Effects of a novel mitochondrial peptide on redox status as measured by EPR in Drosophila melanogaster post-trauma

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Introduction– Trauma resulting from a wide range of injuries impacts skeletal muscle metabolism and is the fifth overall cause of death of Americans, leading to 100,000 deaths in the US annually. The recent advances in electron paramagnetic resonance (EPR) spectroscopy have significantly contributed to the understanding of various pathological changes and we believe it could elucidate metabolic derangement following skeletal muscle trauma and evaluate the effects of potential therapeutic agents (1). Interestingly, the use of *Drosophila melanogaster* as a model organism is of interest due to the remarkable similarities of the metabolism between *Drosophila* and mammals (2,3). Indeed, the study of *Drosophila* metabolism is an emerging field that can potentially give insight to conserved metabolic mechanisms. Furthermore, powerful genetic tools available in *Drosophila* research make the fruit fly a particularly tractable model organism for probing metabolic pathways and possibly leading to a better understanding of human metabolic disorders. Here, we examined the feasibility of a novel, *in vivo* EPR approach towards the investigation of the metabolic derangements in traumatized flies. We hypothesized that these metabolic derangements can be ameliorated by treatment with a novel mitochondria-protective peptide (Szeto-Schiller, SS-31) (4,5).

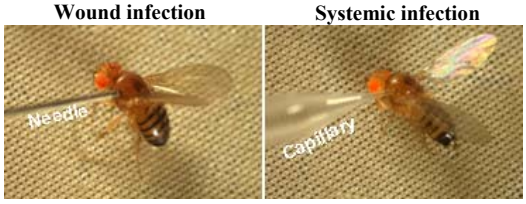

Figure 1. Experimental approach of wound infection by needle and systemic infection by capillary (injection of saline or SS-31) of a *Drosophila melanogaster* prior to in vivo 1.2-GHz EPR spectroscopy.

Materials and Methods– We used male wild-type (WT) and mutant *chico* (exhibiting reduced insulin signaling) *Drosophila* flies (n=6 per group). The flies were first traumatized in the thorax with a needle followed by injection of saline or SS-31 through a capillary (Figure 1). Since nitroxides exist in the biological systems as a redox pair and are reduced to the corresponding hydroxylamine in cell suspensions as well as in vivo by cellular reducing equivalents, the pharmacology of nitroxides reports the redox status of the tissue (6). A 9.2 nl of the nitroxide (3-carbamoyl-2,2,5,5-tetramethylpyrrolidin-1-yloxyl free radical, Sigma-Aldrich) was infused (16 mg/ml) within a few seconds through a capillary (Figure 1) attached to the thorax of the fly. Then the flies were quickly transferred to quartz tube and placed in the cavity of the Bruker X-band (9.2 GHz) EPR spectrometer. The flies were restrained by placing them in between cotton plugs for EPR measurements at room temperature. The optimal spectrometer parameters were: incident microwave power, 0.6mW; magnetic field center, 3487 gauss; modulation frequency, 100 kHz. Modulation amplitude was one-third of the EPR line width, with a scan time of 10 sec. The time difference between the infusion of the nitroxide and the start of EPR acquisition was approximately 1 min. This time was maintained in all the experiments. The change in the signal intensity of the middle component of the nitroxide was recorded over time (Fig. 2) and data were fitted using exponential decay kinetics (Fig. 3) to determine the rate constants. The measurements for the control flies were repeated at identical time points and the same procedures were followed to measure the redox status at various time points.

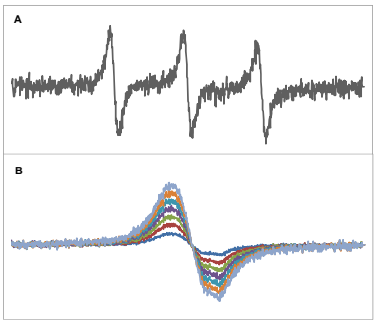

Figure 2. (A) Typical in vivo EPR spectra of the CPA nitroxide injected into flies (B). The middle component of the EPR spectrum was used to follow the reduction of the nitroxide over time *to determine redox status.*

Results– A typical in vivo X-band EPR spectrum acquired from flies injected with the nitroxide is shown in Fig. 2A. The changes in the signal intensity of the middle component of the EPR line of nitroxide (Fig. 2B) were followed each minute to monitor its metabolism. Figure 3A shows that the redox status was corrected in flies 1 h and 6 h post-injury. Moreover, the decay rate of nitroxide at 1 h and 6 h post-injury was 0.0187 ± 0.0040 s^{-1} and 0.0187 ± 0.0043, respectively and did not differ significantly among each other. The decay rate in the post-injury groups differed significantly from the decay rate in the control group (0.0131 ± 0.0027 s^{-1}) with P = 0.018 for the 1 h and P = 0.024 for the 6 h post-injury groups, which is in accordance with our mouse burn trauma findings (7). Infusion of SS-31 alone in control flies (Fig. 3B) significantly increased the decay rate to 0.0187 ± 0.0027 s^{-1}, compared to the control group (P = 0.041). When SS-31 was injected to 6h post-injury flies it significantly decreased the decay rate to 0.0130 ± 0.0036 s^{-1} compared to the 6 h post-injury group (P = 0.040) and normalized to the level of the control group (P = 0.933 when compared to the control group) (Fig. 3B). However, no significant difference was observed between SS-31 and saline treated flies under similar conditions and this warrants further investigation.

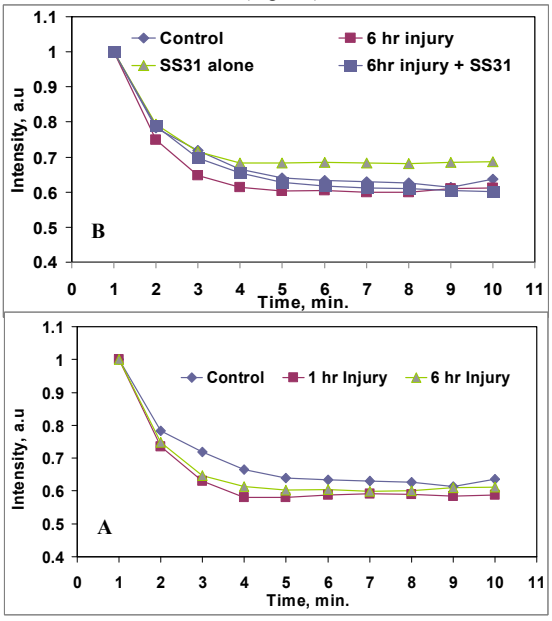

Figure 3. The decay kinetics of the nitroxide (CPA) in the fly (A) control, 1 h and 6 h post injury flies and (B) of control, injected with SS-31 alone, 6 h post-injury plus saline and 6 h post-injury plus SS-31 The redox status of the control group was measured at day 0 (0d) and day 3 (3d). Values are means ± SD: n=6.

Discussion– Thus, the major finding of this study was that the redox status of skeletal muscle is altered in injured flies and is recovered by a novel mitochondria-protective peptide. This change coincides with decreased expression in two major antioxidant systems, sesB and sod2 (data not shown). Moreover, abnormal mitochondrial redox status is indicative of mitochondrial uncoupling, which is in agreement with increased gene expression of uncoupling proteins. These results indicate that EPR is a promising tool for making an *in vivo* assessment of the consequences of injury in well-established Drosophila injury models. Moreover, EPR is complementary to NMR, which has already been shown to be capable of revealing the consequences of mitochondrial dysfunction in vivo (8). Our approach advances the development of novel *in vivo* non-destructive research approaches in *Drosophila*, suggests biomarkers for investigation of biomedical paradigms, and contributes to novel therapeutic development.

References: 1. Swartz HM, Khan N, Buckey J, Comi R, Gould L, Grinberg O, Hartford A, Hopf H, Hou H, Hug E, Iwasaki A, Lesniewski P, Salikhov I, Walczak T. NMR Biomed 17: 335-351, 2004; **2.** Baker, K. D., Thummel, C. S. Cell Metab. 6:257, 2007; **3.** Leopold, P., Perrimon, N. Nature 450:186, 2007; **4.** Szeto, HH. Antioxid Redox Signal. 10(3):601, 2008; **5.** Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, Price JW 3rd, Kang L, Rabinovitch PS, Szeto HH, Houmard JA, Cortright RN, Wasserman DH, Neufer PD. J Clin Invest. 119(3): 573–581, 2009. **6.** Swartz HM, Khan N and Khramtsov VV. Antioxid Redox Signal 9: 1757-1771, 2007; **7.** Khan N, Mupparaju S, Mintzopoulos D, Kesarwami M, Righi V, Rahme L, Swartz H, Tzika A. Mol Med Reports 2008;1:813-819. **8.** Padfield KE, Astrakas LG, Zhang Q, Gopalan S, Dai G, Mindrinos MN, Tompkins RG, Rahme LG, Tzika AA.Proc Natl Acad Sci U S A 2005;102:5368-5373.