Lonidamine reverses the cell membrane pH gradient of human DB-1 melanoma xenografts

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Introduction: As a consequence of their high levels of aerobic glycolysis (1), tumors generally exhibit an acidic extracellular pH (pHe) and a neutral to alkaline intracellular pH (pHi) leading to an acid-outside/neutral to mildly alkaline inside plasmalemmal pH gradient (2). This gradient also impacts tumor response to certain chemotherapeutic agents (3) and to hyperthermia (4). Manipulation of pHe and/or pHi of tumors has a considerable impact on tumor growth and metastasis (5) as well as on response to therapy (3, 6-10). Other laboratories (3, 5) have modified tumor pHe by administering sodium bicarbonate in order to increase the pHe and thereby reduce tumor invasiveness and facilitate uptake of weakly basic drugs such as doxorubicin. In contrast, our aim was to decrease the pHi in order to increase the intracellular activity of N-mustards against DB-1 melanoma xenografts (8, 11). We accomplished this by administering an inhibitor, lonidamine (LND, 100 mg/kg, intraperitoneal) of the monocarboxylate transporter (MCT) that blocks cellular export of lactic acid and also inhibits transport of pyruvate into mitochondria, thereby inhibiting tumor energy production. There has been considerable debate over the mechanism of action of LND (12), but NMR studies have definitively demonstrated that it was a potent MCT inhibitor that impedes cellular lactate export (13-15).

Material and Methods: Tumor-bearing nude mice (n=15) were utilized in this study. Brain (n=3), surgically exposed liver; pHi (n=6), pHe (n=3) and skeletal muscle pHi (n=6), pHe (n=3), were investigated as representative normal tissues under identical conditions utilized for ^{31}P MRS studies of mice with tumors. Xenografts were implanted by subcutaneous (s.c.) injection of one million DB-1 cells into male athymic nude mice. DB-1 melanoma cells were early passage human melanoma cells derived from a lymph node biopsy of a patient with a metastatic melanoma that was excised by Dr. David Berd (Thomas Jefferson University Hospital, Philadelphia, PA). The metastastatic tumor node was excised before administration of any treatment. Cells, which expressed the human melanoma antigen (16) were prepared from the tumor and cryopreserved after the 16th passage. Phosphorus-31 MRS was performed using a home-built dual-frequency (^{1}H/^{31}P) slotted-tube resonator. Magnetic resonance experiments were performed with a 9.4 T/31 cm horizontal bore Varian system in conjunction with physiological monitoring (temperature, respiration, electrocardiogram and pulse-oximetry). Following acquisition of baseline spectra, LND and 3-aminopropylphosphonate (3-APP) were injected through two 26 gauge i.p. catheters inserted into either side of the peritoneum without removing the animal from the magnet. A slice-selective double frequency Hadamard Selective Multiple Quantum Coherence pulse sequence was used to detect lactate and to filter out overlapping lipid signals (17). Measurements of pHi and pHe were made by monitoring the chemical shifts of Pi and 3-APP resonances, respectively, referenced to the α-NTP resonance after calibration of data to the Henderson-Hasselbalch equation. Changes in pH values were evaluated by paired samples t-test (SPSS version 16.0) statistical analysis.

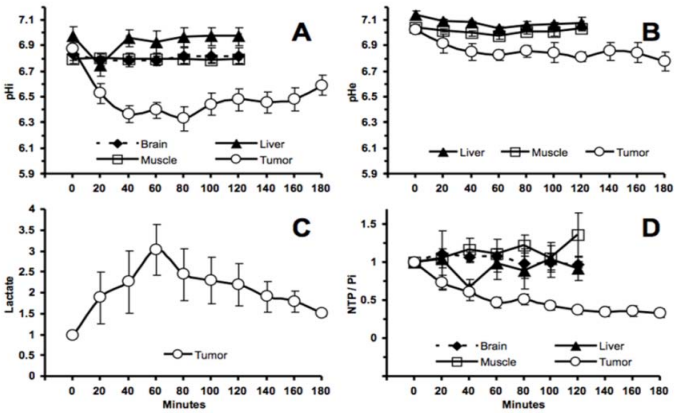

Fig. 1. (A) The intracellular pH (pHi) profile as a function of time of human melanoma xenografts (n=15) and normal tissues [skeletal muscle (n = 6), liver (n = 6), and brain (n = 3)] in response to LND (100 mg/kg; i.p.) administration at time zero. (B) The extracellular pH (pHe) profile as a function of time of human melanoma xenografts (n=4) and normal tissues [skeletal muscle (n = 3) and liver (n = 3),] in response to LND administration at time zero. (C) Change in tumor lactate as a function of time after administration of LND (100mg/kg). Area under the curve at each time point was normalized to baseline levels. (D) The changes of NTP/Pi (ratio of peak area) relative to baseline as a function of time of human melanoma xenografts (n=15) and normal tissues [skeletal muscle (n = 6), liver (n = 6), and brain (n = 3)] in response to LND administration at time zero. The values are presented as mean ± S.E.M. When not displayed, S.E.M. values were smaller than the symbol size.

Results: The pHi significantly decreased 20-180 min after the administration of LND; the maximum decrease in pHi, 0.6 ± 0.1 ($p < 0.001$), occurred 80 min post-LND administration (Fig. 1, A). However, pHe exhibited a smaller decrease of 0.20 ± 0.07 ($p = 0.085$) (Fig. 1, B). Tumor bioenergetics (NTP/Pi) also decreased by 66.8 ± 5.7% ($p < 0.001$) relative to the baseline level (Fig. 1, D). Decreases in tumor bioenergetics and pHi persisted for at least 3 hr following LND treatment. Liver exhibited a small transient intracellular acidification by 0.2 ± 0.1 pH units ($p = 0.027$) at 20 min post-LND (Fig. 1, A); there were no significant changes in hepatic pHe and a small transient decrease in liver bioenergetics, 32.9 ± 10.6 % ($p = 0.027$), at 40 min post-LND. Brain (pHi; $p = 0.423$, bioenergetics; $p = 0.627$) and skeletal muscle (pHi; $p = 0.363$, pHe; $p = 0.328$; bioenergetics; $p = 0.267$) exhibited no significant changes for 120 min post-LND (Fig. 1, A, D). The relative level of steady-state tumor lactate increased ~3-fold reaching a maximum 60 min post-LND (Fig. 1, C).

Discussion: Positron Emission Tomographic imaging with FDG (fluorodeoxyglucose) has utilized the "Warburg Effect" for the noninvasive detection of cancer. We recognized that if we could trap the lactate produced by tumor cells inside the cell, we would potentially have a method to selectively acidify the tumor and make it susceptible to alkylating agents that are sensitized by acid (6, 9, 10) or to heat, whose lethal effect is also enhanced under acidic conditions. Reversal of the pH gradient should also increase uptake of weak bases such as doxorubicin by melanoma cells. Our results provide further support for the MCT inhibitor mechanism of LND activity, demonstrating that LND selectively acidified the tumor by impeding lactate transport across the cell membrane; it also inhibited tumor bioenergetics by blocking transport of pyruvate into mitochondria with no effect on muscle or brain and transient acidification and mild de-energization of the liver. We have demonstrated accumulation of lactic acid in the tumor by ^{1}H MRS and measured the pHe and pHi of the tumor to demonstrate reversal of the pH gradient across the tumor cell membrane. While LND was previously recognized to inhibit export of lactate from the tumor cells of human MCF-7 breast cancer and 9L glioma cells (13-15), it was not clear if it also inhibited transport of pyruvate into mitochondria as α-cyano-4-hydroxycinnamate (CHC) (18) does. However, the similar effect of CHC and LND on the bioenergetics of DB-1 melanomas (8) strongly suggests that transport of pyruvate to the mitochondria is inhibited. If the acidification and reversal of the pH gradient seen in our studies also occurs in other cancers as well as melanomas, improved therapeutic gain should be seen with existing treatment modalities that would take advantage of the altered physiological phenotype of the tumor.

Acknowledgements: This study is supported by grant 1-R01-CA-129544-01A2.

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