$^{1}H/^{31}P$ Polarization Transfer at 7 and 9.4 Tesla for improved specific detection of phosphomono- and -diesters in human breast and breast tumor models.

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Introduction:

^{31}P Magnetic Resonance Spectroscopy (MRS) is a non-invasive tool capable of assessing free phosphorylated phospholipid metabolites such as phosphomonoesters (PME) and phosphodiesters (PDE), which can be used in the evaluation of anti-cancer treatment as shown in preclinical [1, 2] and clinical studies [3]. 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). However, due to shorter T2 relaxation times at high field strength (>7Tesla) the gain in SNR may be small when using PT compared to direct ^{31}P pulse acquire (PA) acquisition, even when considering the more favorable T1 relaxation times of ^{1}H compared to ^{31}P spins. Nonetheless, we tested the hypothesis that 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, PT may also be useful for removing strong disturbing signals from highly concentrated ^{31}P metabolites outside the region of interest (e.g. chest muscle). Particularly in heterogeneous tissues, such as tumor xenografts in preclinical mouse models, and in the human breast, these signals can compromise spectral quality significantly due to susceptibility effects. In this study, we demonstrate that the PT technique results in a flat baseline without any loss of sensitivity, and in uncontaminated detection of PME and PDE only, which are important phospholipid metabolites associated with cancer malignancy [2].

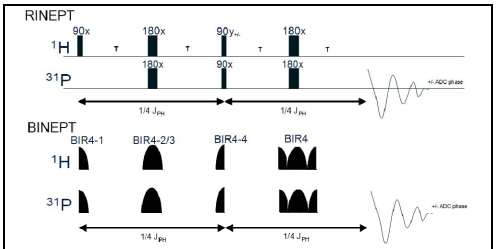

Figure 1: *RINEPT and BINEPT pulse sequence scheme with interpulse timings of ¼ J_{PH} which is optimal for PME and PDE [5].*

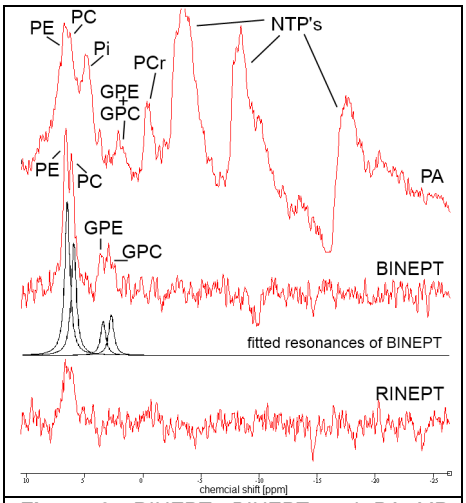

Figure 2: *RINEPT, BINEPT and PA MR spectra of an orthotopic MDA-MB-231 breast tumor xenograft in a mouse at 9.4Tesla. The β-NTP signal is out of phase due to limited band width of the BIR4-90° pulse. The tumor volume was ~240mm^3. (R/B)INEPT: TR 1s, NSA 2048.*
PA: TR 2s, NSA 1024

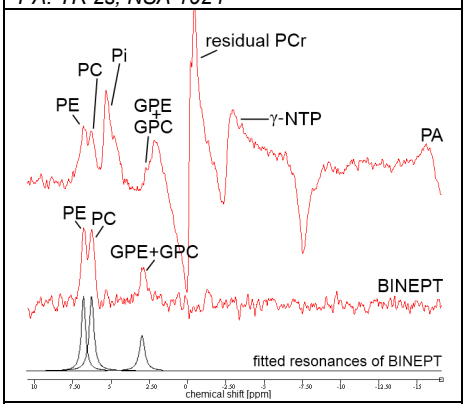

Figure 3: *BINEPT and PA MR spectra of the breast of a healthy volunteer at 7Tesla. The PCr, and NTP signals originate mainly from the muscle tissue and are out of phase due to a frequency offset in the muscle compared to the breast. The glandular tissue volume was ~150cm^3. BINEPT: TR 2.5s, NSA 150.*
PA: TR 1.5s, NSA 249

MRS methods:

RINEPT and its adiabatic equivalent (BINEPT) sequences were implemented on a Bruker 9.4Tesla small animal MR scanner (Fig.1). A home-built solenoid coil tuned to the ^{31}P and ^{1}H frequencies, with an inner diameter of 12mm was used. We used short block pulses (100us each) for the RINEPT, and segmented BIR4 and BIR4-180° pulses for the BINEPT. The segments of the BIR4 were 400us, driven at 10kHz to achieve a flat excitation profile with a broad bandwidth (24ppm), and the full BIR4-180° was 800us driven at 15kHz.

An MDA-MB-231 breast tumor-bearing mouse was anaesthetized with inhalatable isoflurane, and its tumor was positioned inside the coil in the MR scanner. Non-localized B_0 shimming was done manually by changing first order shim gradients. A RINEPT, BINEPT and direct ^{31}P PA (BIR 4-90°) were acquired in 35 minutes each, using SNR optimized values for TR.

For a direct translation to clinical use, these methods were tuned for ^{31}P MRS in a healthy volunteer at 7T. A two-channel double tuned unilateral RF coil was designed for ^{31}P and ^{1}H MRI and MRS of the human breast and interfaced to a whole body 7T MR system (Philips, Cleveland, USA) [4]. Shimming with 2nd order shim gradients was based on a manually segmented region of interest in the breast from a B_0 map. A PA (block pulse 39°) and a BINEPT MR spectrum were obtained in 6.25 minutes each.

Results and Discussion:

BINEPT significantly improved the ^{31}P MRS detection of PME and PDE in a breast tumor xenograft model at 9.4T (Fig.2) as well as in a healthy volunteer at 7T (Fig.3) because the polarization transfer removed all signals originating from PCr, Pi, NTP, and macromolecules that overlap with PME and PDE in PA ^{31}P MRS applications. As a consequence, the BINEPT MR spectrum has a flat baseline, which facilitates PME and PDE analysis using line-fitting algorithms (Fig.2&3). It is even possible to partially resolve the PME signal into phosphoethanolamine (PE) and phosphocholine (PC), and the PDE signal into glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC). The BINEPT MR spectrum displayed higher PME and PDE signals than the RINEPT MR spectrum (Fig.2) as expected from the non-uniform B1 fields of the coil. When comparing the mouse data with the human data, translational research seems very applicable. The significantly smaller volume of the mouse tumor was able to provide sufficient SNR, caused by the smaller coil, higher field and longer scan time. Nonetheless, the smaller volume did not improve the line shape, which reflects dominant T2* effects caused by micro-susceptibility effects within (heterogeneous) tumors.

Conclusion:

PT techniques at high field strengths (>7Tesla) enable the detection of partially resolved PE, PC, GPE and GPC in experimental breast tumor models and human breast tissue *in vivo* due to the removal of unwanted resonances from PCr, Pi, NTP, and ^{31}P-containing macromolecules. No reduction in SNR was detected when comparing PT techniques to regular PA. Our data suggest that BINEPT can be advantageous for studying 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] Glaholm, Leach et al. Lancet 1989: 1326–1327. [4] Klomp, van de Bank. NMR in biomed 2011; Mar 24. doi: 10.1002/nbm.1696. [5] Mancini, Payne, et al. MRM 2003;50:578-8.8

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