Noninvasive in vivo redox status study by fast acquisition proton electron double resonance imaging

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Introduction

It is well proved that by measuring the distribution, clearance and metabolism of nitroxide spin probe, the redox status of a biological sample can be established. EPR imaging (EPRI) so far is the most popular method in this area. In order to map and follow fast metabolism state of many kinds of free radicals *in vivo*, temporal resolution becomes very critical, so is the spatial resolution. Proton electron double resonance imaging (PEDRI) (1) based on the Overhauser effect is a novel and promising double magnetic resonance technique enables imaging free radicals. Enhanced/altered NMR signal (both intensity and phase) is detected during or after EPR irradiation. PEDRI has certain advantages such as high spatial and temporal resolution over traditional CW EPRI technique. After the polarizations of the surrounding unpaired electron spins are transferred to the water protons, many fast pulsed NMR sequences can be utilized in PEDRI. And since NMR signal is detected; the much stronger gradient requirement by EPRI can be replaced by the much lower and regular MRI gradient.

Method and experimental protocol

Fast acquisition PEDRI sequence based on a fast spin echo sequence was developed (2). Mice (body weight ~30 gram) were treated with Cardax (10 mg/kg) delivered via daily i.v. infusion for a period of 4 days. After the last treatment, the animals were anesthetized using ketamine/xylazine and ~ 0.6 mL of the spin probe Tempone (100 mM in PBS) was infused via tail vein over 50 - 55 s. The PEDRI images were acquired immediately following infusion of the redox probe for every 10 - 30 s until the image intensity became "minimum". Tempone (4-oxo-2, 2, 6, 6 -tetramethylpiperidinyl-N-oxy) was chosen as spin probe because of its high metabolic reduction rate and relatively narrow EPR line width. EPR-NMR double resonator for this PEDRI redox study was built. Experimental parameters are as followed: NMR Frequency, 847 kHz; EPR Frequency, 561 MHz; Receiver band-width, 10 kHz; Slice thickness, 25 mm; Field of volume: 8 cm (cropped to 6 cm in the final image); Image matrix, 64x64; EPR irradiation power, ~ 5 W; EPR irradiation time, 4 x 400 ms x 2= 3.2 s; Time of acqusition, 2 x (430 ms + 400 ms) x 4 ≈ 7 s.

Result

Four untreated (control) mice and four treated mice were studied. Time couse images of whole-body mouse were acquired for each mouse (see Figure 1 for a typical example). The image analysis was focoused on the heart region. The data from the heart area were fitted using a single exponential pharmacokinetic function to obtain half-time (**t**) for the decay of the nitroxide signal intensity.The half-time is inversely proportional to the decay rate, thus a larger value of **t** corresponds to a slower rate of decay of the nitroxide probe. The data obtained from the time-course PEDRI images of the heart region demonstrated a significant difference in the **t** values between the control and treated mice. Treated mice had a higher **t** value (0.99±0.14 min) compared to the untreated mice (0.49±0.08 min).This means that the treated mice showed a slower rate of nitroxide reduction in the heart compared to the untreated animals.

Conclusion

Fast acquisition PEDRI pulse sequence made it possible to follow the rapid *in vivo* tissue metabolism status. The decay rate of the spin probe is dependent on the cellular redox environment and is susceptible to both bioreduction and oxidation. Thus, a decrease in cellular oxidizing species (oxidants) would be expected to slow down the decay of the spin probe. It was demonstrated that Cardax treatment (i) significantly altered the tissue redox status of the heart and (ii) significantly reduced the oxidant levels in the heart of treated mice.

Reference

1. Lurie DJ, Bussell DM, Bell LH, Mallard JR. Proton electron double magnetic-resonance imaging of free-radical solutions. J Magn Res 1988; 76:366-3702.

2. H. Li, Z. Sun, Y. Deng, S. Petryakov, P. Kuppusamy, J.L. Zweier. Proc. Intl. Soc. Mag. Reson. Med. 13 (2005), Page 2547.

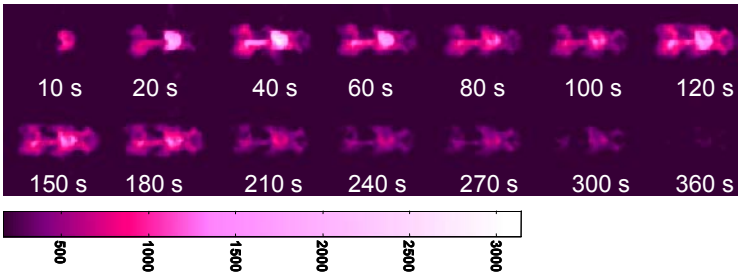

Figure 1. Whole-body time-course fast acquisition PEDRI images of tempone distribution in a treated mouse. Head part is on the right, tail part is on the left side. The distribution in the heart and the kidneys were clear observed, each image took about 7 s.