QUANTITATIVE ANALYSIS FOR HYPERPOLARIZED ^{13}C-PYRUVATE IMAGING: COMPARISON OF METHODS ON A CLINICAL SYSTEM.

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Introduction

Imaging the metabolism of endogenous hyperpolarized ^{13}C-labelled molecules using Dynamic Nuclear Polarization (DNP) has the potential to probe tissue biology non-invasively. The first clinical trial has recently been undertaken in prostate cancer [1] and there are now a number of sites worldwide that are developing this for human use. The most widely studied reaction to date is the conversion of [1-^{13}C]pyruvate to [1-^{13}C]lactate, which in animal models has been shown to detect early treatment response and can be correlated with tumor grade [2,3]. There have been a number of methods used to quantify this exchange reaction. If it is to be used clinically, a simple, informative and robust quantitative parameter is required to characterize the data from hyperpolarized imaging. We assessed four quantitative methods using a clinical polarizer system at 3 T and an *in vitro* model system with pyruvate concentration equivalent to the levels we anticipate in future patient studies. The aim was to determine which quantitative parameters would be most appropriate to use with future clinical data. There has been previous work using pre-clinical models, however we have applied these analysis methods to imaging data on a clinical system in conjunction with a clinical hyperpolarizer and included one previously unpublished analysis method.

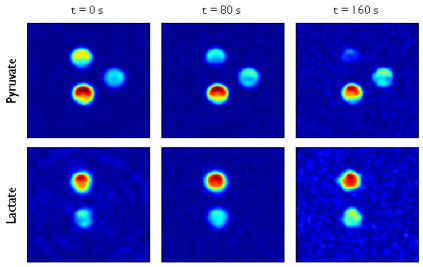

Fig.1: Spiral CSI images at 0, 80 and 160 s showing pyruvate and lactate in phantoms with 0 U (centre), 20 U (bottom) and 40 U (top) of LDH.

Methods

Imaging phantoms were made by filling 15 ml Falcon tubes with 14 ml of 5x PBS containing NADH at 4.4 mM, (sufficient to ensure it is not rate limiting), and between 0 and 120 U of the enzyme L-lactate dehydrogenase (LDH). Non-sterile research fluid paths were filled with 100 µl of [1-^{13}C]pyruvic acid with 15 mM of trityl radical (AH111501); 30 ml of water with 0.1 g/L EDTA was used for dissolution. Samples were polarized in a SPINlab clinical hyperpolarizer (GE Healthcare) to an average polarization of 21% before dissolution. The dissolution fluid was neutralized with NaOH to an average pH of 7.2 (range 6.7-7.4); 1 ml was added to each imaging phantom to give a final pyruvate concentration of 4 mM in the phantom. Three phantoms were imaged simultaneously using an IDEAL spiral CSI imaging sequence [4]: temporal resolution 4 s, in-plane spatial resolution 4 mm, FOV 8x8 cm, slice thickness 2 cm. Custom software was designed in Matlab® to analyze the data using four quantitative methods: fitting for the rate constant k_{pl} using a two-way two-compartment kinetic model [2] with a statistical weighting towards lactate; the lactate/pyruvate (L/P) ratio at peak lactate; the ratio of the L/P area under the curve (AUC) [5] and the time to peak (TTP) of the lactate signal. Each quantitative method was tested for its correlation with enzyme concentration, robustness to artefacts and ease of implementation. R^2 values were used to assess the fit in each case, using a Levenberg-Marquardt fitting algorithm. Each model-free analysis method was also checked for its correlation with theoretically predicted values calculated from the kinetic model fitting results. To assess image homogeneity and distortion, pyruvate and lactate time courses for each phantom were extracted in two ways for comparison: by thresholding and averaging over a region of interest (ROI) and by extracting from a pixel of interest (POI) containing the highest overall lactate signal.

Results

All four quantitative methods tested were able to provide a good correlation with [LDH], with the AUC ratio providing the best linear correlation overall. AUC and the L/P peak ratio were not robust to an outlier at 100 U unlike the other two methods. The TTP, a previously unpublished quantitative method for hyperpolarized carbon-13 analysis, provided a good linear fit and was robust to this error. It also correlated best with the theoretically predicted values (data not shown). Fig. 2 displays the ROI data for all four methods, with a summary of both ROI and POI data given in Table 1. In each case the ROI data correlated better with [LDH] than the POI data, however the difference in R^2 values for fits from the two extraction methods was small. There was no obvious difference in the robustness to any artefacts or errors between the two extraction methods. By plotting the residuals from the linear fits, both the k_{pl} values and the L/P peak ratio, which for a two-way model should theoretically be proportional to the k_{pl}, displayed some non-linearity with enzyme concentration.

Analysis	Theory	R^2 ROI	R^2 POI	Comments
Two-site kinetic model	$P \underset{k_{lp}}{\overset{k_{pl}}{\rightleftharpoons}} L$	0.9498*	0.9438*	Current gold standard; detailed information; non-linear with [LDH]; requires modelling.
L/P peak ratio	$\frac{L(t_{peak})}{P(t_{peak})} = k_{pl}T_1$	0.9199*	0.8967*	Simple to implement; prone to artefacts; non-linear with [LDH].
AUC ratio	$\frac{L_{AUC}}{P_{AUC}} = \frac{k_{pl}}{\rho_l + k_{lp}}$	0.9441	0.9247	Independent of input function; simple; linear.
Time to peak	$TTP = \frac{1}{k_{pl}+k_{lp}} \cdot \ln(1+T_{1,eff}(k_{pl}+k_{lp}))$	0.9435	0.9246	Simple to implement; appears linear with [LDH].

Table 1: Summary of results. * indicates that the logarithm of these values was taken prior to linear fitting.

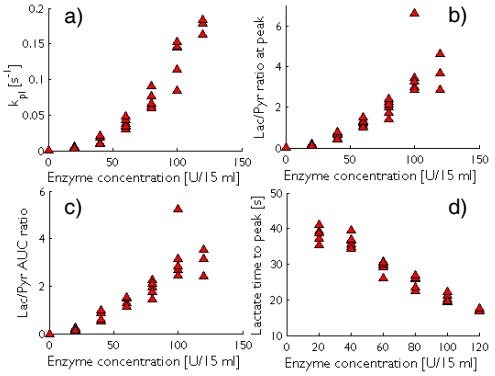

Fig. 2: Correlation with [LDH] for the four quantitative analysis methods a) k_{pl} from kinetic model, b) L/P ratio at peak lac, c) AUC ratio, d) time to peak.

Discussion

For clinical quantification of hyperpolarized imaging data, a standardized method of quantitative analysis is needed to allow for comparison of data within patients at different times, between patients and between different sites. The analysis should be robust to errors, simple to implement and representative of the exchange reaction. Kinetic modeling is the current gold standard of analysis however its implementation is complicated. Here, we have developed a clinically relevant model and custom image analysis software to compare the kinetic model to three simple approaches; AUC ratio, L/P peak ratio and TTP, which were each shown to be robust methods for analyzing the imaging. A POI extraction method could provide a simple and reproducible approach to image analysis analogous to methodology used in Positron Emission Tomography. Although the ROI method of data extraction provided data that correlated better with known enzyme concentration, the POI method appears no less robust in these homogeneous phantoms. In human tumors, analysis will be further complicated by an arterial input function (see Table 1) and tumor heterogeneity but similar simple approaches for PET analysis, such as the use of a maximum Standardized Uptake Value (SUVmax), have proved to be very powerful as routine clinical tools. In conclusion, both the AUC ratio and TTP provided excellent linear correlations with enzyme concentration, and are both simple and robust. In addition, the AUC ratio is independent of inflow function making it an excellent approach as a clinical standard for the analysis of hyperpolarized imaging data.

References: [1] Nelson, S.J. et al. 2013 *Science Translational Medicine,* 5(198):198ra108. [2] Day, S.E. et al. 2007 *Nature Medicine* 13(11):1382-7. [3] Albers, M.J. et al. 2008 *Cancer Research,* 68(20):8607-8615 [4] Wiesinger, F et al. 2012 *Magnetic Resonance in Medicine,* 68:8-16. [5] Hill, D.K. et al. 2013 *PLoSOne* DOI: 10.1371/journal.pone.0071996