Application of PEDRI to Measure the In vivo Distribution and Clearance of a Triaryl Methyl Radical in Mice

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Introduction

Proton-Electron Double-Resonance Imaging (PEDRI) provides a method for *in vivo* or *ex vivo* imaging of free radicals in biological tissues and living animals [1]. This technique is based on the Overhauser effect with irradiation and saturation of the electron spin system during the acquisition of a proton magnetic resonance image. An enhancement of the NMR signal of up to two orders of magnitude is possible and this ehancement enables mapping of the spatial distribution of the free radical.

Recently, a new class of radical labels have been developed which are especially well suited for *in vivo* and potential clinical applications of PEDRI [2,3]. These triaryl methyl radicals (TAM) exhibit a sharp single line EPR spectrum (anaerobic linewidth 15 mG) and possess unusually long values of T_{1e} and T_{2e}. The labels have the potential to provide important information regarding tissue perfusion and tissue oxygenation. Using these labels a specific PEDRI technique has been developed to enable mapping of tissue oxygenation [2].

To utilize these labels for a particular *in vivo* experiment, it is important to know the distribution of the label and the pharmacokinetics of its clearance. With the development of genetically engineered mice where specific molecular alterations can be made to explore the molecular mechanisms of disease, there has been a great need for noninvasive techniques to measure *in vivo* metabolism in murine models. In order to utilize the new TAM radicals for PEDRI based measurement of tissue perfusion and oxygenation, it is first necessary to determine the process of their *in vivo* distribution and clearance. In this study, we apply PEDRI with a modified clinical MR imaging instrument [4] to determine the *in vivo* distribution and clearance of a TAM radical label in mice following intravenous administration.

Methods

The PEDRI instrument was developed based on modification of a 0.4 T resistive-magnet based clinical MRI system. This system was modified to provide appropriate conditions for PEDRI measurements in mice. The system parameters and resonator designs used are:
Magnetic field (fixed): 201 Gauss (20.1 mT)
EPR frequency: 564 MHz, NMR frequency: 856 kHz
PEDRI double-resonance coil: NMR coil (a solenoid, diameter 4 cm length 6 cm) and EPR resonator (Alderman-Grant type, diameter 7 cm, length 7 cm) combined inside a cylindrical shield (diameter 14 cm, length 26 cm).
Scan parameters: FOV: 8x8 cm, TE (Time of Echo delay): 12 ms (using gradient echo pulse sequence), TR (Time of Repetition): 1200 ms, NEX (Number of Excitation): 1, Time of Acquisition: 2 min 40 s, Slice thickness: 30 mm, EPR Power: 10 W (gating, 400 ms on, 800 ms off).
Radical used: TAM, triaryl-methyl free radical (symmetric trityl) provided by Nycomed Innovation, Malmo, Sweden.

Results

Mice of approximately 40 gram weight were anesthetized with pentobarbital then a tail vein catheter was inserted and the mice were placed in the resonator. Initial sets of images were obtained in the absence of EPR excitation. The TAM probe was then infused, approximately 0.7 mmol/kg over 2 minutes and a series of MRI images obtained with EPR excitation. The image data was transferred from the HP-UNIX based acquisition computer to a PC and analysis of the image data was performed using laboratory-developed software. Figure 1 shows an example of the PEDRI image observed after loading with the TAM probe. In this image the anatomical structures of the heart, major vessels, kidney and bladder are clearly seen. Each of these organs was contoured as shown and the intensity of the images at these anatomic locations was plotted as a function of time in order to provide information regarding the distribution and clearance of the TAM probe [Fig. 2]. As expected, following intravenous loading TAM is initially seen primarily within the vascular space (heart and great vessels) and then is increasingly taken up by the kidney and excreted into the bladder. Clearance from the vascular space is almost complete within 10 minutes.

Conclusions

Thus, PEDRI enabled measurement and mapping of the distribution and clearance of the TAM radical within the body of mice. It was observed that TAM was rapidly cleared from the vascular space. In spite of the low field (201 Gauss) used, good quality images were obtainable even with a single excitation. With incorporation of field-cycling (FC-PEDRI) enabling measurements at higher field as well as faster imaging pulse sequences, it should be possible to further enhance the obtainable signal to noise ratio, image quality and speed of image acquisition.

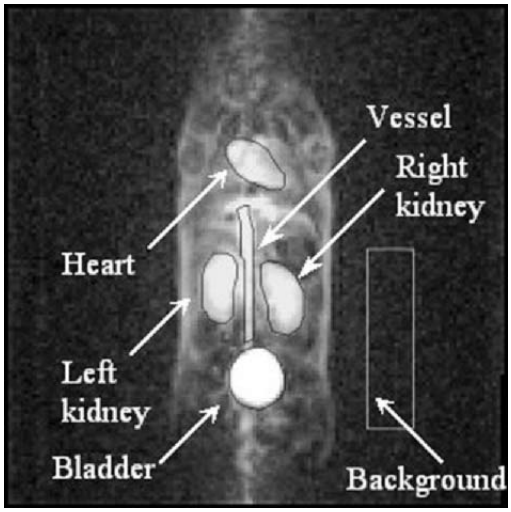

Fig. 1

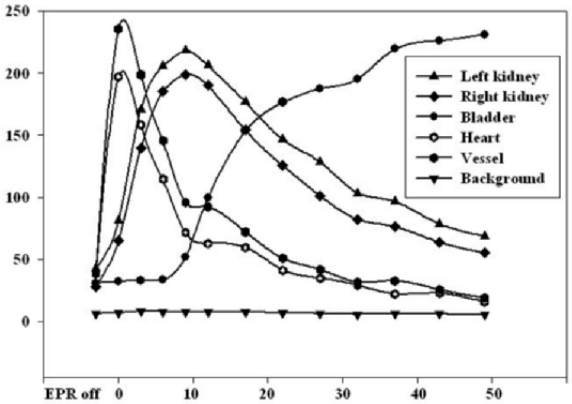

Fig. 2 Average signal intensity vs time (minutes)

References

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