Proton Electron Double Resonance Imaging Enables Real-time Mapping of Risk Region and Necrosis in the Postischemic Heart

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Introduction

Proton electron double resonance imaging (PEDRI) is a powerful technique enabling imaging of free radicals based on the Overhauser effect whereby saturation of the EPR resonance results in up to 300-fold enhancement of the proton NMR signal [1]. It combines the advantages of proton MRI (high spatial and temporal resolution with relatively low gradient field) and EPR (enabling detection of free radicals in biological samples). Early coronary artery reperfusion is critical to limit heart muscle death in acute myocardial infarction. But it is also well known that further myocardial injury occurs during reperfusion. So it is very important to have a way to perform real-time monitoring of myocardial injury. Myocardial redox state is a critical parameter in the evaluation of the tissue viability and injury, and can be evaluated using nitroxide spin probes. Nitroxide radicals have been proven to be versatile spin probes and were widely used to study tissue metabolism. In isolated beating rat hearts the absorbed non-resonance energy from the EPR irradiation used in PEDRI can be readily compensated by the perfusion buffer. All of these make PEDRI a very suitable technique to perform real-time mapping the free radicals in the isolated heart under normal, regional ischemia and reperfusion conditions. In our study, by comparing time course change of metabolism characteristic inside the risk region with normal myocardium, the risk and necrotic area were evaluated and mapped. TEMPONE (4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl) was chosen in this study because of its high metabolic reduction rate and relatively narrow EPR linewidth.

Materials and Methods

The imaging system is a lab-developed low field PEDRI system based on a clinical RESONEX 0.4T system [2], the field in these experiments was 20.9 mT, with NMR frequency of 892 KHz and EPR frequency of 592 MHz. A better performance EPR-NMR double resonator (inner diameter of 22 mm for imaging with NMR coil of 40 mm Length and EPR resonator of 60 mm length) was built. Specifically the EPR resonator was modified from a typical Alderman-Grant resonator: High temperature stability was achieved by using two quartz tubes (inner diameter of 60 mm and 55 mm respectively) with thick (0.8 mm) copper foil. Matching was achieved using an electronic capacitance coupling circuit, which resulted in easy tuning and coupling with a wide range (50 MHz). Rat hearts were retrogradely perfused at 37 ºC with an oxygenated modified Krebs-Heinseleit solution [3]. Regional ischemia and reperfusion were performed by tightening and loosing the thread around the left anterior descending coronary artery (LAD) correspondingly. Heart temperature was monitored by LUXTRON "FOT Lab Kit" fluoroptic thermometer. Experimental protocol is shown in Table 1. The LAD occluded heart was infused with Evans blue (2% solution) through the cannulated aorta and then the heart was frozen, cut into 2 mm thick transverse slices and incubated in a 1% triphenylterazolium chloride (TTC) solution for 20 min to visualize the risk region and infarcted myocardium.

Table 1. Protocol for PEDRI Imaging of Myocardial Infarction in Isolated Rat Hearts during Regional Ischemia-Reperfusion

Buffer Perfusion Control Heart			Buffer Perfusion LAD-occluded Heart			Buffer Perfusion LAD-released Heart		
2D PEDRI TEMPONE uptake images 10 min	3D PEDRI image 5 min	2D PEDRI TEMPONE clearance images 10 min	TEMPONE uptake 5 min	2D,3D PEDRI images 15 min		2D PEDRI TEMPONE uptake images 10 min	3D PEDRI image 5 min	2D PEDRI TEMPONE clearance images 10 min
0 ↔ 30 min			30 ↔ 60 min			Repeat: 60 ↔ 90 min, 90 ↔ 120 min, 120 ↔ 150 min, 150 ↔ 180 min		

Results and Conclusion

Good quality PEDRI 2D images (TR: 0.20 s; TE: 12 ms; matrix: 64x128; NEX: 2; slice thickness: 6 mm; FOV: 8 cm; Acq. Time: 35 s) and 3D images (TR: 0.07 s; TE: 26 ms; flip angle: 40.0; matrix: 128x128x16; NEX: 2; FOV: 9 x 9 x 3.2 cm; Acq. Time: 4.5 min) were acquired. Fig.1 through Fig.3 are results of one heart; this heart was subject to 55 min regional ischemia before reperfusion. In Fig.2 the regionally ischemic area (void in the left ventricular wall) was clearly seen and the size of the ischemic risk area is close to the histology result in Fig.3. After 90 min reperfusion, the myocardial redox state was studied by time course measurements from 2D PEDRI images of TEMPONE clearance in Fig.1. By following the localized signal intensity over time, mapping of redox rates in different ROI was performed (results not shown). Following reperfusion infarcted myocardium within the risk zone reduces the TEMPONE much slower than before ischemia or compared to that outside the risk zone. as a result, the infarcted myocardium within the risk zone appears brighter than the non-risk zone, and the image intensity decreases much slower. Each set of 2D PEDRI clearance images after different ischemic and reperfusion times were studied. The infarction within the risk zone was evaluated through the whole experiment. The EPR power of 12 W applied at the resonator induced only slight reversible changes in heart temperature (~1°C) and function (<10%).

In conclusion, PEDRI was demonstrated to be a novel and non-invasive technique enabling real time visualization of alterations in free radical metabolism, redox state, risk region and viability following the onset of myocardial ischemia.

Reference

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2. Lurie DJ, Li H, Petryakov S, Zweier JL. Development of a PEDRI free-radical imager using a 0.38 T clinical MRI system. Magn Reson Med 2002;47:181-186.
3. Liebgott T, Li H, Deng Y, Zweier JL. Proton electron double resonance imaging (PEDRI) of the isolated beating rat heart. Magn Reson Med 2003;50:391-399.

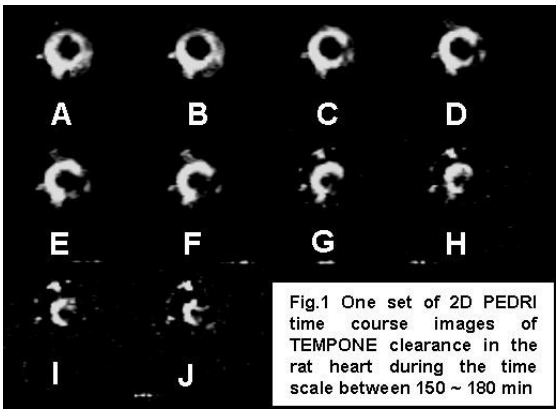

Fig.1 One set of 2D PEDRI time course images of TEMPONE clearance in the rat heart during the time scale between 150 ~ 180 min

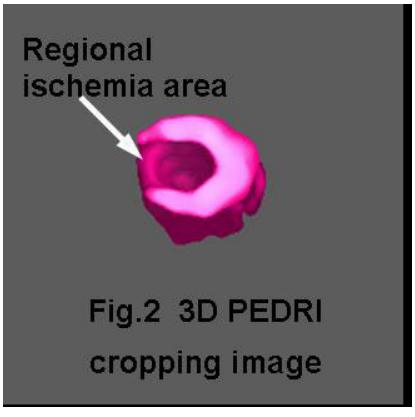

Fig.2 3D PEDRI cropping image

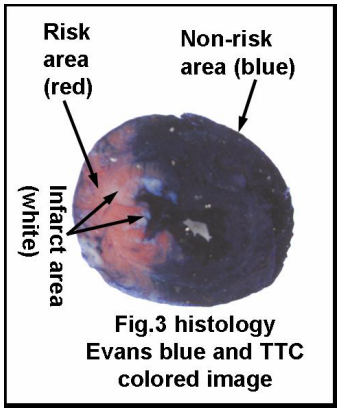

Fig.3 histology Evans blue and TTC colored image