1H/31P Polarization Transfer at 9.4T to Detect Phosphomono- and -diesters in Breast Tumor Models

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Introduction: Free phospholipid metabolites such as phosphomonoesters (PME) and phosphodiesters (PDE), which can be detected non-invasively with ^{31}P Magnetic Resonance Spectroscopy (MRS), are potential biomarkers for cancer diagnosis [1] and anti-cancer treatment evaluation [2]. Because of the intrinsically low sensitivity of the phosphorous nucleus, applying a high magnetic field strength can increase the signal-to-noise-ratio (SNR) of ^{31}P MRS. Another way of increasing SNR is to apply polarization transfer (PT) methods such as refocused insensitive nuclei enhanced by polarization transfer (RINEPT) [3]. PT techniques transfer the polarization of the excited proton spins through J-coupling to the phosphorous spins during the time period of TE^{1}H (Fig. 1). However, due to the small J_{PH}-coupling constants, the optimal duration of TE^{1}H for polarization transfer is relatively long, which in high field causes substantial signal loss due to T2 relaxation. However, PT may be advantageous even at high field strengths for removing broad resonances from macromolecules that do not posses H-P J-coupling. In addition, it may be useful for removing strong disturbing signals from highly concentrated ^{31}P metabolites even outside the region of interest (chest muscle). In this study, we have tested this hypothesis by comparing the PT technique with direct ^{31}P pulse acquire (PA) at 9.4T in both less aggressive MCF-7 and more aggressive MDA-MB-231 breast cancer models. We have validated our *in vivo* PME and PDE quantification results with *ex vivo* ^{31}P high resolution (HR) MRS of tumor extracts.

MRS methods: Approximately 2x10^{6} MDA-MB-231 or MCF-7 cells were inoculated in the mammary fat pad of female athymic nude mice to grow tumors. *In vivo* ^{31}P MRS was performed on a 9.4T Bruker Biospec spectrometer. A home-built double tuned solenoid coil with an inner diameter of 12 mm was used. A RARE image was acquired with echo time (TE) of 7.2ms, repetition time (TR) of 500ms, RARE factor of 4. Non-localized PA ^{31}P MR spectra were acquired with an adiabatic excitation (BIR4 45°, 200µs, 120ppm band width), repetition time of 1s, and 2000 scans. Subsequently, an MR spectrum was acquired using the adiabatic version of the refocused insensitive nuclei enhanced polarization transfer technique (BINEPT) with a repetition time of 1s and 2000 scans. Segmented BIR4 pulses (400µs per segment, 35ppm band width) and a full BIR4 180° (400µs, 35ppm band width) pulse were used (Fig. 1). Both TE^{1}H and TE^{31}P were set to the optimal echo time of 34ms for detection of phosphocholine (PC) and phosphoethanolamine (PE). Metabolite levels were fitted and quantified using JMRUI 4.0 software. Metabolite levels of both PA and BINEPT spectra were corrected for ^{1}H and ^{31}P T1 and T2 relaxation accordingly. Tumors were taken out directly after *in vivo* MRS measurements, and methanol/chloroform/water based dual-phase extraction [4] was performed to extract water-soluble intracellular metabolites. ^{31}P HR-MRS was performed on the tumor extracts with a Bruker 11.7T spectrometer. The HR-MRS data were processed using the MestReC 4.9.9.6 software, and the metabolite levels were corrected for differences in ^{31}P T1 relaxation time.

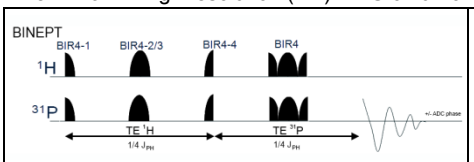

Figure 1: *Schematic overview of the adiabatic version of the refocused insensitive nuclei enhanced by polarization transfer (BINEPT) sequence. Segmented BIR4 pulses are used instead of conventional 90 and 180 degree pulses. The echo time 1/4J is set to 34 ms, which is optimal for detecting PE and PC.*

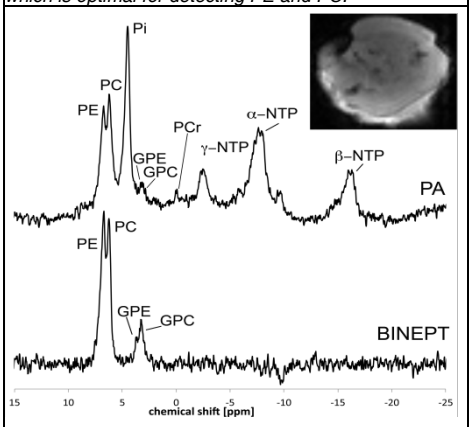

Figure 2: *Example of in vivo ^{31}P MR spectra of an MCF-7 tumor showing the pulse acquire (PA) spectrum on the top and the BINEPT spectrum on the bottom. An image of the tumor is shown in the upper right corner. In the PA spectrum all phosphorylated signals are visible, in the BINEPT spectrum only signals from metabolites with H-P-coupling are visible. In the BINEPT spectrum the GPE and GPC signals are clearly visible with high SNR.*

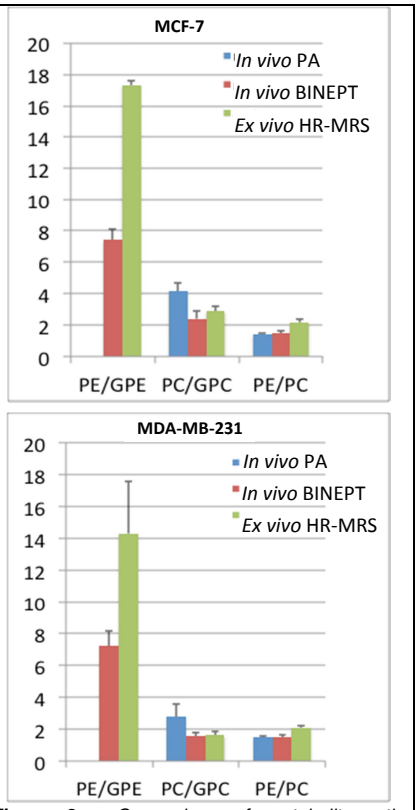

Figure 3: *Comparison of metabolite ratios between in vivo and ex vivo ^{31}P MRS measurements in both MCF-7 (n=7) and MDA-MB-231 (n=4) breast cancer models.*

Results and Discussion: Seven MCF-7 mice and four MDA-MB-231 mice were studied in this experiment. Besides signals of PME and PDE, the signals of inorganic phosphate (Pi), PCr, and α-, β-, and γ-nucleotide triphosphates (NTP) were identified in the PA ^{31}P MR spectra (Fig. 2). The BINEPT MR spectrum has a flat baseline, which facilitates PME and PDE analysis using line-fitting algorithms. It is even possible to partially resolve the PME signal into PE and PC, and the PDE signal into GPE and GPC (Fig. 2). When comparing the ratios of metabolites from three different types of measurement (*in vivo* PA, *in vivo* BINEPT, and *ex vivo* HR-MRS, see Fig. 3), the PE/GPE ratio cannot be assessed accurately in the PA measurement since GPE is hardly visible, particularly in MDA-MB-231 tumors. The PC/GPC ratio in *in vivo* BINEPT measurement is similar to that obtained from *ex vivo* HR-MRS data, whereas PC/GPC in *in vivo* PA data is somewhat higher. The PE/PC ratios in both PA and BINEPT measurements match the *ex vivo* measurement equally well. Higher PC/GPC levels were reported previously in more aggressive cancer cell lines [5], however, we found no significant differences but a trend towards a higher PC/GPC ratio in less aggressive MCF-7 compared to more aggressive MDA-MB-231 tumors. This observation could be related to effects of the tumor microenvironment on the PC/GPC ratio in tumor xenografts, which were not present in cell lines in culture. Furthermore, metabolite levels measured in cell cultures depend on the proliferative stage of the cells, which affects phospholipid metabolite and transcript levels as reported previously [6]. In conclusion, our data suggest that BINEPT can be advantageous for studying the individual compounds in phospholipid metabolism in translational research in breast cancer and other cancers *in vivo* at high magnetic field strength.

References: [1] Krishnamachary et al., Cancer Res 2009; 69(8):3464-71. [2] Podo. NMR Biomed 1999; 12(7):413-439. [3] Klomp, et al., Magn Reson Med 2008; 60(6):1298-1305. [4] Glunde et al., Cancer Res 2005; 65(23):11034-11043. [5] Aboagye et al., Cancer Res 1999; 59(1):80-84. [6] Kalra et al., British Journal of Cancer 1993; 67(5):1145-1153. ***Acknowledgement:*** *This work was funded by NIH R01 CA134695 and the Niels Stensen foundation.*