Real Time Assessment of Krebs Cycle Metabolism with Hyperpolarized [2-13C]Pyruvate

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Introduction

The Krebs cycle plays a fundamental role in cardiac energy production and is often implicated in the energetic imbalances characteristic of heart disease. Previous measurements of Krebs cycle metabolism have used carbon-13 MR spectroscopy (MRS) combined with isotope analysis but these techniques have generally been limited to steady-state experiments. Metabolic imaging with hyperpolarized 13C MRS has enabled unprecedented visualization of real-time mechanisms of normal and abnormal metabolism. However, no hyperpolarized MR tracer, or other experimental technique, has routinely monitored instantaneous Krebs cycle metabolism in whole organs. The aim of this work was to use hyperpolarized [2-13C]pyruvate as an alternative metabolic tracer to enable the direct monitoring of Krebs cycle metabolism in normal and ischaemic hearts.

Methods

[2-13C]pyruvate was hyperpolarized in a HyperSense system (Oxford Instruments, UK) according to the method of Ardenkjaer-Larsen et al. Six rat hearts were perfused in the Langendorff mode and placed in the bore of an 11.7T vertical bore MR scanner. Hyperpolarized [2-13C]pyruvate was infused while the heart was functioning normally and spectra were acquired with 1s temporal resolution. A 10 min period of no-flow ischaemia was then initiated. A second dose of the same hyperpolarized tracer was infused immediately upon reperfusion. Peaks arising from hyperpolarized [2-13C]pyruvate were identified using high resolution 13C NMR and by examination of cross-peaks arising from 2D H/13C NMR on tissue extracts. Whole heart cardiac 13C MR spectra were analyzed using the AMARES algorithm and the results plotted against time to generate metabolic progression curves, as previously described.

The area under the metabolic progression curves for each metabolite was then compared between healthy and post-ischaemic states.

Results

[2-13C]pyruvate was found to polarize similarly to [1-13C]pyruvate and was successfully dissolved to provide polarization levels greater than 20%. The relaxation time was measured to be 34s, slightly reduced when compared to [1-13C]pyruvate at 45s. Figure 1 shows an example metabolic time course over 60s of spectral acquisition in a healthy heart. The appearance and subsequent decay of [2-13C]pyruvate can be seen at 205ppm. Peaks visible with 1s resolution were attributed to [5-13C]glutamate, [2-13C]citrate, [1-13C]acetyl-carnitine, [1-13C]pyruvate, [2-13C]pyruvate-hydrate, [2-13C]lactate and [2-13C]alanine. In the post-ischaemic heart, the [2-13C]lactate peak was seen to increase by 70% and the [5-13C]glutamate and [2-13C]citrate peaks were reduced by 43% and 41% respectively (Figure 2), when compared with the healthy state.

Discussion

These results demonstrate the first example of direct monitoring of instantaneous Krebs cycle metabolism. The entry of [2-13C]pyruvate into the Krebs cycle has been monitored with 1s temporal resolution and been shown to reveal differences between the healthy and the post-ischaemic heart. [2-13C]pyruvate may have great potential in assessment of impaired metabolism in heart disease.

References

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