In-vivo proton HRMAS detects effects of trauma in Drosophila Melanogaster

D. Mintzopoulos[1,2], Y. Apidianakis[3], M. N. Mindrinos[4], R. G. Tompkins[3], L. G. Rahme[3], and A. A. Tzika[2,5]

[1]NMR Surgical Laboratory, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, [2]Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, [3]Department of Surgery, Burn Trauma Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, [4]Department of Biochemistry, Stanford University, CA, United States, [5]NMR Surgical Laboratory and Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

Introduction

Trauma is the leading cause of death (COD) of Americans under age 45, mostly because of car crashes, and ranks fifth overall COD nationwide causing 160,000 deaths annually. Trauma is multiple types of injuries ranging from car-crash, gunshot, to burn trauma. A leading complication of trauma is the onset of multiple-organ failure several days post-injury, responsible for a quarter of overall trauma-related deaths. In mammals, both acute and sustained responses are induced after trauma to orchestrate tissue repair. The highly evolutionarily conserved cJun-N-terminal Kinase (JNK) regulatory signaling cascade, which is quickly activated upon injury [1], plays an important role in development, wound healing, and tissue repair in mammals as well as in insects [2]. For example, JNK signaling is implicated in embryonic dorsal closure and thoracic disc fusion in Drosophila, and has been linked to apoptosis, stress and immune responses in both insects and mammals [1, 5]. Drosophila as a simpler genetic organism has allowed the multiple biological functions of a few signaling pathways to be examined in the whole organism. For example, this model host has provided important insights into the role of JNK *in vivo*, including a conserved function for JNK signaling in wound healing [2]. Physiological processes such as apoptosis, necrosis, and inflammation that occur after trauma likely perturb muscle lipid content, resulting in observed NMR changes of metabolites; lipids, in particular, are associated with these processes. High resolution Magic-Angle Spinning (HRMAS) ^{1}H NMR spectroscopy studies in mice burn models have shown lipid accumulation in skeletal muscle in response to burn trauma-induced apoptosis, most likely in response to JNK pathway [3]. Polyunsaturated fatty acid (PUFA) peaks increased, in accordance with evidence that PUFAs accumulate following apoptosis [3, 4]. In addition, ICMLs reported biomarkers of insulin resistance also increased [3,6]. Here, we use Drosophila model of trauma to detect possible biomarkerks using *in-vivo* ^{1}H HRMAS spectroscopy and validate our findings with gene-expression studies.

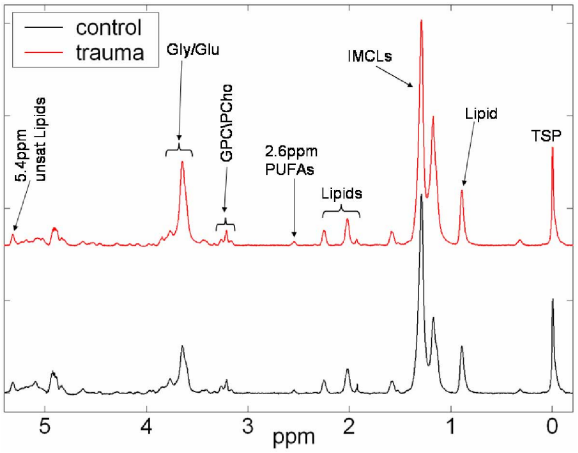

Figure 1: Average single-fly ^{1}H HRMAS MR spectra of the control group and the injured group. Spectra were acquired with the CPMG sequence, rotor-synchronized at 2kHz spinning rate (τ = 500 µs) withspin echo time TE = 20 ms (n=20). The reference peak at 0 ppm is TSP.

Materials and Methods

Experiments were performed on two groups of male Drosophila flies: (a) control healthy, uninjured flies; and (b) traumatized flies, injured with thoracic non-lethal, needle puncture. All traumatized flies were placed in the spectrometer at least one and up to three hours after trauma. All flies, healthy and traumatized, were anesthetized prior to insertion in the spectrometer by dropping their body temperature to 4°C and were kept at 4°C while in the spectrometer. Special care was taken to avoid further injury during placement in and extraction from the rotor.

^{1}H HRMAS 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 temperature was controlled at 4°C by a BTO-2000 unit in combination with a MAS pneumatic unit (Bruker). The flies were placed into 4mm zirconium oxide (Zirconia, Bruker) rotors with spherical inserts. 10 µl D_2O (deuterium lock reference) containing 10 mM TSP (trimethylsilyl propionic-2,2,3,3-d_4 acid, M_w=172, δ=0ppm, external chemical shift reference) was added to the rotor with the sample. The MAS rotation frequency was stabilized at 2.0 ± 0.001 kHz by a MAS speed controller. One-dimensional ^{1}H NMR single-fly spectra were acquired on all samples using a rotor synchronized Carr-Purcell-Meiboom-Gill (CPMG) spin echo pulse sequence, [90°-(τ-180°-τ)$_n$-acquisition], which works as a T_2 filter to remove the spectral broadening. The inter-pulse delay (τ = 500µs) was synchronized to the MAS rotation frequency. One sample was acquired at 4kHz (τ=250µs) for reference with high-spin-rate spectra. The number of transients was 256 with 32,768 (32k) data points. The 1D fully-relaxed spectra were also obtained using a single pulse sequence with water signal suppression. A line-broadening apodization function of 1.0Hz was applied to all HRMAS ^{1}H FIDs prior to Fourier transformation. Spectra were referenced with respect to TSP, manually phased, and a Whittaker baseline estimator was applied to subtract the broad components of the baseline prior to peak area calculations (using MestReC software, Mestrelab Research). Peak heights were scaled with respect to TSP for each acquired spectrum (TSP peak height = 1). Within-group peak-height ratios were averaged and between-groups ratios were compared with t-tests (two-tailed, $p<0.05$). RNA from Drosophila thoracic muscle was extracted and hybridized onto MOE430E oligonucleotide arrays, which were stained, washed, and scanned. All procedures followed standard Affymetrix, Inc (Santa Clara, CA) protocols.

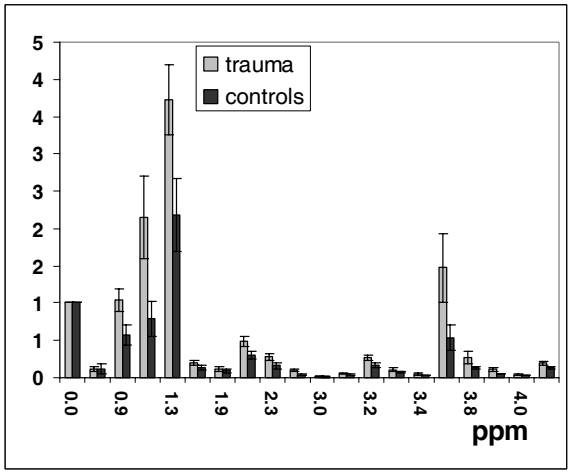

Figure 2: Average scaled ratios of metabolites for the control group (N=5) and the injured group (N=4). TSP is scaled to 1 by definition. The errorbars represent standard error (SE).

Results

We found differences between the two groups, traumatized and control, at the 0.9 and 1.2-1.3 ppm IMCLs, the 2.6 ppm PUFAs and the 5.3ppm unsaturated lipids. We also found difference at 2-3.33 ppm (lipids/Cho peaks) and at 3.7ppm (Glucose/Glycine peaks). Averaged representaive spectra and results for averaged ratios of scaled peak heights are shown in Figures 1 and 2. 67% of flies survived up to three hours after the experiment, which was the time of last observation.

Discussion

We suggest that the common fruit fly (Drosophila Melanogaster) is a useful model for high-throughput NMR and gene-expression studies of trauma and cascaded physiological processes that stimulate the highly evolutionarily conserved JNK pathway. We also demonstrate the ability to perform *in-vivo* ^{1}H HRMAS experiments using fly models, which is important inasmuch as flies can be observed after NMR and subjected to other procedures for high-throughput studies to evaluate agents inhibiting traumatic responses. To our knowledge, this is the first literature report of an *in-vivo* ^{1}H HRMAS experiment.

References

1. Tsuda M, *et al,* EMBO Rep 2005; 6:1082-1087
2. Galko MJ, and Krasnow MA, PLoS Biol 2004; 2, E239; Zhang JY *et al,* Genes Dev 2004; 18:17-22
3. Astrakas LG *et al,* The FASEB Journal 2005; 19:1431-1440
4. Hakumaki JM *et al,* Nat Med 1999; 5:1323-1327
5. Stronach BE, and Perrimon N, 1999; *18*, 6172-6182; Papa S *et al,* J Cell Sci 2004; *117*, 5197-5208; Boutros M *et al,* Dev Cell 2002; 3:711-722
6. Kuhlman J et al, Magn Reson Med 2005; 53:1275-1282