Monitoring kidney viability before transplantation by means of 31P CSI and oxygenated hypothermic perfusion

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Introduction

Monitoring viability of kidney before transplantation has become critical with the increasing need of marginal organs. 31P MRS is able to assess energy metabolites such as nucleotide triphosphates (NTP), inorganic phosphate (Pi) and cell phosphomonoester (PME). During cold storage, ATP decreases rapidly and is partially metabolized in PME. Therefore PME/Pi has been proposed as useful indicator to assess kidney viability [1]. In this paper, we show that ATP can be directly detected in the presence of O2, which provides direct evidence of cell integrity. This new approach can be used for kidney monitoring during cold storage before transplantation. In this paper, we have tested the effect of cold static storage (CSS) versus oxygenated perfusion on kidney metabolic status.

Material and Methods

HPP perfusion MRI compatible oxygenated hypothermic pulsatile perfusion (O2+HPP) system was used to maintain the kidney in cold storage (4° C) using 25 mmole phosphorus medium (KPS-1). Oxygen tension of pO2 ≥ 100kPa was maintained during HPP. Two storage conditions were tested: In the first experience, kidney was perfused (O2+HPP) immediately after resection for 8 hours, than was maintained in cold static storage (CSS) for 10 hours, and reperfused (O2+HPP) for 10 hours. In the second experiment, the kidney was maintained under CSS for 18 hours after resection, following by 10 hours of O2+HPP.

MR acquisition MRI/MRS was performed at 3T (Trio, Siemens), with home made 31P interface and surface coil. 1H imaging and shimming were performed with the body coil. T2 sequence (TSE, TR 5000ms, TE 108 ms, 3 mm slices) was used as localizer. 31P MRS consisted of 3D CSI, 16x16x8, resolution 1.56x1.56x2.5 cm^3, TR 1000 ms, weighted k-space, acquisition time 44 min.

Data Analysis Processing was performed with SAGE software (General Electric Medical Systems) and consisted of zero filling, 10 Hz exponential apodization, and FFT. Acquisition delay (2.5 ms) was corrected with a first order phase correction and *sinc* deconvolution. Individual spectra were corrected for frequency shift and averaged. The assessment of the metabolites concentrations was performed using the Pi peak as internal reference (25 mMolar).

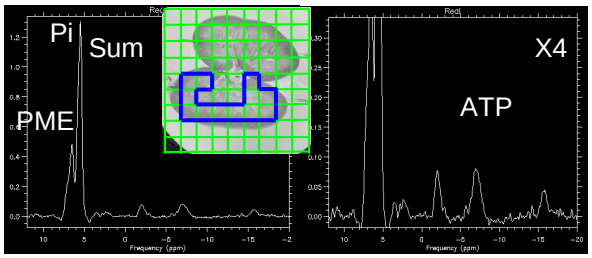
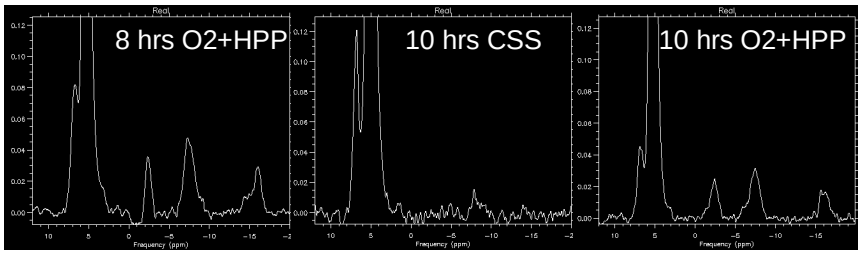


Figure 1: *31P CSI sum spectrum (selection in blue) during O2+HPP (100 kPa). The 3 ATP resonances are detected at -16 ppm, -7.5 ppm and at -2.3 ppm. PME peak is identified at 7 ppm.*

Results

Figure 1 demonstrates that ATP is resynthesized with oxygenated HPP (pO2 ≥ 100 kPa). In addition, the peak at 7 ppm representing PME is clearly visible. Intracellular Pi cannot be distinguished from the Pi of the perfusion medium. Normalization to Pi peak area yields an ATP concentration of 1.5mMolar and a PME level of 3.9 mMolar. Figure 2 illustrates the first preservation condition: we note a complete disappearance of ATP when CSS alone (no perfusion) is applied. Only a slight resonance at -8 ppm is visible, which may correspond to nicotinamide nucleotides (NAD). ATP is resynthesized when O2+HPP is reinitiated for 10 hours. Normalization to Pi (right) shows that PME level remains high and ATP returns at 25% of its initial value. Figure 3 represents the second preservation condition: again ATP is not visible upon CSS alone (no perfusion). Only a slight amount of ATP can be resynthesized when O2+HPP is applied for 10 hours after 18 hours of CSS. In this condition, PME level declines gradually (figure 3, right).



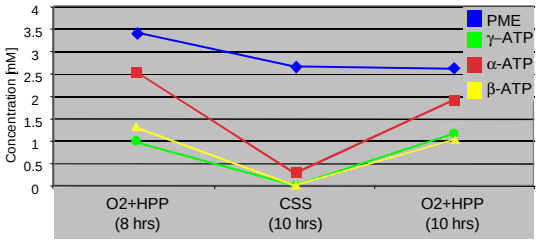
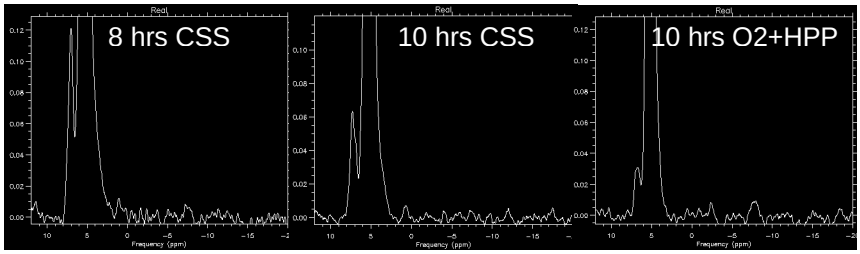


Figure 2: *31P CSI sum sectra (left) of the same kidney obtained at three different time points of the first preservation condition: at 8 hours of O2+HPP (applied immediately after kidney harvesting), following by 10 hours of CSS and again by 10 hours of O2+HPP reperfusion. Right: metabolite levels using Pi as reference.*



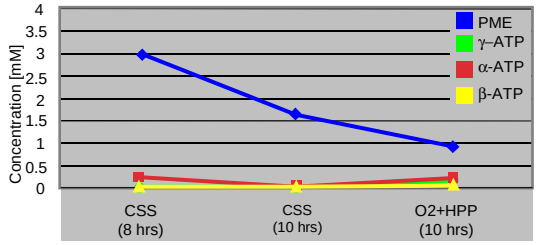


Figure 3: *31P CSI sum sectra (left) kidney monitoring corresponding to the second preservation condition: at 8 and 10 hours of CSS (no perfusion), following by 10 hours of O2+HPP reperfusion. Only slight ATP (< 0.5 mM) can recover. Right: metabolite levels using Pi as reference.*

Discussion and Conclusion

We have first demonstrated that ATP resynthesis during HPP is possible with O2 tension of 100 kPa. Our data show that ATP is resynthesized if O2+HPP is applied immediately after kidney harvesting and recover in a large extent after 10 hours of CSS. In opposition, only little ATP is visible if no perfusion is applied after kidney removal. This could reflect damage of the kidney, that may explain delayed graft function as frequently observed after CSS. PME level drops during CSS, in agreement with published data [2]. PME peak includes membrane phospholipids, sugar phosphate and AMP, which is a precursor of ATP. Thus, a reduction of PME during CSS may reflect in part a reduction of AMP, which in turn limits the ATP generation. Because the measurement of ATP provides direct evidence of cell bioenergetics, it is likely to provide a more robust marker for organ viability. Finally, our results demonstrate the benefit of oxygenated pulsatile perfusion for cold storage, which might become the standard for marginal donors organ preservation.

References [1] Bretan PN et al, Am Surg 1993, 59:182-87. [2] von Elverfeldt D et al, NMR Biomed 2007, 20:652-57.