Chronic Administration of MRSI agent IEPA Increases Tumor pH; has Potential to Bias pH Measurement

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Introduction Noninvasive measurement of pH has been of scientific interest for the past several decades, because this type of physiological information would be useful for diagnosis and evaluation of therapy in many diseases, including cancer. Multiple methods of monitoring pH have been developed using MR, because it has the advantages being noninvasive and having inherent contrast between soft tissues resulting in high spatial resolution anatomical images. Among the methods for monitoring pH, PARACEST agents [1-3], relaxation agents [4] and spectroscopic methods using ^{31}P [5] are popular options. Additionally, 2-imidazole-1-yl-3-ethoxycarbonylpropionic acid (IEPA) has been used to quantitatively measure the pH in tumors with good spatial resolution [6]. In a parallel project, we have shown that chronic ingestion of IEPA (200 mM ad lib in water) leads to reduced incidence of spontaneous metastases and furthermore results in increased tumor and urine pH (Fig 1,2). Thus, there is the potential that use of this compound as a pH indicator actually influences the tissue pH being measured.

Materials and Methods The high resolution spectra of IEPA were obtained using a single pulse sequence on a 9.4T Varian for reference. All other imaging and spectroscopy were completed on an ASR 7T Varian system in the Small Animal Models and Imaging (SAMI) at the Moffitt Cancer Research Institute. The titration curve for IEPA was obtained on using a solution containing 200 mM IEPA, 20 mM EDTA (ethylenediaminetetraacetic acid) and 10% D2O in FBS (fetal bovine serum); the pH of 1.5 mL aliquots of this solution was adjusted with NaOH or HCL to values from 4 to 9. Spectra for each pH solution were obtained using a PRESS (point resolved spectroscopy) sequence on a 2x2x2 (mm^3) voxel, and the chemical shift of the pH sensitive peak was recorded. Initial investigations of chronic administration of IEPA were performed by obtaining the IEPA signal *in vivo* in the bladder of a mouse that had been drinking a 200 mM solution of IEPA for 2 days. Animal imaging was completed to obtain T2 weighted images using an fsems sequence, with FOV = 40x90(mm), 21 coronal slices, 1 mm thick, no gap, TR ≈ 4 seconds, effective TE = 72 ms. The spectrum was obtained using a STEAM (stimulated echo) sequence on a 2x2x2.5 mm3 voxel in the bladder. Next, studies were performed on mice that had been consuming water with 200 mM IEPA chronically (over two weeks). These animals were placed on IEPA treatment four days prior to subcutaneous injection with either PC3M (Prostate) or LL2 (Lewis Lung) xenograft tumors. The tumor pH measurements were taken directly, using a reference and needle microelectrode (MI-301F and MI-408B, MicroElectrodes, Inc. Bedford, NH), in subcutaneous xenograft tumors on SCID animals under anesthesia.

Results Initial results indicate that the concentration of IEPA in the bladder after two days of chronic treatment with IEPA is sufficiently high to be MRS-visible. Using the titration curve, the pH of the urine was calculated to be 7.37, which is notably higher than the average pH of mouse urine, 5.3 [7], suggesting that IEPA is alkalinizing. Under these conditions, the tumor pH was shown to be substantially increased in the LL2 tumors, and slightly increased in the PC3M tumors.

Current Work We are completing follow up studies to directly measure the pH (via microelectrode) on animals with short term administration of IEPA versus a negative control (administration of saline) to determine the viability of using IEPA to measure the pH *in vivo* without altering the results. Additionally, the pH will be measured via MRSI in the short term IEPA treated animals to compare the pH results, to determine the accuracy of the measurement.

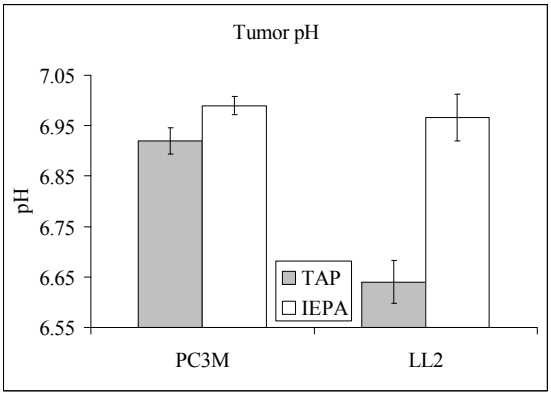

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Figure 1 (Left). IEPA alters tumor pH when administered chronically.

Figure 2 (Below). IEPA as MRS agent. The high resolution spectrum of IEPA was obtained using a single pulse sequence on a 9.4T Varian for reference [A]. The titration curve for IEPA [E] was obtained at 7T, pH range: 4 – 9, 200 mM IEPA, 20 mM EDTA and 10% D2O in FBS (sample spectrum pH 7.42 [B]). Initial studies to determine the viability of finding the IEPA signal *in vivo* were performed on a mouse that had been drinking 200 mM IEPA for 2 days. The spectrum [C] was obtained using a stimulated echo sequence on a 2x2x2.5 mm3 voxel [D] in the bladder.