Effects of hyperglycemia on lonidamine-induced acidification and de-energization of human melanoma xenografts treated with melphalan

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Introduction: Melanoma is the most rapidly increasing form of human cancer in the United States, exhibiting a 4% increase in incidence per year since 1970 (1). In the US, melanoma ranked fifth in incidence among males and sixth among females in 2006 but was not among the top ten causes of cancer death for either gender (2). Surgical excision is the only proven therapy that leads to cure if the cancer is detected early. However, if recurrence occurs with metastasis, the prognosis is very poor since effective methods for treating the systemic disease are not available. Specifically, we seek to employ the natural tendency of melanomas and other 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. We performed this study to evaluate whether hyperglycemia induced selective intracellular acidification following lonidamine (LND) administration and substantial activity of melphalan (LPAM) by applying P-31 and hydrogen-1MR spectroscopy to monitor intra- (pHi) and extracellular pH (pHe), tumor bioenergetics, and lactate levels respectively. We have reported before (9) that while LND alone had no significant effect on tumor growth delay, it substantially enhanced the activity of LPAM and did not substantially increase the toxicity of this antineoplastic agent. These findings point to the potential utility of nitrogen mustards and LND in the systemic treatment of disseminated melanoma.

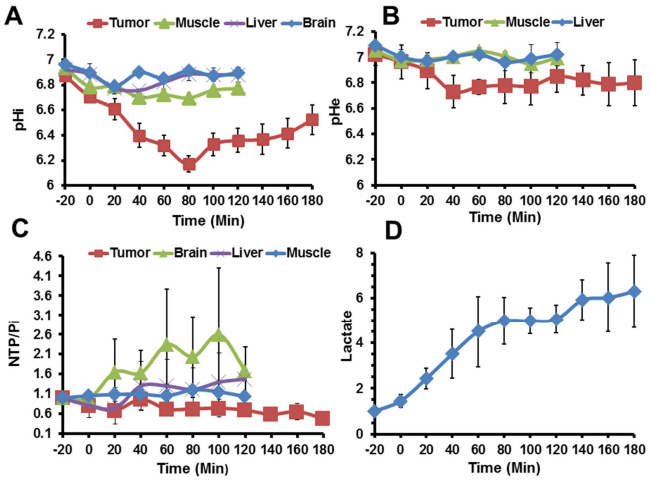

Fig. 1. (**A**). The intracellular pH (pHi) profile as a function of time (n =10) and normal tissues [skeletal muscle (n = 3), liver (n = 3), and brain (n = 3. (B). The extracellular pH (pHe) profile as a function of time (n = 7) and normal tissues [skeletal muscle (n = 3) and liver (n = 3),]. (C). The changes of NTP/Pi (ratio of peak area) relative to baseline (n=10) and normal tissues [skeletal muscle (n = 3), liver (n = 3), and brain (n = 3)] (D). Change in tumor lactate as a function of time. Area under the curve was compared to baseline at each time points and was normalized to baseline levels as a function of time in response to LND (100 mg/kg; i.p.) administered at time zero after 20 min i.v. infusion of glucose (26mM). The values are presented as mean ± S.E.M. When not displayed, S.E.M. values were smaller

Material and Methods: Human melanoma xenografts development (n=10), pHi, pHe and bioenergetics (βNTP/Pi) estimation as well as lactate levels were performed as described elsewhere (9). LND (100 mg/kg; i.p.) was injected after 20 min following glucose infusion. A stock solution of D-glucose (2.5 M) diluted to 0.6 M and then delivered through a tail vein catheter with a variable rate using a syringe pump to maintain a blood concentration of 26 mM, as follows: (10ml/hr, 1min; 3ml/hr, 4min; 2.5ml/hr, 2min; 2.0ml/hr, 2min; 1.5ml/hr, 2min; 1.0ml/hr, 2min; 0.5ml/hr, 167min). In addition to glucose infusion four cohorts having five animals were treated with same protocol as described before (9). ANOVA with Bonferroni and Tukey multiple comparisons were used for statistical analysis. To assess the significance of treatment effects, we fit spline models to the longitudinal tumor growth data (10). The models described log tumor volume as linear throughout the period of observation in the control arm and prior to treatment in the active arms, and as a quadratic spline in the period immediately after treatment in the active arms. **Results:** LND exhibit a decrease in intracellular pH (pHi) from 6.87 ± 0.03 to 6.17± 0.06 ($p < 0.001$), a slight no significant decrease in extracellular pH (pHe) from 7.02 ± 0.09 to 6.73 ± 0.12 ($p > 0.05$), and a monotonic decline in bioenergetics (βNTP/Pi) by 51.4 ± 0.09% ($p > 0.05$) relative to the baseline level after LND administration following glucose infusion (Fig. 1). Liver exhibited a minimal transient no significant intracellular acidification by 0.17 ± 0.01 pH units ($p > 0.05$) at 40 min post-LND with no significant change in pHe and a small no significant transient decrease in bioenergetics, 25.5 ± 0.4 % ($p > 0.05$), at 20 min post-LND (Fig. 1). No changes in pHi or ATP/Pi were detected in the brain (pHi, bioenergetics; $p > 0.1$) or skeletal muscle (pHi, pHe, bioenergetics; $p > 0.1$) for at least 120 min post-LND (Fig. 1). Steady-state tumor lactate monitored by ^{1}H MRS with a selective multiquantum pulse sequence with Hadamard localization increased ~6-fold ($p = 0.04$) (Fig. 1). Treatment with LND increased systemic melanoma response to melphalan (LPAM; 7.5 mg/kg, i.v.) producing a growth delay of 4.47 ± 0.6 d (tumor doubling time = 3.14 ± 0.26d, log10 cell-kill = 0.429 ± 0.10, cell-kill = 62.8 ± 2.03%) compared to LND alone of 0.34 ± 0.1 d and LPAM alone of 2.88 ± 0.51 d (Fig. 2). **Discussion:** The inclusion criteria behind infusing exogenous glucose that most tumors exhibit high levels of aerobic glycolysis (11, 12), methods have been explored to utilize the "Warburg Effect" for the detection and treatment of cancer. We recognized that if we could trap the lactate produced by tumor cells inside the cell, we would have a method to selectively acidify the tumor and make it susceptible to alkylating agents that are sensitized by acid or to heat, whose lethal effect is also enhanced under acidic conditions (3-9). Since the MCT is the main mechanism by which the DB-1 melanoma (13) and most other tumors (14) maintain intracellular pH homeostasis, inhibition of this co-transporter would suffice to acidify the tumor. By feeding exogenous glucose (26 mM) the pHi dropped to 6.17 and lactate increased ~ 6 fold. However, without glucose pHi was 6.33 and lactate~3 fold from baseline following LND administration (9). Tumor cells maintain pH homeostasis by buffering capacity of CO_2 having the pKa = 6.4. In turn cell kill activity of LPAM in presence of glucose didn't improve (cell kill = 62.8%) compared to without glucose (cell kill = 89.4%). This study provides the insight not to provide extra load of glucose to the patients while applying these methods in clinics. **Acknowledgements:** This study is supported by grant 1-R01-CA-129544. **References:** (1) Cancer facts and figures, 1994. (2) Jemal A, et al Ca-a Cancer Journal for Clinicians, 2006; 106-130. (3) Chu GL, et al. Radiat Res. 1988; 576-585. (4) Atema A, et al. Int J Cancer 1993; 166-172. (5) Canter RJ, et al. Ann Surg Oncol 2004; 265-273. (6) Jahde E, et al. Cancer Res 1989; 2965-2972. (7) Kuin A, et al. Br J Cancer 1999; 793-801. (8) Wong P, et al. Clin Cancer Res 2005; 3553-3557. (9) Nath K, et al NMR Biomed 2012 (Epub ahead of Print). (10) Corbett THaV F. A (Pergamon Press, New York), 1987. (11) Warburg, Constable and Co.; 1930. (12) Weinhouse S, Z Krebsforsch Klin Onkol Cancer Res Clin Oncol. 1976; 15-126. (13) Wahl ML et al. Mol Cancer Ther. 2002; 617-628. (14) Webb SD et al. J Theor Biol 1999; 237-250.

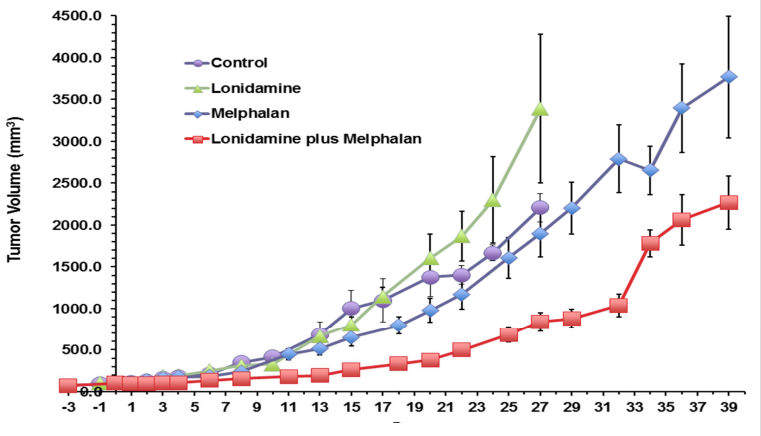

Fig. 2. Growth delay experiments performed on DB-1 human melanoma xenografts in nude mice treated with 7.5 mg/kg LPAM with i.v. infusion of glucose (26mM). Mice were treated on Day 0 as follows: Control (sham i.p. tris/glycine buffer + sham i.v. PBS), LND, LPAM, LND + LPAM. Values shown are means ± SEM for n = 5 animals, Control and LND groups; n = 5 animals, LPAM and LND + LPAM groups. When not shown, error bars are less than symbol size