Endogenous ^{31}P CEST: A tool for monitoring Glycolysis without contrast agents

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INTRODUCTION:

The dysregulation of glycolysis stands as a metabolic anomaly observed across various pathologies. Among these dysregulations, foremost is the well-documented phenomenon occurring in numerous solid tumors, recognized as the Warburg effect. In clinical and pre-clinical settings, multiple imaging techniques have been successfully used to detect aberrant glycolysis such as ^{18}FDG-PET, HP ^{13}C MRI, DMI (Deuterium Metabolic imaging) and ^{1}H-CEST-MRI. All these techniques share the limitation of relying on exogenous contrast agents to observe the *in vivo* fate of injected metabolites. Indeed, the MRI approach owns a detection limit that does not permit the visualization of metabolites from the glycolytic pathway at their endogenous concentrations, even when the detection deals with a magnetically active species such as ^{31}P whose nat. abundance is 100%. On this basis, we deemed it interesting to design a novel method for detecting relevant glycolysis substrates at endogenous concentration. In principle, Heteronuclear CEST-MRI allows to increase the detectability threshold of the irradiated molecule of some order of magnitude as demonstrated by Ammon Bar-Shir with ^{19}F(1). Dealing with endogenous molecules the natural choice is to use ^{31}P signals belonging to Pi (inorganic phosphate)-PCr or -y ATP as bulk while saturating the signals of the phosphate groups belonging to the phosphorylated molecules of the glycolytic cascade. The feasibility of using saturation transfer measurements to detect the fluxes of Pi→ATP was already reported By K. Brindle and many others (2). The innovation here relies on the possibility of exploiting the amplification effect of the CEST contrast modality to detect signals from phosphorylated metabolites, which are below the S/N threshold in MR spectroscopy, via the most abundant signal of the bulk that can be detected by MRS.

METHODS: In vitro experiments were performed on three mammary carcinoma cell lines from mouse tissues, well known in the literature for their varying aggressiveness: 168FARN, TS/A, and 4T1 (3). Cells were cultured to reach about 40-60 x 10^6 cells, detached, washed in HEPES buffer, and centrifuged into a capillary for the NMR measurement. Z-spectra (Irradiation power: 3 µT, Irradiation time: 2s, ns: 64 per point), centered on the 31P inorganic phosphate signal in a range comprised between ±5, were performed on a Bruker Avance 600 operating at 14T at a temperature of 37°C. Control experiments were conducted by incubating cells with glycolysis inhibitors. For in vivo experiments, 4 mice were inoculated subcutaneously with the mammary breast cancer cell lines TS/A or 4T1. After two weeks, localized ^{31}P-CEST MRI experiments were performed in the tumor mass (voxel size about 0.8 cc) with the ISIS sequence implemented with the saturation transfer module. Z-spectra were acquired centered on the PCr signal intensity in a range of ppm comprised between ±9, using the following parameters (TR=3s; ns=8; Irradiation time=2s; irradiation power 2 µT; total acquisition time 1h 29', 3 minutes per point) in a Bruker Pharma scan 7T equipped with a 1H/31P dual-tuned volume coil.

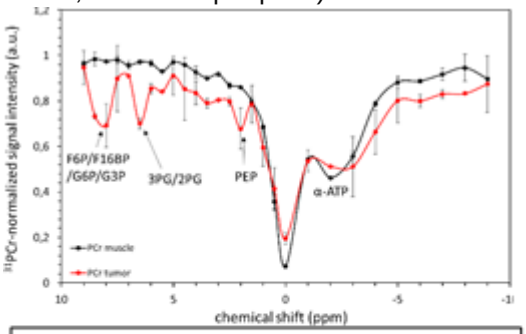

Fig. Localized 31P Z-spectra on the tumor mass (red circle) or in the hindlimb muscle (black circles) acquired on a Bruker Pharmascan 7T

RESULTS: Z-spectra acquired on the 4T1, TS/A and 168-Farn displayed different amounts of saturation transfer in correspondence to the chemical shifts of the glycolytic substrates (G6P, F6P, F1,6BP, G3P, 2PG, 3PG and PEP). Control cells treated with glycolysis inhibitors (NaF) displayed significant differences from untreated cells. In vivo (fig), it was possible to observe saturation transfer coming from glycolytic substrates in the breast cancer models (either 4T1 or TS/A). As control Z spectra on the muscle of the hindlimb were acquired and no ST have been measured in correspondence to that metabolites.

DISCUSSION: The herein reported results prove that ^{31}P CEST-MRS is a powerful methodology able to detect indirectly glycolysis metabolites. Different saturation transfers were measured according with the different aggressiveness of the breast cancer cells analyzed either in vitro or in vivo in a mouse model. These differences were ascribed to a different rate of glucose consumption and metabolism. The sensitivity and resolution of the method is the same as ^{31}P-MRS, but the detection threshold is at least three orders lower. The advantage of this method over the existing ones to monitor glycolysis is related to the possibility of performing the analysis without the administration of contrast agents.

CONCLUSION. The proposed approach might be of great interest because the absence of contrast agents will allow for repeated analysis also in fragile patients (renal failure or diabetes).

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