Tumor Transmembrane pH Gradient Measurement with 6-Fluoropyridoxol by ^{19}F NMR Spectroscopy.

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It has been suggested that pH measurement may be of prognostic value and that many tumors exhibit a reversed pH gradient, which could be a basis for therapeutic exploitation(1). We had previously shown that 6-Fluoropyridoxol (6-FPOL) 6-FPOL, a vitamin B_6 analog could be used to observe the pH gradient in red blood cells(2) and the perfused rat heart(3). Given the exceptional chemical shift sensitivity to changes in pH ($\Delta\delta$ ~ 10 ppm) we have now investigated the feasibility of measuring the pH gradient in a rat tumor *in vivo*.

Methods: Dunning prostate adenocarcinoma R3327-AT1 (potential volume doubling time 5 days) was implanted in a skin pedicle on the foreback of adult male Copenhagen rats. Once tumors had grown to ~ 15 mm diameter each rat was placed under general gaseous anesthesia (0.3 dm^3/min. O_2, 0.6 dm^3/min. N_2O, and 0.5% methoxyflurane). 6-FPOL (200 mg) was dissolved in a mixture of water (1.5 ml) and DMSO (0.5 ml) and injected IP together with NaTFA (20 mg) as an intratumoral chemical shift standard. A tunable ($^1H/^{19}F$) 2 cm single turn solenoid coil was placed around the tumor. The rat was placed in a 4.7 T magnet and shimming was performed on the water resonance. Data were processed using 40 Hz line broadening prior to Fourier Transformation. NMR experiments were immediately followed by pH electrode measurements along three tracks in the tumor (5 determinations per track).

Results: ^{19}F NMR signal was detected from tumors within 15 mins and persisted for several hours. ^{19}F spectra were acquired in 4 minutes and rapidly showed two well resolved peaks assigned to the intra- (I) and extra- (E) cellular compartments as shown below. A SNR of 10 could be obtained for 6-FPOL (extracellular) in 8 mins. Two signal attributable to the intra- and extracellular compartments were always visible resolved by ~ 1 ppm. Typical peaks occurred at δ = -10.37 ppm and δ = -11.32 ppm corresponding to pHe = 6.97 and pHi = 7.40. By comparison, ^{31}P NMR showed Pi at δ = 5.19 ppm corresponding to pHi = 7.34. ^{31}P spectra required 8 min (na = 512). pHi estimated by ^{19}F NMR (7.40) was in corresponded to the traditional ^{31}P estimate (7.34). pHe measurement by ^{19}F NMR (6.97) corresponded to the electrode measurement (6.93 ± 0.02).

Conclusion: Two resolved signals were obtained corresponding to the intra- and extracellular compartments. The pH values confirmed a reversed pH gradient, as also determined independently on the basis of combined ^{31}P NMR and electrode polarography. This further substantiates literature reports and demonstrates the feasibility of measuring tumor pH gradient in vivo on the basis of a single molecule. However, given its relatively basic pKa and poor aqueous solubility, we continue to investigate new analogies to provide enhanced ^{19}F NMR pH probes.

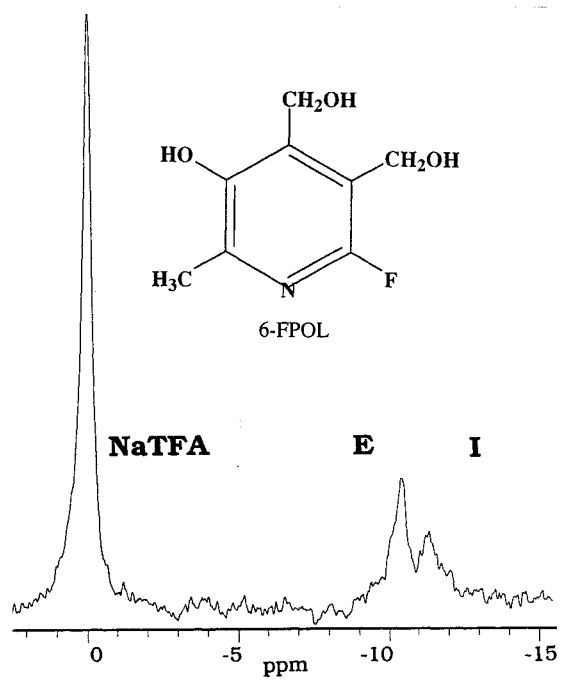

Figure 1: ^{19}F NMR intratumoral signal after IP injection of 6-FPOL solution. Two distinct signals were seen: intracellular (I) and extracellular (E).

References:
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