# In vivo Electron Paramagnetic Resonance detects oxidative stress in skeletal muscle after burn trauma

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## Introduction

Electron paramagnetic resonance (EPR) is a magnetic resonance-based technique that detects species with unpaired electrons such as free radicals (organic or inorganic) and transition metal ions. The development of low frequency (1200 MHz and below) EPR spectrometers has led to the *in vivo* application of the technique in a variety of animal models. Often, EPR may also be complementary to nuclear magnetic resonance (NMR) [1]. For assessing oxidative damage after burn trauma, *in vivo* EPR using nitroxides is complementary to NMR since NMR cannot measure redox status and reactive oxygen species (ROS), while EPR can. Here, for the first time, we report tissue partial pressure of oxygen (pO2), redox status and ROS measurement by *in vivo* EPR in intact proximal skeletal muscle tissue following burn trauma in mice. The potential significance of our findings includes the *in vivo* non-invasive nature of the EPR measurements, which can serve to follow tissue pathology and to monitor the effectiveness of antioxidant agents in order to alleviate the symptoms of severe burn trauma. The development and application of *in vivo* EPR oximetry [2-5] in the clinical management of burn injury alongside NMR might also prove to be very useful.

## Material and Methods

Male 6-week-old CD1 mice weighing 20-25 g were anesthetized by intraperitoneal (i.p.) injection of 40 mg/kg pentobarbital sodium and were randomized into burn or control groups. The left hind limb of all mice in both groups (control and burn) was shaved. Each burned mouse was subjected to a nonlethal scald injury of 3-5% total body surface area (TBSA) by immersing its left hind limb in 90°C water for 3 sec [6]. The gastrocnemius muscle was excised from the hind limbs of both control and treated mice and was immersed in 1 ml Trizol. All animal experiments were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital, Boston, MA, and by the Institutional Animal Care and Use Committee of Dartmouth Medical School, Hanover, NH. EPR measurements were carried out with a 1.2-GHz EPR spectrometer equipped with a microwave bridge and external loop resonator specially designed for *in vivo* experiments. The optimal spectrometer parameters were: incident microwave power, 10 mW; magnetic field center, 400 gauss; modulation frequency, 27 kHz. We measured partial pressure of oxygen (pO2) levels, redox status and oxidative stress following a non-lethal burn trauma model to the left hind limb of mice.

## Results

The results obtained for redox measurements are shown in Fig. 1. The control mice had the same skeletal muscle redox status on days 0 and 3. No significant difference was found in the decay rates of the nitroxide at day 0 (immediately post burn) and day 3 between the control and burn groups. The decay rate of the nitroxide in the burn group at 6 h (0.000604± 0.00008 sec-1) differed significantly from the decay rate in the control group at day 0 (0.00165±0.00024 sec-1, P=0.027) and from the burn group at time 0 (0.00143±0.00151 sec-1, P=0.018, t-test).We measured ROS production with EPR *in vivo* using of the oxidation of hydroxylamine (CP-H). Before burn (baseline), a low-intensity EPR signal was detectable from the mouse hind limb. Twelve hours after burn, the oxidized CP-H signal increased, indicating an increase in ROS generation by the burn. The oxidized CP-H signal increased between 12 and 24 h, suggesting a further increase in ROS formation (Fig. 2). No such increase in the EPR signal of the nitroxide was observed in the control group at all time points. The EPR results confirmed genomic results, which indicate a down-regulation of antioxidant genes.

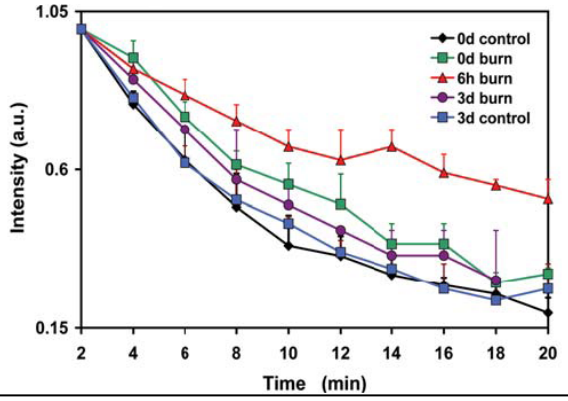


**Figure 1.** Decay kinetics of the nitroxide (CPA) in the gastrocnemius muscle in control and burn mice. The redox status of the control mice was measured at day 0 (0d) and day 3 (3d). The redox status of the burn mice was measured at day 0, 6 h and day 3. Values are means ± SE; *p<0.05, n=4.

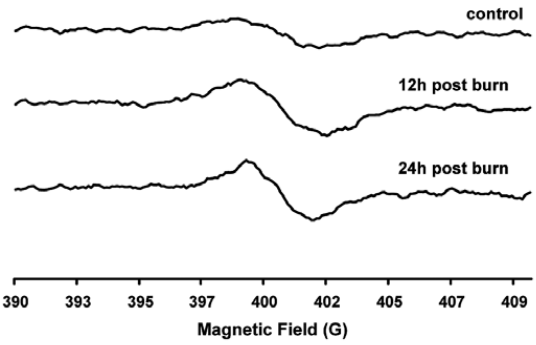


**Figure 2.** Typical *in vivo* EPR spectra of the oxidized CPH spin trap, collected over the gastrocnemius tissue of the mice pre-, 12 h and 24 h post burn trauma. The oxidized CPH spin trap was injected via the tail vein. The oxidation of the hydroxylamine to the nitroxide occurs due to its oxidation by ROS in the tissue.

## Discussion

Our results strongly suggest the dysfunction of the mitochondrial oxidative system. We believe that the direct measurement of tissue parameters such as pO2, redox and ROS by EPR may be used to complement measurements by nuclear magnetic resonance (NMR) in order to assess tissue damage and the therapeutic effectiveness of antioxidant agents in severe burn trauma.

## References

1. Gallez B and Swartz HM: In vivo EPR: when, how and why? NMR Biomed 17: 223-225, 2004.
2. Khan N, Williams BB, Hou H, Li H and Swartz HM: Repetitive tissue pO2 measurements by electron paramagnetic resonance oximetry: current status and future potential for experimental and clinical studies. Antioxid Redox Signal 9: 1169-1182, 2007.
3. Towner RA, Sturgeon SA, Khan N, Hou H and Swartz HM: *In vivo* assessment of nodularin-induced hepatotoxicity in the rat using magnetic resonance techniques (MRI, MRS and EPR oximetry). Chem Biol Interact 139: 231-250, 2002.
4. Swartz HM, Khan N and Khramtsov VV: Use of electron paramagnetic resonance spectroscopy to evaluate the redox state *in vivo*. Antioxid Redox Signal 9: 1757-1771, 2007.
5. Khan N, Williams B and Swartz H: Clinical applications of *in vivo* EPR: rationale and initial results. Appl Magn Reson 30: 185-199, 2006.
6. Tomera JF and Martyn J: Systemic effects of single hindlimb burn injury on skeletal muscle function and cyclic nucleotide levels in the murine model. Burns Incl Therm Inj 14: 210-219, 1988.