Mitochondria-targeted antioxidant promotes recovery of skeletal muscle mitochondrial function after burn trauma

Valeria Righi[1,2], Caterina Constantinou[1,3], Dionyssios Mintzopoulos[1,2], Laurence G. Rahme[3], Hazel H. Szeto[4], Ronald G. Tompkins[5], and Aria A. Tzika[1,2]

[1]NMR Surgical Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, Massachusetts, United States, [2]Department of Radiology, Athinoula A. Martinos Center of Biomedical Imaging, Boston, Massachusetts, United States, [3]Molecular Surgery Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, Massachusetts, United States, [4]Department of Pharmacology, Joan and Sanford I, Weill Medical College of Cornell University, New York, NY, United States, [5]Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, Massachusetts, United States

Target Audience: Radiologists, NMR Scientists.

Introduction– Severe burn injury is associated with anatomical, physiological, endocrinological and immunological changes, which lead to generalized cachexia, and metabolic alterations which play a central role in pathophysiological progression (1). Mitochondrial oxidative stress and dysfunction are also associated with skeletal muscle wasting caused by mechanical ventilation and immobilization (2). Treatment with a mitochondria-targeted peptide antioxidant (SS-31) attenuated mitochondrial ROS production, normalized mitochondrial respiration, and prevented myofiber atrophy following mechanical ventilation and immobilization (2), and treatment with SS-31 after burn injury reduced oxidized proteins and prevented increase in caspase-3 activity and apoptosis in skeletal muscles (3). We investigated the effects of SS-31 on oxidative stress and ATP production in skeletal muscle in a mouse model of burn injury using ^{31}P NMR spectroscopy. With this methodology, it is possible to measure the rate of skeletal muscle ATP synthesis catalyzed by mitochondrial ATPase (4), which by definition is proportional to oxygen consumption via the P/O ratio (the ratio of the net rate of ATP synthesis by oxidative phosphorylation to the rate of oxygen consumption). Our results revealed that treatment with SS-31 reduces mitochondrial oxidative stress and improves ATP production that are likely to play a role in reducing skeletal muscle apoptosis and insulin resistance after severe burn trauma. **Materials and Methods–** *Experimental Animals*. Male 6-week-old CD1 mice were anesthetized by intraperitoneal injection of 40 mg/kg pentobarbital sodium and the left hind limb of all mice was shaved. Burn injury was produced by a nonlethal scald injury of 3-5% total body surface area by immersing the left hind limb in 90°C water for 3 sec . Control animals were prepared in the same way with the exception that limb immersion was performed with room temperature water. The mice were randomized into 4 groups: burn (B), burn+SS31 peptide (B+P), control (C) and control+SS31 (C+P). Two types of experiments were performed: 1) SS31 (3 mg/kg) was injected intraperitoneally immediately after burn and 2) SS31 (3 mg/kg) was injected intraperitoneally 30 min before burn and repeated immediately after burn. *^{31}P NMR Experiments.* Each animal's left hind limb was placed into a solenoid coil (four turns; length, 2 cm; diameter, 1 cm) tuned to ^{31}P frequency. All *in vivo* ^{31}P NMR experiments were performed in a horizontal bore magnet (proton frequency at 400 MHz, 21 cm diameter, Magnex Scientific, Varian, Palo Alto, CA, USA) using a Bruker Advance console. A 90° pulse was optimized for detection of phosphorus spectra (repetition time 2 sec, 192 averages, 4,000 data points). Saturation 90° selective pulse trains (duration, 36.534 ms; bandwidth, 75 Hz) followed by crushing gradients were used to saturate the γATP peak. The same saturation pulse train was also applied downfield of the inorganic phosphate (Pi) resonance, symmetrically to the γATP resonance. T1 relaxation times of Pi and phosphocreatine (PCr) were measured using an inversion recovery pulse sequence in the presence of γATP saturation. An adiabatic pulse (400 scans; sweep width, 10 kHz; 4,000 data points) was used to invert Pi and PCr, with an inversion time between 152 and 7,651 ms. *NMR data analysis.* The theoretical basis of saturation transfer experiments has been described previously by Forsen and Hoffman. ^{31}P NMR spectra were analyzed using the MestRe-C NMR software package (Mestrelab Research, NMR solutions, website: www.mestrec.com). The intramyocellular pH was calculated using the formula pH = 6.75 + log[(s - 3.27)/(5.69 - s)], where s is the chemical shift difference between the Pi and the PCr peaks. **Results–** Mean ATP concentration was significantly lower in B than in C mice by approximately 50%. Figure 1 shows representative ^{31}P NMR spectra acquired from control, B and B+P group of mice before (upper panel) and after (low panel) saturation of the γ-ATP resonance. We observed a decrease in PCr peak and ATP resonances from control to burn mice. Moreover the figure shows an increase of PCr and ATP peaks in Burn+P, probably due to the partial regeneration of ATP synthesis. The calculation of ATP synthesis rate was performed with both the NMR data and results of a biochemical assay (ATP concentration measurement) (5), and both were significantly decreased in the burned mice. The NMR-measured fractional change ΔM/M0 was decreased by 7.9% in the B group and increased by 39.9% in C+ P group and by 56.7% in B+Pa (1 dose of SS-31 immediately after burn) and by 24% in B+Pb (1 dose of SS-31 before and 1 dose immediately after burn), relative to the C group. ATP synthesis rate was significantly reduced in burned mice (-76 % lower than in C), (P=0.026). The ATP synthesis rate was then obtained as the product of kf and Pi concentration. Figures 2 and 3: ATP synthesis rate (Pi → γATP) at 6 hours after burn was significantly reduced in burned mice (P=0.026); and SS-31 injection resulted in significantly increased ATP synthesis rate in control mice (P=0.008). Importantly, ATP synthesis rate was significantly increased in burned mice injected with SS-31, as compared to burned mice alone (P=0.002 for B vs B+Pa and P= 0.00006 for B vrs B+Pb). Also, a single injection of SS-31 in burned mice normalized the ATP synthesis rate, while 1 dose of peptide SS-31 before and 1 dose immediately after burn increased the ATP synthesis rate and this increase was statistically significant (P= 0.046), (C vrs B+Pb). Moreover, when the ATP synthesis rate (reaction PCr → γATP) was compared in burned mice and mice injected with SS-31 the increase was also statistically significant (P=0.002 for B vrs B+Pa and P= 0.0001 for B vrs B+Pb).

Discussion– In this study, we proved the concept that SS-31 administration increases ATP synthesis rate in murine skeletal muscle. This proof was accomplished by injecting mice before and immediately after burn. More importantly, this increase remained significant even after one single injection of SS-31 immediately after burn, a period of critical importance for clinical intervention. We also observed that SS-31 administration in burned animals lowered mitochondrial aconitase activity to levels that were not significantly different from controls, indicating that SS-31 lowers TCA cycle flux which is significantly increased in burn due to a futile TCA cycle. Given that a mitochondrial coupling index is the ratio of ATP synthesis rate to the TCA cycle flux (30). **Conclusion-** This results suggest that SS-31 administration increases mitochondrial coupling, which is abnormally decreased in burn injury. These findings suggest that SS-31 administration is effective as a mitochondrial protective agent to alleviate the symptoms of severe burn trauma.

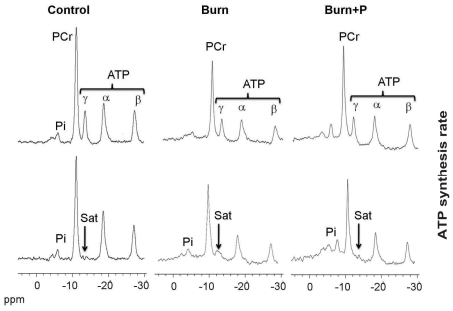

Figure 1. Figure 1. NMR spectra using 31P NMR saturation-transfer on the hind limb skeletal muscle of live mice. Representative summed 31P NMR spectra acquired from Control, Burned and Burned+P (peptide) group of mice before (upper spectra) and after (lower spectra) saturation of the γ-ATP resonance. The arrow indicates the position of saturation (Sat) by rf irradiation (-13.2 ppm, γ-ATP).

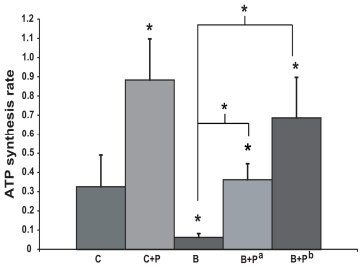

Figure 2. ATP synthesis rate (μmol/g/s) from Pi in control (C), control+SS-31 peptide (C+P), burned (B), and burned+peptide SS-31: B+Pa = 1 dose immediately after burn; B+Pb = 1 dose before and 1 dose immediately after burn, by 31P NMR at 6 hours after burn (* P<0.05).

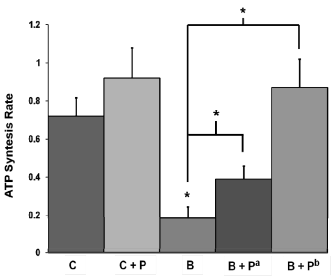

Figure 3. ATP synthesis rate (μmol/g/s) from PCr in control (C), control+SS-31 peptide (C+P), burned (B), and burned+peptide SS-31: B+Pa = 1 dose immediately after burn; B+Pb = 1 dose before and 1 dose immediately after burn, by 31P NMR at 6 hours after burn (* P<0.05).

References

1. Yu, YM. *et al*. (1999) *JPEN J Parenter Enteral Nutr* 23, 160-168, **2** Min, K. *et al*. (2011) *J Appl Physiol* 111, 1459-1466, **3**. Lee, HY. *et al*. (2011) *Shock* 36, 580-585, **4.** Brindle, KM. *et al*. (1989) *Biochemistry* 28, 4887-4893, **5**. Padfield, KE. *et al*. (2005). *Proc Natl Acad Sci U S A* 102, 5368-5373