Phosphomonoester Levels in Drug Resistant and Drug Sensitive Human Breast Cancer Xenografts via Fully-Relaxed ^{31}P MRS

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Introduction

Phosphomonoesters (PMEs), specifically phosphoethanolamine (PEtn) and phosphocholine (PCho), are generally elevated in tumors [1], and are useful indicators of proliferation, metastatic potential, and therapy response [2,3,4]. PMEs have been shown to decrease in response to effective therapy [1]. Hormones and media composition also contribute to PME concentrations in cell culture [1, 6]. Quantification of individual PMEs via ^{31}P MRS *in vivo* has been difficult due to poor resolution of the PEtn and PCho peaks. Recently, quantification of these peaks in human brain has been accomplished by proton-decoupled ^{31}P MRS [8]. In mouse tumor xenografts on our system, decoupling has little effect on spectral linewidths, which are more dependent on B_0 homogeneity and shimming. One method to improve shimming has been susceptibility matching in tumors implanted in legs or feet [7]. For xenografts in isotopic sites, e.g. mammary fat pad, susceptibility matching is impractical, and hence the PMEs are more difficult to resolve. We describe here a method for quanitfying PMEs in mouse tumors using ^{1}H-coupled ^{31}P MRS.

Methods

Drug-sensitive (MCF-7/S) and drug-resistant (MCF-7/D40) human breast cancer cells were implanted in the mammary fat pad of SCID mice and allowed to grow into tumors ranging in volume from 111 mm^3 to 678 mm^3. For MRS, mice were anesthetized, immobilized and the tumor placed in a home-built solenoid coil. ISIS-localized, fully-relaxed ^{31}P spectra were acquired with a Bruker Biospec, 4.7 T system, 14 G/cm self-shielded gradients, 90° adiabatic pulsing and a 10.5 s recycle delay. Data analysis included zero-filling and resolution enhancement with a -30 Hz Lorentzian exponential window and a Gaussian factor of 0.1 Hz, manual phasing and baseline correction. Following Fourier transformation, the broad lipid resonance underlying the PEtn/PCho, Pi and GPE/GPC resonances was subtracted from the spectrum by baseline correction. A deconvolution routine based on the Levenberg-Marquardt algorithm was applied. A 50% mixture of Lorentzian to Gaussian lineshapes was used with the PEtn/PCho peaks grouped together. Data were expressed as ratios of the PEtn/γ-NTP and PCho/γ-NTP integrals. Error was expressed as standard deviation (s.d). To verify the accuracy of the deconvolution algorithm, unlocalized ^{31}P MR spectra of phantoms of known PME and Pi concentrations in 25% BSA were used.

Results and Discussion

As a test of the deconvolution algorithm, phantoms of known PEtn and PCho concentrations were analyzed. Five spectra were generated from phantoms with a 1:1 PEtn/PCho ratio. Following deconvolution, with peak areas normalized to 1, the s.d. was 0.099 for PEtn and 0.084 for PCho. A correction factor of 0.689 (±0.034) was applied to the phantom PEtn peak areas to correct for partial saturation. Spectra were then generated from phantoms with 10:1, 5:1, 2:1, 1:2, 1:5 and 1:10 ratios of PEtn/PCho. As expected, the deviation from the expected value was greater as ratios moved away from 1.1. Since the PEtn/PCho ratios of the *in vivo* spectra were all near 1:1, the method is relevant for analyzing these data.

Figure 1 is an ISIS-localized, fully-relaxed ^{31}P MR spectrum with an overlay of the deconvolution line-fit. Five drug-resistant and four drug-sensitive tumors were analyzed. PEtn/γ-NTP and PCho/γ-NTP ratios were determined for both cell lines (Fig. 2).

For drug-resistant MCF-7 cells, the PEtn/γ-NTP ratio was 1.16±0.17 and PCho/γ-NTP ratio was 0.96±0.055. For drug-sensitive cells, the PEtn/γ-NTP ratio was 0.71±0.081 and PCho/γ-NTP ratio was 0.75±0.069.

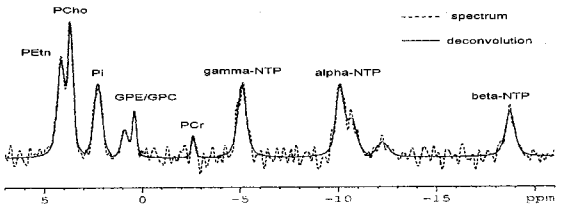

Figure 1. Fully-relaxed ^{31}P MR spectrum of a MCF-7/D40 tumor with deconvolution line-fit overlay.

The PME to γ-NTP ratios were higher in the drug-resistant tumors compared to the drug-sensitive tumors: $p = 0.0020$ for PEtn/γ-NTP; and $p = 0.0014$ for PCho/γ-NTP. Since the γ-NTP concentrations are nearly equal between these two cell lines in cell culture [9], the difference may be due to alterations in the PME levels alone. However, differing levels of γ-NTP between the cell lines *in vivo* can not be ruled out as a factor. The PEtn/PCho ratios were: 1.21±0.24 for drug-resistant tumors; and 0.95±0.17 for drug-sensitive tumors. The PME/γ-NTP ratios remain unchanged through the entire range of tumor volumes.

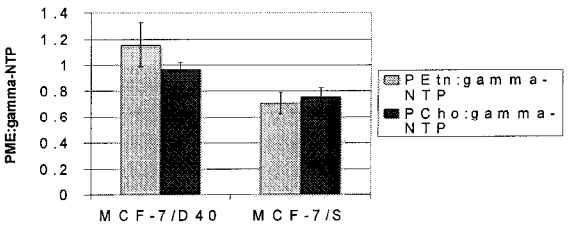

Figure 2. PME/γ-NTP ratios of drug-resistant and drug-sensitive human breast cancer xenografts in SCID mice.

Conclusions

As determined by analysis of phantoms, this method of quantifying PMEs is reliable for spectra with nearly equal amounts of PEtn and PCho. *In vivo* PME concentrations were then quantified for both drug-sensitive and drug-resistant tumors. PME/γ-NTP ratios are higher in drug-resistant tumors when compared to drug-sensitive tumors; and PME concentrations did not vary within the range of tumor volumes analyzed. Tumor pH varies with tumor volume. Therefore observations that PME concentrations are independent of tumor pH are supported [10]. Future experiments will include the quantification of PMEs in response to chemotherapy.

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