Ratio of lactate-to-pyruvate apparent diffusion coefficients is an indicator of necrosis in tumor cells

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Introduction:

In the past, diffusion measurements with ^{13}C-metabolites were very time extensive and therefore prone to errors due to macroscopic motion and flow. However, taking advantage of the > 10,000-fold amplified hyperpolarized ^{13}C signal (1), we have shown that it is possible to measure metabolite apparent diffusion coefficients (ADCs) in combination with metabolic rate constants in real-time (2,3). ADCs were measured in MCF-7 tumor cell spheroids at concentrations below 2 mM and for lactate below 100 µM within 25s, whereas in a conventional thermally polarized ^{13}C diffusion experiment, this sensitivity would require experiment times of several months. After injection of pyruvate and uptake into the cell, the hyperpolarized label is intracellularly exchanged from pyruvate to lactate by LDH activity. In contrast to proton diffusion measurements, ^{13}C-metabolite ADCs allow to probe the intracellular compartment and are, therefore, sensitive to changes in compartment structure, e.g. due to breakdown of the plasma membrane. In a recent study, pyruvate-to-lactate conversion has been shown to decrease in breast but not in prostate cancer cells upon treatment, indicating the diversity of factors affecting pyruvate-to-lactate exchange (4). In contrast to a characterization of response-to-treatment solely by exchange measurements, we suggest that the combination of both kinetic and diffusion measurements gives an added benefit in detecting cell death upon treatment.

Methods:

A diffusion-weighted NMR pulse sequence based on a pulsed gradient spin echo (PGSE) sequence was developed for measuring ADCs of hyperpolarized ^{13}C nuclei (2,3). It relies on the principle of signal loss through diffusion dependent phase dispersion in the presence of a magnetic field gradient. Cultures of MCF-7 tumor cell spheroids were incubated with 0.015 % Triton X-100 in the assay medium**.** At this concentration of Triton X-100, membrane permeabilization was a gradual process over several hours, as confirmed by fluorescent staining (Fig. 1 A''-D''), and was observed by ^{13}C diffusion NMR spectroscopy (Fig. 1 A-D, A'-D').

Results and Discussion:

Tumor cell spheroids were analyzed by hyperpolarized diffusion NMR under detrimental conditions. Such conditions were given by treating cells with Triton X-100 to gradually permeabilize the cell membrane to mimic a necrotic process. The ADC_{lac}/ADC_{pyr} ratio increased with time. This can be explained by an increased blending of the intra- and extracellular compartments with time. In the last time steps, both ADC_{lac} and ADC_{pyr} decreased again, presumably due to further cell lysis, which leads to molecular leaching through the disrupted membrane and eventually exposes the metabolites to a common molecular environment. Fluorescent staining showed that an increase of the ADC_{lac}/ADC_{pyr} ratio correlates to an increase in the fraction of dead cells, until a complete blending of the intra- and extracellular compartments is reached and ADC_{lac}/ADC_{pyr} ratio approaches unity (Fig. 2 A,B). Simultaneously, pyruvate-to-lactate exchange decreased with time (Fig. 2 C). In conclusion, we have demonstrated that hyperpolarized ^{13}C diffusion-weighted spectroscopy is feasible. As a new technique, ^{13}C ADCs can provide information on the viability of tumor cells. Since an increase in ADC_{lac}/ADC_{pyr} ratio correlated with the fraction of dead cells, these metabolite ADCs are indicators of necrosis, making them useful tools for monitoring the pathological state of tumors in vivo.

References:

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(4) Lodi, A. et al. NMR Biomed (2012), DOI: 10.1002/nbm.2848.

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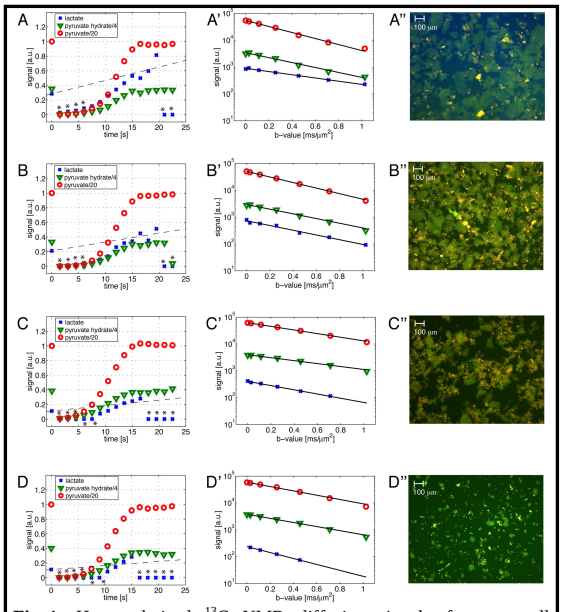

Fig. 1: Hyperpolarized ^{13}C NMR diffusion signal of tumor cell metabolites and label exchange rate from pyruvate to lactate in cancer cells at 37°C after various times of incubation with Triton X-100 (0.015 %). *(A, B, C, D)* Lactate, pyruvate hydrate and pyruvate signal corrected for excitation and relaxation as a function of time, as well as label exchange rate fit (dashed line) from pyruvate to lactate, for hyperpolarized pyruvate injected into cell medium after 1:00 h, 2:20 h, 3:45 h, and 5:00 h of treatment with Triton X-100. Black asterisks mark signals too weak to be included in further analysis. *(A', B', C', D')* Lactate, pyruvate hydrate and pyruvate signals and the corresponding fits to the *Stejskal-Tanner* signal equation on a semi-logarithmic scale as function of the b-value. *(A'', B'', C'', D'')* Fluorescent microscopy images of live cells (green) and dead cells (yellow).

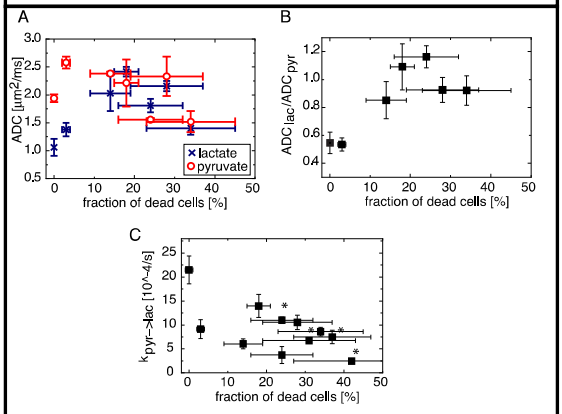

Fig. 2: (A) ADCs of pyruvate and lactate, (B) ADC_{lac}/ADC_{pyr} ratio, and (C) pyruvate-to-lactate label exchange rates related to the fraction of dead cells determined by fluorescence microscopy. Data from an experiment with untreated tumor cell spheroids (no dead cells) was combined with that from further experiments, where tumor cell spheroids were treated with membrane permeabilizing Triton X-100. Black asterisks mark kinetic rate constant values for which no diffusion information could be obtained due to low signal.