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Free radicals imaged *in vivo* in the rat by using proton–electron double-resonance imaging

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A new technique called proton–electron double-resonance imaging is described for imaging free radicals in aqueous samples. The method is a combination of proton NMR imaging with nuclear electron double resonance.

The results of using this technique to image free radicals *in vivo* in the rat are presented. Rats were injected intravenously with a nitroxide free radical solution and a series of images was obtained from which the clearance of the free radical through the liver and kidneys could be observed.

1. Introduction

Free radicals may be defined as molecules which have an unpaired electron. The ability to image their distribution *in vivo* has many potential applications in biology and medicine.

Naturally occurring free radicals have been implicated in many disease states including carcinogenesis and inflammatory disease (Slater 1984). Oxygen-derived free radicals are thought to cause 'reperfusion injury', for example following myocardial infarction (Zweier *et al.* 1987).

Although endogenous free radicals generally occur at concentrations less than 1 μM, making their direct detection difficult, certain 'stable' free radicals can be introduced into animals at sub-toxic levels and these may generate useful information. For example, the electron paramagnetic resonance (EPR) characteristics of nitroxide free radicals are known to depend on the concentration of dissolved oxygen (Morse & Swartz 1985); this phenomenon might be exploited for *in vivo* oximetry. Pharmacokinetic studies may be possible by labelling a molecule of interest with a nitroxide free radical and following its progress through the body.

Free radicals are conventionally detected directly by using EPR. EPR imaging is being developed with the aim of detecting free radicals *in vivo*, but has so far been confined to small samples of dimension 1 cm or less, mainly because very large magnetic field gradients must be used (Ohno 1986).

2. Proton–electron double-resonance imaging

We have recently developed a new technique called proton–electron double-resonance imaging (PEDRI) for imaging free radicals in large aqueous samples (Lurie *et al.* 1988). In this work we have used PEDRI to image the distribution of a nitroxide free radical injected into the bloodstream of the rat.

PEDRI is a combination of proton NMR imaging with nuclear electron double

Figure 1. NMR signals obtained at 0.04 T from a 2.5 mM aqueous solution of TEMPOL: (*a*) without EPR irradiation; (*b*) with EPR irradiation at 1.12 GHz (same vertical scale as (*a*)).

resonance (Dwek *et al.* 1969): a proton NMR image is collected while the EPR resonance of a free radical solute is irradiated. In parts of the sample where cross relaxation is occurring between the proton and electron spins the NMR signal is enhanced, and these regions appear with greater intensity in the final image, revealing the distribution of the free radical.

The enhancement of the NMR signal upon irradiation of the solute's EPR may be written empirically as

$$E = A_z/A_0, \tag{1}$$

where A_z and A_0 are the NMR signal amplitudes with and without EPR irradiation. The maximum possible enhancement factor is given by $-|\gamma_s|/2\gamma_p = -330$ where γ_s and γ_p are the electron and proton gyromagnetic ratios. (A negative enhancement factor means that the NMR signal changes phase by 180° when the EPR is irradiated.)

The observed enhancement factor depends on a number of factors including the type and concentration of free radical and the power applied in the EPR irradiation. Assuming that the interaction between proton and electron spins is mainly via a dipole–dipole interaction, applying a high power EPR irradiation will result in a large negative enhancement factor. As the RF power is reduced to very low levels E passes through zero and becomes positive but less than unity. The effect on the NMR signal of an intermediate-power EPR irradiation is illustrated in figure 1.

For *in vivo* studies the power applied in the EPR irradiation must be minimized. Thus a low magnetic field B_0 should be used, since the power required to achieve a given enhancement factor is approximately proportional to B_0^2 (Lurie *et al.* 1989).

3. Apparatus

PEDRI was implemented on our home-built, whole-body proton NMR imager which has a four-coil, vertical-field, resistive magnet (Mallard *et al.* 1980). The magnet was operated at 0.01 T, giving NMR and EPR frequencies of 425 kHz and 238 MHz respectively, the latter being the lowest frequency EPR line of the nitroxide's hyperfine triplet. A solenoidal NMR coil (diameter 7.5 cm) was used for both transmission and reception while the EPR irradiation was applied using an Alderman–Grant resonator (Alderman & Grant 1979) placed coaxially outside the NMR coil.

4. Experimental

Adult Sprague–Dawley rats (300–350 g) were given an intraperitoneal injection of 50 mg kg^{-1} xylazine plus 50 mg kg^{-1} ketamine to produce surgical anaesthesia before cannulation of the external jugular vein. Once positioned inside the imager the

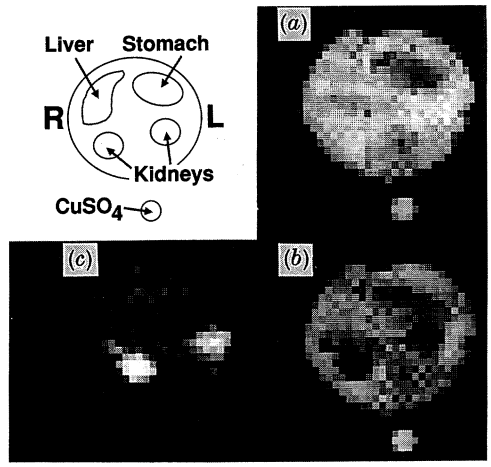

Figure 2. Images of a rat obtained 3 min after intravenous administration of PCA free radical solution (see text). Field-of-view 7.5 cm square; slice thickness 1.5 cm: (a) image obtained without EPR irradiation; (b) image obtained with EPR irradiation at 238 MHz; (c) 'difference' image showing only regions containing free radical. Line drawing (upper left) shows main anatomical features.

animals were given a 1 ml dose of 150 mM bicarbonate-buffered nitroxide free radical, administered over the course of 1 min through the cannula. In this study the free radical used was 3-carboxy-proxyl (PCA), which had been found by other workers to be broken down only relatively slowly *in vivo* (Ehman *et al.* 1985). The quantity administered here was less than 4% of the LD50 for PCA in the rat (Ehman *et al.* 1985).

An interleaved saturation-recovery spin-warp pulse sequence was used in which the EPR irradiation was applied only before alternate NMR 90° pulses, allowing images with and without EPR irradiation to be collected simultaneously. The repetition time (between 90° pulses) was 500 ms and the length of the EPR irradiation was 300 ms, the instantaneous and average applied RF power being 30 W and 9 W respectively. Images were collected on a 32×32 matrix and two averages were taken so that each image pair (EPR on, EPR off) took 64 s to collect. During image processing the data-sets for the two images were subtracted, yielding a 'difference' image showing only those regions of the sample containing free radical.

5. Results

Following injection of the free radical a series of 17 images was obtained, from which the fate of the free radical was followed over *ca.* 25 min. Figure 2 shows the image set obtained 3 min after injection. Only the stomach can be distinguished from the 'without-EPR' image, while the kidneys can be seen clearly as low-signal regions in the image obtained with EPR irradiation. This is due to the presence of the free radical, the enhancement factor in this case being close to zero. In the difference image the kidneys are immediately visible as intense regions, while the liver can also be seen at lower intensity. A sample tube containing copper-sulphate-doped water and placed immediately below the animal was a check on the correct operation of the pulse sequence: it cannot be seen in the difference image.

6. Conclusions

We have demonstrated that PEDRI can be used to follow the progress of nitroxide free radicals injected intravenously into experimental animals. The same technique has recently been used to image Fremy's salt injected into the peritoneum of a rat (Grucker 1990). Our results indicate that useful pharmacokinetic studies can be performed using PEDRI.

Although the signal-to-noise ratio (SNR) is relatively poor at present, the use of field-cycled PEDRI (FC-PEDRI) should improve this, also increasing the sensitivity of the technique (Lurie *et al.* 1989). (In FC-PEDRI B_0 is changed throughout the pulse sequence, being lowered for the EPR irradiation and increased for the NMR detection, giving low RF power levels and good SNR.)

Future work will include the study of other free radicals as tracers and their use to monitor oxygen concentrations *in vivo*. At present the lowest detectable concentration of free radicals in our apparatus is approximately 50 μM. If the sensitivity is increased it may eventually be possible to detect endogenous free radicals, perhaps with the aid of spin trapping.

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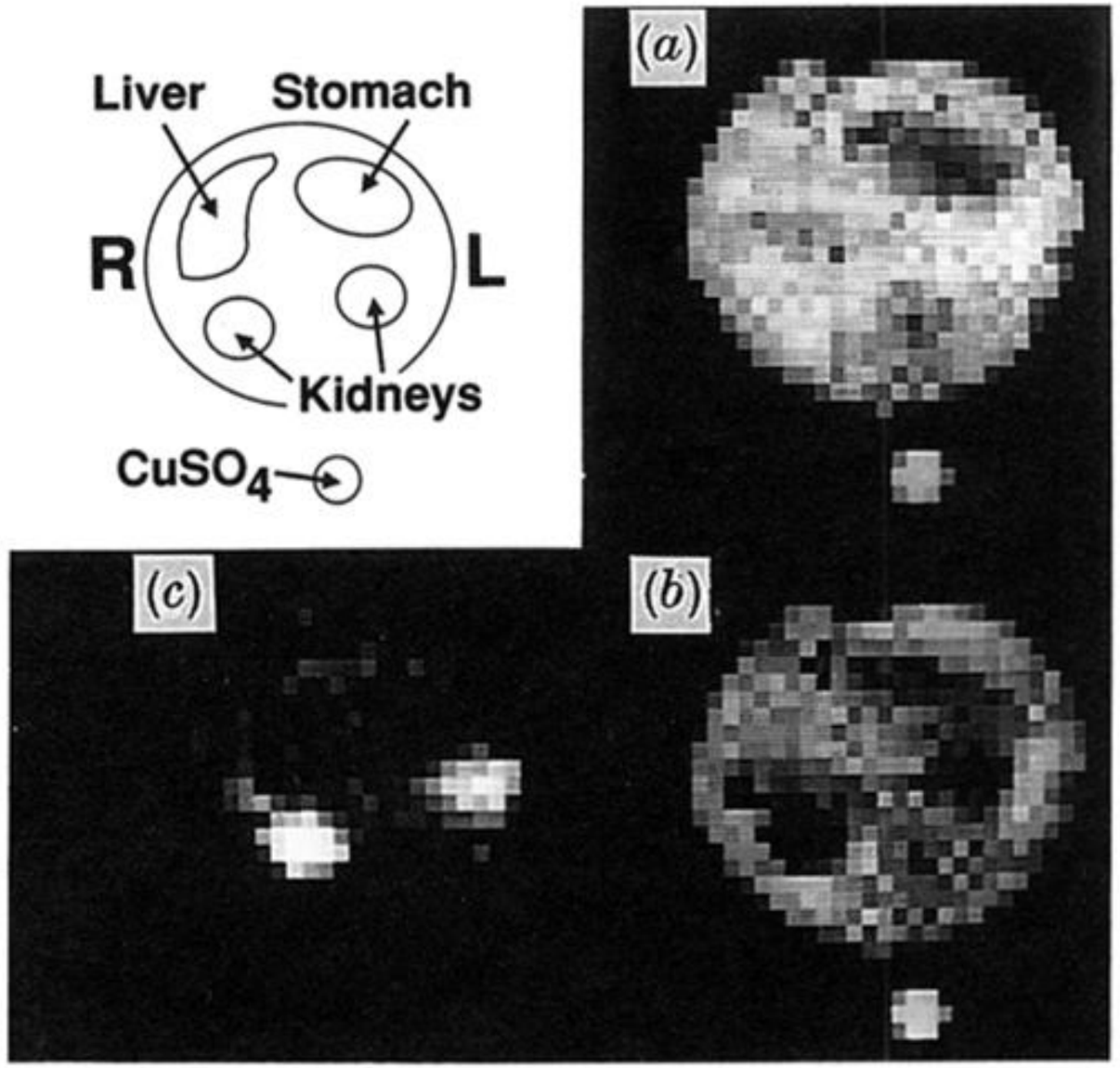

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