31P NMR of Phospholipid Metabolites in Prostate Cancer and Benign Prostatic Hyperplasia

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Introduction: 1H MRSI is increasingly being used to diagnose prostate cancer noninvasively (1), although a recent meta-analysis suggested that substantial technique development is still warranted (2). Typically a composite resonance attributed primarily to choline and choline-containing phospholipid (PL) metabolites [phosphocholine (pc), glycerophosphocholine (gpc)] is elevated in cancer, while the resonances from citrate and polyamines in normal prostate or benign prostatic hyperplasia (BPH) are reduced (1). At high magnetic field 31P MRS may be an attractive possibility for studying prostate cancer, although very little 31P work in vivo or in vitro has been done recently. Here we report an in vitro 31P NMR study of prostate cancer and BPH, focusing on the levels of the major PL metabolites.

Methods: Residual pathology specimens (0.25-2 g, from transurethral resections or radical prostatectomies) from patients undergoing evaluation for prostatic neoplasia were immediately (<2 min) frozen in liquid N2 and stored at -70°C. Pathology reports confirming the diagnoses were obtained in all cases. Frozen tissues were extracted with 6% cold perchloric acid, lyophilized, and stored at -70°C. Immediately prior to analysis the lyophilized specimens were reconstituted in 20% D2O with 0.1 M EDTA/0.05 M MOPS buffer, the pH adjusted to 7.0, and the sample transferred to a 10-mm NMR tube. The 31P NMR spectra were acquired at ambient temperature at 121.7 MHz on a GE GN-300WB (7.05T) spectrometer using a broadband probe with gated 31P decoupling (no NOE). Typical conditions were: 45° rf pulse, 4.5 µs; pulse delay, 3 s; spectral width, 6024 Hz; line broadening, 2 Hz; variable number of acquisitions. Internal chemical shift reference was made to gpc at 0.58 ppm (from 85% H3PO4), which is relatively independent of pH and solution conditions (3). Individual peak areas were determined by integration using the GN-300 software and ratioed to the total of the four PL metabolites (tPLM) of interest (see the Table). T-tests for independent samples and Pearson correlations were performed with Statistica (Statsoft, Tulsa, OK).

Results: A typical 31P NMR spectrum of a BPH sample is shown in the Figure. The results for comparison of PL metabolites of the cancer and BPH groups are given in the Table. The metabolites phosphoethanolamine (pe) and glycerophospho-
ethanolamine (gpe) (and their ratio) were significantly different between cancer and BPH. These results are similar to those seen in an early 31P NMR study (4). However, unlike Cornel at al. (4) we saw no difference for pc. As expected, resonances from high-energy phosphate metabolites such as ATP and phosphocreatine (PCr) were greatly reduced, and the resonances of inorganic phosphate (Pi), ADP, and 5'-AMP elevated, due to sample degradation between excision and freezing. PCr was seen in most spectra and was significantly lower in the cancer group (p=0.002, results not shown). This difference probably arises from different average times to freezing for the two groups, and not from tissue pathology. PL metabolites are considerably less prone to degradation than high-energy phosphate metabolites under the circumstances of our sample acquisition (5). Similar to our previous 1H NMR in vitro study of prostate cancer (6), none of the individual PL metabolite ratios to tPLM correlated with the summed Gleason score for the cancer group (range 5 to 8, results not shown). However, the ratio gpc/pc did correlate with the summed Gleason score (r=0.69, p<0.05). Of the nine cancer samples, two were from different regions of tumor in the same subject. Metabolite levels (relative to tPLM) varied substantially between the two regions (pe: 0.36, 0.57; pc: 0.11, 0.09; gpe: 0.32, 0.20; gpc: 0.22, 0.14).

Discussion: Recently Swanson et al. (7) measured various metabolites in benign and cancerous prostate tissues by 1H MAS total correlation spectroscopy at 11.7T. Although they also found increased gpc and gpe in cancer, unlike us they found a significant increase in pc with cancer. Like us they found high levels of pe relative to other PL metabolites. Unlike our study the difference in pe between cancer and benign tissue was reversed, and not statistically significant (7). We attribute the differences between our study and theirs (7) to methodology and perhaps differences in the makeup of benign tissues. They concluded that ethanolamine-containing metabolites may contribute as much to the in vivo total choline peak in 1H MRSI as choline-containing metabolites. Our 31P results, for which total phosphoethanolamines are substantially larger than total phosphocholines, also suggest this. It was recently reported (8) that radiolabeled ethanolamine is taken up to a much greater extent than choline by proliferating tumor cells. The above results suggest that 31P MRS, with its ability to distinguish the major PL metabolites, including pe and gpe, may be a useful approach both in vivo and in vitro for diagnosing prostate cancer, if the sensitivity limitations can be overcome, as expected at high magnetic field. Differences within tumors may be distinguishable, as demonstrated above in one case. Additional in vitro 31P NMR work employing normal and cancerous tissues from different regions of the prostate, as well as from cancers of varying Gleason scores, is warranted.