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) 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 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 MRS 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. Demonstrated were 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 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. 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 increased 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.

References