Initial Studies of Ifosfamide and Tumour Metabolism using High-Resolution 31P MAS

G. S. Payne[1], Y-L. Chung[2], S. J. Vaidya[3], M. O. Leach[1]

[1]CR UK Clinical Magnetic Resonance Research Group, Royal Marsden NHS Trust and the Institute of Cancer Research, SUTTON, Surrey, United Kingdom, [2]St George's Hospital Medical School, Tooting, London, United Kingdom, [3]Royal Marsden NHS Trust and the Institute of Cancer Research, SUTTON, Surrey, United Kingdom

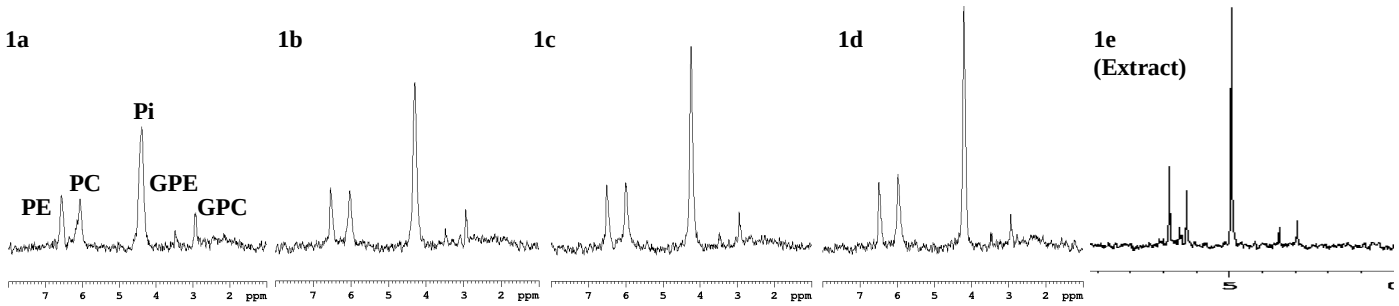
Introduction

^{31}P MRS measurements *in vivo* have demonstrated early changes in the phosphomonoester (PME) and phosphodiester (PDE) signals in response to treatment (1). Work in cells suggests that some changes may relate to specific signalling pathways (e.g. (2)). ^{31}P MRS can also detect uptake and metabolism of anticancer drugs such as ifosfamide in patients following treatment (3). *Ex vivo* MRS measurements are normally performed on tissue extracts, although the process has the potential to lose some metabolites. Recently high-resolution ^{1}H magic-angle spinning (MAS) spectroscopy has been introduced for the study of small (~ 50 μg) tissue samples directly at high field (4). Here we explore the potential of high-resolution ^{31}P MAS to study tissue samples, in particular looking at the visibility and integrity of the PME and PDE regions. We also begin to address it's value as a tool to help interpret the broad ifosfamide-related peaks observed in clinical studies, by looking at MAS spectra of liver and tumour samples from mice bearing rhabdomycosarcoma (Rd) tumours following treatment with ifosfamide.

Methods

Animal Models Rd xenografts were grown to 582 ± 47mg in nude mice, and then treated i.v. with 400mg/kg ifosfamide. Tumours (n = 11) and liver (n = 4) samples were snap-frozen at 0.5 – 3 hours after treatment (following *in vivo* studies described elsewhere (5)) and stored under liquid nitrogen. Control tumour and liver were obtained from 4 saline-treated animals. Samples were divided for (i) direct study using high-resolution MAS, or (ii) PCA extraction and analysis by conventional high-resolution ^{31}P NMR (with methylene diphosphoric acid added as reference).

NMR measurements All measurements used a Bruker wide-bore 500 MHz Avance spectrometer. MAS samples were placed in 4mm ZrO_2 rotors and spun at 3 kHz in a wide-bore MAS probe, tuned to ^{1}H and ^{31}P. To avoid biochemical degradation sample temperature was maintained at 4^{o}C. Shimming was performed on either water or the lactate doublet (where visible). Following acquisition of a water-suppressed ^{1}H spectrum (TR = 3.8s; NS = 64), 8 sets of ^{1}H-decoupled ^{31}P spectra were obtained (NS = 256 scans; TR = 2.8s), interleaved with single-scan ^{1}H acquisitions to check for field drift. Tissue extracts were measured in a 5mm BBO probe (^{1}H and ^{31}P with deuterium lock), using TR = 5.7s, and 2000 scans. ^{31}P spectra were processed with exponential apodisation (3 Hz for MAS; 1 Hz otherwise), Fourier transformation, and phase correction.



Results

Appearance of HR-MAS ^{31}P spectra. Fig 1a shows a ^{31}P MAS spectrum from a control sample of RD tumour (NS = 512). Well-resolved peaks are seen from PE, PC, Pi, GPE and GPC. The larger linewidth of PME resonances (22 Hz for PE) relative to PDE peaks (14 Hz for GPC) includes a contribution from a small pH change during the measurement (see below). Interleaved ^{1}H spectra show that the field drift for the entire study only contributes 0.7 Hz to the ^{31}P linewidth. As expected there is no significant signal from high-energy phosphates (ATP or PCr). Chemical shifts imply a pH of ~6.8. Fig 1e shows a high-resolution ^{31}P MR extract spectrum from the same tumour. GPE concentration in the tumour extracts was 0.28 ± 0.18 mM (mean±sd, n=6). This suggests that the threshold of sensitivity of the MAS measurements is approx 0.1 mM (for a 25-minute acquisition).

Stability and reproducibility. Figure 1a-d shows a series of 4 sequential data sets, each of 512 scans (approx 25 minutes). These show an increase in Pi amplitude and reduction in chemical shift of Pi (4.39 → 4.21ppm), PE and PC, but little change in the amplitudes of the PME and PDE resonances. A preliminary investigation of one sample from each of the four control livers showed good reproducibility in 3 cases, but absence of GPE and GPC in the fourth case. Continuing studies are further investigating issues related to reproducibility.

Ifosfamide-treated tumours.

Peaks in the region expected for ifosfamide (18 ppm) appear in 7/11 of the tumour MAS spectra, with no clear correlation with excision time. Several ifosfamide metabolites resonate in this region (6,7), so more work is required to assign these signals precisely. Surprisingly no ifosfamide signals were seen in the 4 liver spectra (all excised at < 2 hours) but were observed in some extracts (and *in vivo* at times up to 3 hours (5)). It may be that the environment in liver makes the ifosfamide more unstable, but further measurements are required to confirm this.

Discussion and Conclusions

Well-resolved high-resolution spectra have been obtained using ^{31}P MAS of tissue samples. While less sensitive than ^{1}H MRS, ^{31}P MRS produces much better resolved resonances from choline-containing compounds (of especial interest currently), while MAS of the intact tissue sample avoids loss of information during extraction. The PME and PDE resonances are relatively stable over 2 hours. In addition, peaks related to the anti-cancer drug ifosfamide have been detected in tumour following treatment. Absence of a field frequency lock does not adversely affect the spectra. Important applications include measurement of drug and drug metabolite levels in target tissues, e.g., tumour (PK measurement) and measuring the effect of drug on target tissues (PD measurements). The method is very straightforward and potentially biopsy samples could be used.

References.

(1) W Negendank. NMR Biomed. **5:** 303(1992). (2) YL Chung J Nat Canc Inst **95:** 1624 (2003) (3) GS Payne Mag Res Med **44:** 180 (2000) (4) LL Cheng Mag Res Med **36:** 653 (1996) (5) SJ Vaidya Br J Cancer 88 (Suppl 1) S17 (2003) (6) GS Payne Proc ISMRM 2003, p2144 (7) R Martino J Pharm &Expt Ther **260:** 1133 (1992)

Acknowledgements. The work was funded by Cancer Research UK (Grant SP1780/0104)