tube (4mm diameter, 50 µl), containing an external standard (trimethylsilyl propionic-2,2,3,3-d4 acid (TSP), Mw=172, d=0.00 ppm) that functioned as a reference both for resonance chemical shift and quantification. The HRMAS ^{1}H MRS was performed at -8°C with 3 kHz MAS speed to minimize tissue degradation. For the 2D experiment the same acquisition data: 200 t1 increments (TPPI), 45 ms mixing time, 2 s repetition time (1 s cw water suppression), 8 scans, 2 dummy scans, 50 min total acquisition time) and the same processing parameters (zero filling to 1k in F1, QSINE = 2 window function, base line correction) were used.

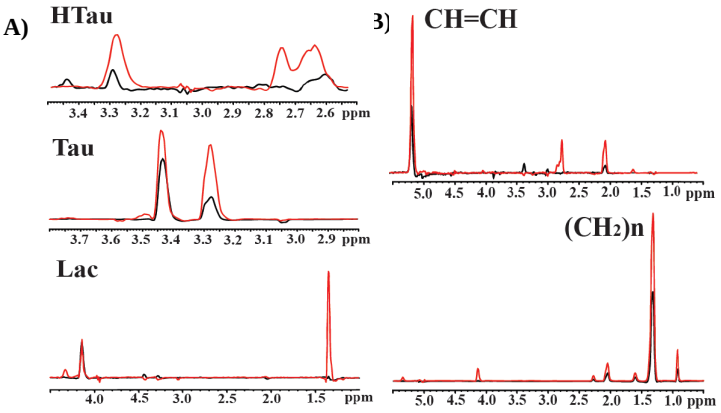

Figure 1. 1D slices processed from 2D experiments overlay of C9$^1_{15}$ (red) and MLEV-16 (black). **A**) HTau, hypotaurine; Tau, Taurine; Lac, Lactate. **B**) unsaturated acids (CH=CH) and acyl chain methylene $(CH_2)n$.

We refined the C9$^1_{15}$ (TOBSY) symmetry-based ^{13}C MAS solid-state NMR pulse sequence for 2D HRMAS ^{1}H-MRS use and compared the magnetization transfer efficiency and SNR to MLEV-16 (TOCSY) on burn mice specimens. C9$^1_{15}$ cancels the 1st order average Hamiltonian and minimizes the higher orders contributions from CSA, D and offset terms, retaining only the isotropic J-coupling. In both cases, WURST-8 adiabatic inversion pulses were employed for their efficient use of r.f. power to compensate pulse offsets, in-homogeneity and miscalibration with reduced r.f. heating. By design C9$^1_{15}$ is rotor-synchronized, and we rotor synchronized MLEV-16 according to. The 2D TOBSY pulse sequence using C9$^1_{15}$ is shown in Figure 1 (a cw water suppression block is omitted) (4).

Results– Figure 1 shows 1D slices extracted along the indirect dimension of 2D TOBSY (red) and TOCSY (black) experiments respectively. 1D slices were scaled to the same noise levels and integral values are given. Figure 1A shows that the SNR of low molecular weight biomarkers such as Hypotaurine (HTau), Taurine (Tau) and Lactate (Lac) is increased by 50-60% (↑50-60%). Figure 1B shows that the SNR of high molecular weight such as unsaturated acids (CH=CH) and intramyocellular lipids (CH2)n is increased by 60% (↑60%). 2D TOBSY spectra of control and burned skeletal muscle are reported in figure 2. Several small metabolites and also lipids were identified. We identified the following metabolites: Alanine (Ala), Lac, OH-Butyrate (OH-But), Glutamine (Gln), Glutamate (Glu), Glutathione (GSH), Tau, HTau, Proline (Pro), Lysine (Lys), myo-inositol (Myo), α-, β-Glucose (α-Glc, β-Glc), Carnosine (Cnr). We detected an altered concentration in many water-soluble metabolites in burned samples as compared to controls (data not shown). Some metabolites such as glutamate (Glu) and Glutathione (GSH) were absent in burned skeletal muscle.

Discussion– In this study we used a novel 2D TOBSY HRMAS ^{1}H MRS and observed increased in SNR for both small metabolites and lipids. Our results confirmed our expectations regarding TOBSY to detect biomarkers with high SNR and decreased acquisition time. Our data analysis showed that with TOBSY we detect an improved metabolic profile of burned skeletal muscle. Thus, 2D TOBSY HRMAS ^{1}H MRS is well suited for simultaneously qualitative and quantitative analysis of metabolites concentration in burned tissues and this can help us to better evaluate and understand the metabolic dysfunction due to burn.

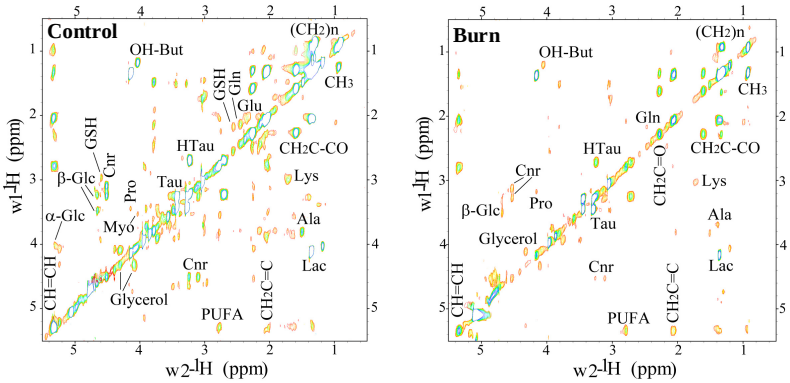

Figure 2. ^{1}H-^{1}H TOBSY HRMAS spectra of control and burned skeletal muscle.

References

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