Characterization of a hepatoma cell line in a novel 3D bioreactor flow system using hyperpolarized 13C MRS

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INTRODUCTION: Recent studies of hyperpolarized ^{13}C labeled compounds, by way of the DNP method [1], have been used to investigate metabolic processes associated with the Warburg effect. The Warburg intermediates (elevated lactate, alanine and pyruvate) have been used as a way to characterize cancer aggressiveness [2] and response to therapy based on changes in glycolysis [3]. With this in mind, the purpose of this study was to apply the DNP hyperpolarization method to a cancer cell line that will lead to potentially different metabolic cycles and behavior (Fig. 1). Hepatocytes (liver cells) have the potential to exhibit metabolism of ^{13}C labeled pyruvate outside of the predominant Warburg intermediates. In this study, JM1 (isolated rat hepatoma) cells were cultured in a 3D NMR compatible bioreactor to investigate the differential metabolism of this cell type and to determine the feasibility of applying this method to primary hepatocyte cultures.

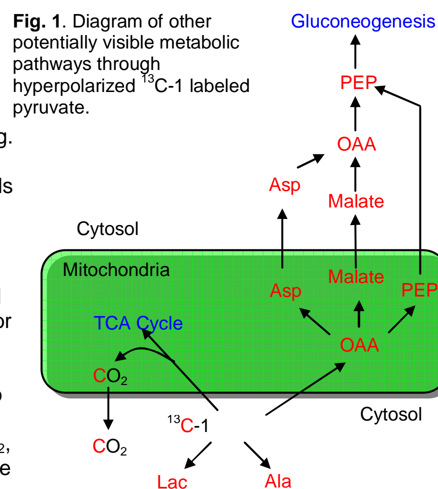

Fig. 1. Diagram of other potentially visible metabolic pathways through hyperpolarized ^{13}C-1 labeled pyruvate.

METHODS: $^{13}C_1$ pyruvate was hyperpolarized using the Hypersense (Oxford Instruments) as described previously (ref) and 1mL of 4mM or 2mM pyruvate was injected into a custom designed 10mm NMR compatible bioreactor flow system. The bioreactor is a completely contained 3D culture system (Fig. 2) with a continuous flow of 37°C media (containing RPMI, 10% FCS, and Penn/Strep) heated by water-jacketed inlet lines. Prior to entering the bioreactor, media is oxygenated using a Gas Exchange Module (GEM), which is filled with 95% Air/5% CO_2, to preserve physiologic conditions. At the time of injection, the flow system was set to flow 4mL/min. The bioreactor contained JM1 cells electrostatically encapsulated in 500µm alignate beads, with a total cell concentration of 2.5 x 10^7 cells/mL. ^{13}C NMR spectra were acquired in intervals of 3 sec using a 5° pulse for 300 secs on a narrow-bore 11.7T Varian INOVA (125MHz ^{13}C, Varian Instruments) equipped with a 10mm triple tune direct detect broadband probe. Prior to injection of the hyperpolarized compounds, a 16 hour time course of ^{31}P spectra (202MHz ^{31}P) was acquired with a 90° pulse, nt=2048, and at=1s to assess the b-NTP resonance as a function of time and infer cell health. The relative intensities of pryuvate and lactate were plotted as a function of time to determine the degree of saturation as a result of varying concentrations of injected pyruvate.

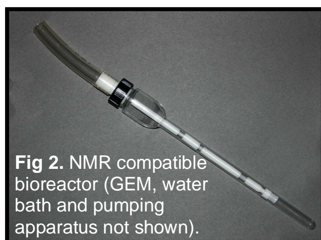

Fig 2. NMR compatible bioreactor (GEM, water bath and pumping apparatus not shown).

RESULTS: JM1 cells were encapsulated and the bioreactor was inoculated immediately after trypsinization. The plotted increase in ^{31}P b-NTP (Figure 3) prior to hyperpolarization studies is characteristic of JM1 cell growth during the first 24 hours post trypsinization and is indicative of healthy cell growth [4]. Figure 4 demonstrates the metabolism of 4mM $^{13}C_1$ pyruvate 60s after injection. Resonances corresponding to $^{13}C_1$ pyruvate, lactate, alanine, and pyruvate hydrate are shown analogous to previous studies of cancer [2]. These metabolites are readily visualized in the bioreactor with high signal to noise. Aside from these metaboites, resonances corresponding to intermediates PEP, malate, and OAA are visualized with low signal to noise. With the injection of varying concentrations of pyruvate, the production of lactate was measured as a function of time. Figure 5 demonstrates the differences in lactate production, or analogously lactate dehydrogenase activity with pyruvate concentration. Maximal lactate production is achieved 60 secs after injection. Injections of 2mM pyruvate yielded approximately half the relative concentration of lactate in comparison to 4mM pyruvate. These injections were done within 1 hour of each other and demonstrate the LDH activity of the same cell concentration.

Fig. 3

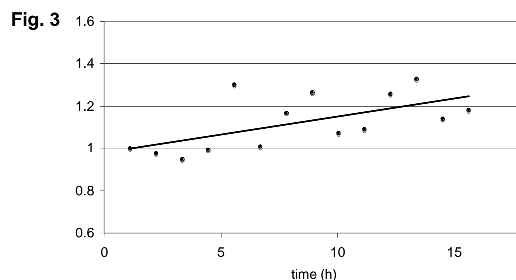

Fig. 4

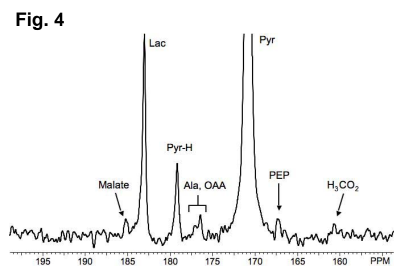

Fig. 5

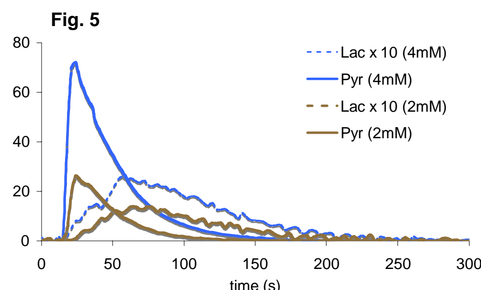

Fig. 3. Above is a time course of β-NTP measured by ^{31}P in the bioreactor. The increase in the triphosphorylated NTP is indiciative of cell health.

Fig 4. 4mM pyruvate metabolism in JM1 cells 60 secs after injection. Characteristic resonances of pyruvate, pyruvate-hydrate, lactate and alanine are shown.

Fig 5. Shows the metabolism of C1 labeled pyruvate to lactate at pyruvate concentrations of 2 and 4mM.

DISCUSSION AND CONCLUSIONS: This study demonstrates for the first time the application of hyperpolarized ^{13}C NMR spectroscopy to an NMR compatible 3D culture system. Injections of 2 and 4mM pyruvate demonstrated metabolism in the range of spectroscopic detection without saturation. JM1 time course data exhibited characteristic pyruvate metabolism analogous to previously presented time course data of tumor metabolism [2]. JM1 cell metabolism of labeled pyruvate also displayed metabolic intermediates corresponding to gluconeogenesis, which have not been previously visualized. This data suggests that through further study of this cell line and primary hepatocyte cultures, it is possible to visualize other metabolic processes, specifically intermediates of normal metabolism at higher resolution.

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ACKNOWLEDGEMENTS: National Institute of Health, NIH R21 EB007588 and R21 GM075941-01