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- (pH) 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.

Material and Methods: Human melanoma xenografts development (n=10), pH, pHe and bioenergetics (jNTP/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.

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 (jNTP/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 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). 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 = 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) Kjin 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) Corbetti THaV A. F (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. 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 (26M). The values are presented as mean ± S.E.M. When not displayed, S.E.M. values were smaller than the symbol size.

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 (26M). 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.