In vivo Electron Paramagnetic Resonance detects oxidative stress in 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 non-lethal scaldf 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 control mice was measured at day 0 (0d) and day 3 (3d). The redox status of the muscle 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 1. Decay kinetics of the nitoxide (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
