

Investigation of Cell Membrane Transport and Compartmentalisation of Hyperpolarised Metabolites using a GdDO3A Relaxation Agent

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Introduction

Injection of hyperpolarised pyruvate has enabled real-time analysis of cancer metabolism with the possibility to derive apparent rate constants. However, those constants are subject to biological parameters other than enzymatic conversion such as perfusion, cell membrane transport, back reaction, label exchange, and enzyme saturation. Especially the role of monocarboxylate transporters (MCTs) has gained importance recently since it was demonstrated that transporter activity can have a major impact on the quantification of metabolic activity [1][2]. In this work, an extracellular relaxation agent (GdDO3A) was used to quench the extracellular compartment of the metabolites which enables in vivo quantification of MCT activity.

Materials and Methods

Previous work dealt with the use of commercially available Gd-based contrast agents [3] but the observed effect was limited since only an outer sphere interaction was possible. A much higher efficiency in destroying the hyperpolarisation in the extracellular compartment can be achieved using a coordinatively unsaturated Gd(III)DO3A complex which binds selectively to pyruvate and its metabolites. In vitro T_1 measurements were conducted in serum at 3T for pyruvate, alanine and lactate with a concentration of GdDO3A of 1:10 (GdDO3A:pyr/lac/ala). In vivo experiments were performed in male Fisher F344 rats (n=3) with subcutaneous MAT BIII tumour in the neck region. Experiments were carried out on a 3 T HDx clinical scanner (GE Healthcare, USA) using a quadrature ^{13}C volume coil. 2.5 ml/kg bodyweight of 80mM hyperpolarised [^{13}C]pyruvate solution was injected into the tail vein. For each animal, two pyruvate injections were applied: The first without and the second with administration of highly concentrated GdDO3A solution (dose compared to pyr about 1:10) after 16s. The measurement was started at 20s at the supposed plateau of lactate. To be able to analyse metabolite transport, a substrate saturation sequence was developed which applies a frequency selective saturation pulse on the entire rat to quench all pyruvate magnetisation. Subsequently, spectra of lactate and alanine (256 points, 5kHz readout bandwidth) are acquired in an axial tumour slice using a spectral-spatial excitation pulse (see Fig. 1.). Relaxation rates were determined by using a non-linear least squares fit on the time evolution of the metabolite peak signal. Quenching the pyruvate allows to exclude effects of inflowing pyruvate, pyruvate transport across cell membranes and reaction to downstream metabolites which otherwise could not be separated from metabolite transport. The only difference in signal decay should thus be due to

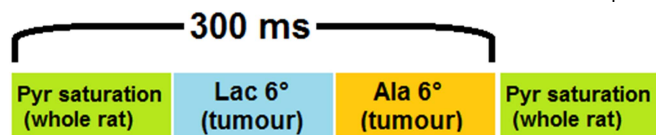


Fig. 1. Sequence scheme of substrate saturation sequence

transport out of the cell, where the hyperpolarised metabolite is quenched immediately when GdDO3A is present. The transporter rate therefore manifests as an additional relaxation term which can be calculated by comparing both measurements. For one rat, the pyruvate saturation pulse was alternated between slice selection and no slice selection to study the (apparent) back reaction to pyruvate.

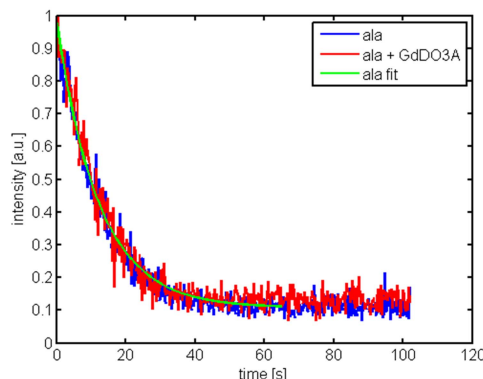
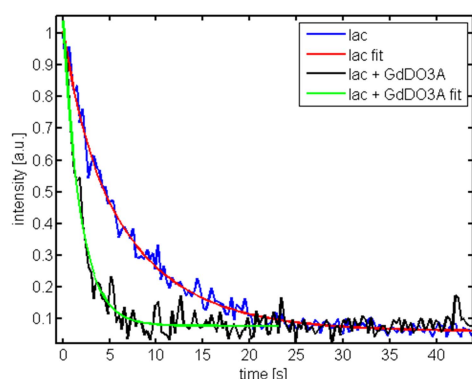


Fig. 2. Lactate and alanine curves in Mat BIII tumour slice with and without additional GdDO3A injection. A significant difference is observed for lactate, which indicates that it is rapidly transported out of the cell.

different T_1 , the difference in relaxation between lactate and alanine could reflect the different compartmentalisation, outflow and back reaction. For the (apparent) back reaction of metabolites to pyruvate, an upper limit of 0.12s^{-1} could be obtained.

Conclusion

Cell membrane transporter rates for hyperpolarised metabolites could be quantified for the first time in vivo. These results demonstrate the feasibility of GdDO3A to study transport of hyperpolarised compounds between extra- and intracellular compartments. The substrate saturation approach allows a simple and robust quantification of metabolite transport which only requires exponential fitting of relaxation rates. Furthermore, the findings suggest that transport out of the cell plays an important role for lactate kinetics in tumour tissue which has to be accounted for in metabolic studies.

References

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Results and Discussion

In serum, a 1:10 concentration of GdDO3A achieved T_1 relaxation times of pyruvate and its metabolites in the range of 50ms (Pyruvate: 90ms, Lactate: 40 ms, Alanine: 30 ms). This verifies that GdDO3A can indeed be used to achieve almost instantaneous quenching of extracellular hyperpolarisation. Regarding the in vivo experiments, a considerable difference between the two measurements was observed for lactate (T_1 without GdDO3A: $9.3 \pm 2.0\text{s}$, T_1 with GdDO3A: $2.51 \pm 0.40\text{s}$). This indicates that lactate is rapidly transported out of the cell. The transporter rate was estimated to be $0.33 \pm 0.06\text{s}^{-1}$. For alanine, the curves are virtually identical within the SNR limits ($T_1 = 13.3 \pm 1.7\text{s}$), which means alanine stays mostly within the cells. Besides an intrinsically