Detecting tumor cell response to therapy using hyperpolarized ^{13}C MRS

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Objective: Dynamic Nuclear Polarisation (DNP) offers dramatic gains in sensitivity for the liquid state NMR experiment, with a greater then 10,000x gain demonstrated for the ^{13}C nucleus (1). The introduction of this technique creates the possibility to image ^{13}C-labelled molecules *in vivo* and, more importantly, their enzymatic interconversion into other cellular metabolites. This technique promises new insights into tissue function *in vivo*. The aim of the present study was to determine whether measurements of the uptake and metabolism of hyperpolarised [1-^{13}C]pyruvate by murine lymphoma cells could be used to detect the response of these cells to treatment with a DNA-damaging chemotherapeutic drug, etoposide.

Methods: [1-^{13}C]pyruvate was hyperpolarised in a 3.35 T hyperpolariser (GE Healthcare) as described previously (1) and added at a concentration of 40 mM to a suspension of murine lymphoma cells (EL-4) cells at 37ºC and a density of 2.5 x 10^7 cells/ml in a 10 mm NMR tube. ^{13}C NMR spectra were acquired at 1 s intervals for 250 s using a low flip angle pulse on a Varian INOVA spectrometer interfaced to a wide-bore Oxford Instruments 9.4 T magnet (100.6 MHz ^{13}C NMR frequency). The exchange rate constants and spin lattice relaxation times of the ^{13}C label in lactate and pyruvate were determined by fitting the lactate and pyruvate peak intensities to the modified Bloch equations for two-site exchange. Apoptosis was induced by treating the cells for 16 h with 15μM etoposide and was assessed by staining them with acridine orange/ propidium iodide (2). Cell extracts were prepared for ^{31}P NMR spectroscopy from 6 x 10^8 cells, as described previously (3).

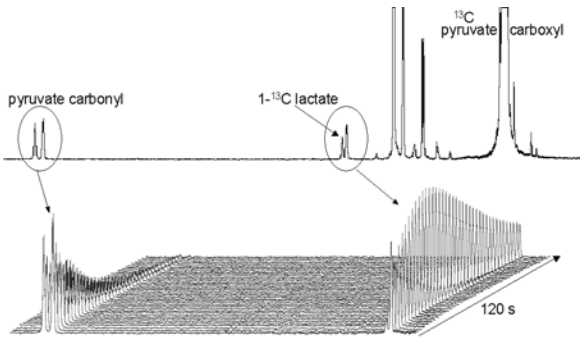

Figure 1: Stacked Plot showing ^{13}C NMR spectra. An increasing signal in the carboxyl carbon of lactate is visible, as is the decay of the hyperpolarisation from the natural abundance carbonyl signal.

Results and Discussion: Addition of 40 mM hyperpolarised [1-^{13}C]pyruvate to a suspension of EL-4 cells resulted in observable transfer of label to lactate (Fig. 1). Addition of exogenous lactate increased the amount of hyperpolarised ^{13}C label appearing in the lactate pool (Fig. 2). This latter result is inconsistent with net chemical flux from pyruvate to lactate and suggests instead that we are observing exchange of ^{13}C label between pyruvate and lactate in the reaction catalysed by the enzyme lactate dehydrogenase. In effect, this experiment measures the *in situ* activity of this enzyme (and also the activities of the lactate and pyruvate plasma membrane transporters). Induction of apoptosis, using the DNA-damaging drug etoposide, resulted in an approximately seven-fold decrease in the exchange flux (decreased from 0.013 mM s^{-1} to 0.002 mM s^{-1}, n=3, p<0.001). DNA damage activates the enzyme polyADPribose polymerase (PARP), which can deplete cells of their NAD(H) pool (3). Since NAD(H) is a coenzyme for LDH, this could explain the decrease in exchange flux. ^{31}P NMR of extracts of EL4 cells prepared 16 h after the addition of etoposide showed that there was substantial depletion of NAD+ (11 ± 25% of control cells) and that this could be largely prevented by addition of the PARP inhibitor nicotinamide (20 mM) to the cell suspension (81 ± 24% of control cells). Addition of 20 mM nicotinamide was shown to partially rescue the LDH-mediated exchange of label between the pyruvate and lactate pools (Fig. 3).

To summarise, addition of hyperpolarised [1-^{13}C]pyruvate to a murine lymphoma cell suspension results in LDH-catalyzed exchange of label with lactate. Loss of this exchange in cells treated with a DNA-damaging drug appears to be due to PARP-mediated depletion of the cellular NAD(H) pool. Thus measurements of this exchange in a tumor *in vivo* may be used to report on the response of the tumor to DNA-damaging chemotherapeutic drugs.

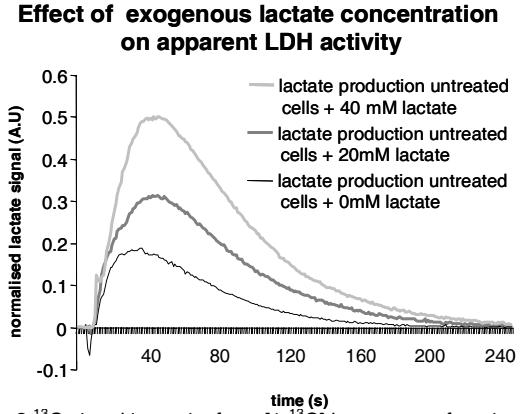

Fig. 2 ^{13}C signal intensity from [1-^{13}C] lactate as a function of time following addition of 40 mM hyperpolarised [1-^{13}C] pyruvate and varying concentrations of unlabelled lactate to an EL-4 cell suspension.

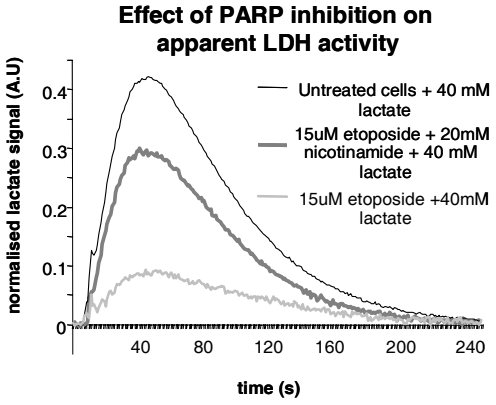

Fig. 3 Addition of 20mM nicotinamide partially rescues the loss of lactate-pyruvate exchange induced by incubation of EL-4 cells with etoposide.

1. Ardenkjaer-Larsen et al, (2003) Proc. Natl. Acad. Sci. USA 18, 10158-10163
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3. Williams, S. N. O., Anthony, M. L., and Brindle, K. M. (1998) Magn. Reson. Med. 40, 411-420.