Selective acidification and de-energization of LNCaP prostate cancer xenografts using lonidamine

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Introduction: Prostate cancer is the sixth leading cause of cancer-related death in men (1) [second in the United States (2)] and is the most common cancer in the developed world with increasing rates in the developing world (1). Surgical excision is the most common curative method if the cancer is detected early. Chemotherapy is sometimes used if prostate cancer has spread outside the prostate gland and if hormone therapy is not effective. Specifically, we seek to employ the natural tendency of tumors to convert glucose to lactate as a method for selective intracellular acidification of the tumor, which has been reported to potentiate tumor response to hyperthermia (3) as well as to chemotherapy with platinum (4) and N-mustard (5-9) alkylating agents. This study monitors intracellular pH (pHi), extracellular pH (pHe) and bioenergetics (βNTP/Π) in LNCaP prostate cancer xenografts by 31P magnetic resonance spectroscopy (MRS) following administration of lonidamine (LND), a putative inhibitor of transmembrane monocarboxylate transporters (MCT) (10-13). These findings point to the potential utility of chemotherapeutic drugs and LND in the systemic treatment of disseminated prostate cancer.

Material and Methods: 4-6 weeks old male 01B74 athymic nude mice (n=3) were used in the tumor study. LNCaP prostate cancer cells (5x10^6) in a mixture of 75 μl matrigel and 75 μl of RPMI 1640 medium were inoculated subcutaneously into the right thigh of each animal. Prostate cancer xenografts were allowed to grow until they reached 7-10 mm in diameter along the longest axis of the tumor. Mice were anesthetized using 1% isoflurane in oxygen, and the MR studies were performed on a 9.4 T/31 cm horizontal bore Varian system. In-vivo 31P MR spectra were acquired with a homemade resonator (10 mm in diameter). The animal was mounted in the coil such that the subcutaneous tumor projected into the resonator. Oxygen was delivered through a custom-built nose cone. A rectal thermistor and respiration pillow were placed and connected to a small animal monitoring device for measuring vital signs, core body temperature and respiration. The animal’s core temperature was maintained at 37±1°C during the scan. After acquisition of baseline spectra, LND (100 mg/kg) was injected i.p. through a 26 ga×3/4inch catheter without removing the animal from the magnet. Data were processed offline by using NUTS (Livermore, CA, USA) and MestRec (Mestrelab Research, Spain) software. The pH and pHe were determined from the Henderson-Hasselbalch equation using the chemical shifts of Pi and 3-amino-phenylphosphonate (3-APP) referenced to the α-NTP resonance. Analysis of variance with Tukey multiple comparisons was used for statistical analysis (SPSS 16). The data of pHi, pHe and bioenergetics at time points following LND administration were compared by ANOVA and t-test analysis.

Results: In vivo 31P MRS (Fig. 1) demonstrates that LNCaP prostate cancer xenografts in immunosuppressed mice treated with the MCT inhibitor (LND) exhibit a sustained and tumor-selective decrease in pH from 6.87 ± 0.04 to 6.40 ± 0.07 (p = 0.02), pHe from 6.97 ± 0.04 to 6.46 ± 0.09 (p = 0.18) and/or improving tumor response to antineoplastic agents. The concept of manipulating tumor pH with metaboliodbenzylguanidine (MIBG) and α-cyano-4-hydroxycinnamate (CNCn) has been proposed in our previous studies (14, 15). LND appears to inhibit the MCT on the plasma membrane and may also block the mitochondrial pyruvate carrier thereby impeding the delivery of pyruvate to produce acetyl-CoA for the tricarboxylic acid cycle. Thus, LND is very attractive because it may simultaneously cause selective tumor acidification and tumor de-energization without the need for MIBG. While LND clearly inhibits export of lactate from the tumor cells of human (DB-1 and MCF-7) and rat origin (9L), it is not clear if it is inhibiting transport of pyruvate into mitochondria as α-cyano-4-hydroxycinnamate (CHC) does (11-13). However, the similar effect of CHC and LND on the bioenergetics of DB-1 melanomas (9, 15) and prostate cancer here strongly suggest that it is. Therefore, the decline of bioenergetics that was evident both from the decrease in NTP/Pi and from direct monitoring of NTP by 31P MRS vs. time in each animal could be explained by a profound decrease in mitochondrial metabolism following LND administration.

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