P31 NMR Detects Mitochondrial Dysfunction in Burn Trauma

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Introduction

To date, altered mitochondrial function due to burn has not been well studied in skeletal muscle, which accounts for 90% of ATP production in respiring cells (1). *In vivo* NMR spectroscopy allows measurements of physiological biomarkers in intact systems (2, 3). In addition, GeneChip microarrays have greatly advanced physiological studies, by providing a snapshot of the transcriptome in a specific organ. Combining NMR and microarray data provides the opportunity to perform "functional genomics". Here we evaluate mitochondrial dysfunction in skeletal muscle following burn trauma in the mouse hind limb burn model by employing *in vivo* NMR on intact mice to assess alterations in ATP synthesis, and by characterizing the concomitant gene expression patterns in burn versus control muscle tissue.

Materials and Methods

Mice were studied with *in vivo* ^{31}P NMR spectroscopy three days after non-lethal burn trauma caused by immersion of the left hind limb of anesthetized mice in 90°C water for 3 seconds. All ^{31}P NMR experiments were performed in a horizontal bore magnet (proton frequency at 400 MHz, 21cm diameter, Magnex Scientific) using a Bruker Advance console. Saturation 90^{o} selective pulse trains (duration 36.534 ms, bandwidth 75 Hz) followed by crushing gradients were used to saturate the γ-ATP peak. The same saturation pulse train was also applied downfield of the Pi resonance, symmetrically to the γ-ATP resonance. T1 relaxation times of Pi and PCr were measured using an inversion recovery pulse sequence in the presence of the γ-ATP saturation. An adiabatic pulse (400 scans, sweep with = 10 KHz, 4K data) was used to invert the Pi and the PCr, with an inversion time between 152 ms and 7651 ms. Total RNA was extracted from muscle and hybridized onto MOE430A oligonucleotide arrays, which were subsequently stained, washed, and scanned. All procedures followed standard Affymetrix, Inc. protocols (Santa Clara, CA).

Results

NMR-measured unidirectional ATP synthesis flux primarily reflects flux through the F1F0-ATP synthase enzyme, with the coupled glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase reactions being negligible. Our in vivo 31P-NMR saturation-transfer spectra data (Figure 1) showed that burn trauma reduces ATP synthesis (Table 2), to suggest a significant reduction in the rate of mitochondrial phosphorylation. Our microarray data validated our NMR findings.

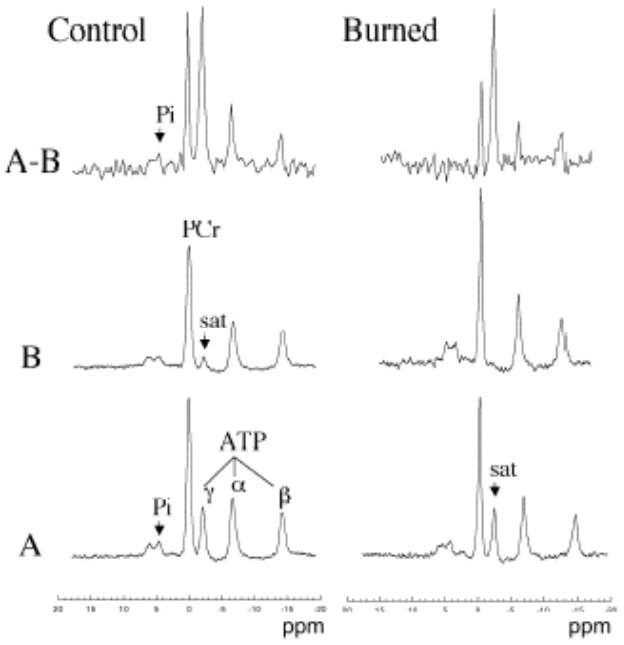

Figure 1: MR spectra of in vivo ^{31}P-NMR saturation-transfer performed on the hind limb skeletal muscle of awake mice. Representative summed 31P-NMR spectra acquired from normal and burned mice before (A) and after (B) saturation of the γATP resonance, with the difference spectrum between the two (A-B). The arrow indicates the position of the saturation (sat) by rf irradiation (-2.4 ppm). Pi, inorganic phosphate.

	Control (n=5)	Burn (n=6)	P-value §
ATP synthesis flux (reaction Pi → γATP)			
$\Delta M/M_o$	0.261±0.047 *	0.045±0.015 (-82.8%) ‡	0.007
$T1_{obs}$ (s)	1.31	6.62	
κ (s^{-1})	0.200±0.036	0.007±0.002	0.005
P_i † (*μmol/g*)	0.379±0.032	0.461±0.090	0.377
ATP synthesis (*μmol/g/s*)	0.089±0.021	0.004±0.002 (-95.5%)	0.015
ATP synthesis flux (reaction PCr → γATP)			
$\Delta M/M_o$	0.234±0.084 *	0.132±0.096 (-43.6%) ‡	0.446
$T1_{obs}$ (s)	2.3	1.9	
κ (s^{-1})	0.121±0.044	0.070±0.051	0.464
PCr † (*μmol/g*)	3.324±0.132	3.347±0.316	0.947
ATP synthesis (*μmol/g/s*)	0.390±0.142	0.225±0.168 (-42.3%) ‡	0.472

Table 2: ΔM/Mo, the fractional change in Pi or PCr magnetization as a result of saturation transfer; T1obs, observed spin lattice relaxation time of Pi or PCr during γATP saturation in seconds; *κ*, rate constant (s^{-1}). ATP synthesis is calculated as P_i (or PCr) concentration x *κ*. A Bioluminescence Assay Kit was used to assess ATP concentration.

Discussion

The NMR results, in conjunction with our genomic results showing down-regulation of mitochondrial oxidative phosphorylation and related functions, strongly suggest alterations in mitochondrial-directed energy expenditure reactions and indicate mitochondrial dysfunction in skeletal muscle accompanies burn pathogenesis. As such dysfunction likely contributes in whole or in part to the muscle atrophy observed in burn patients, future clinical treatments might be targeted to limit this dysfunction.

References

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