DB-1 human melanoma xenograft pH and energy state changes during treatment with lonidamine plus melphalan

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Introduction: Melanoma, the most deadly of all skin cancers, is the most rapidly increasing form of human cancer in the United States (1) and is rapidly increasing among Caucasian populations throughout the world (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. Since acidification has been reported to enhance the activity of platinum compounds and alkylating agents such as nitrogen (N)-mustards (3-8), we have evaluated the effect of lonidamine (LND)-induced acidification on two representative agents, cisplatin (CPT) and melphalan (LPAM). We found that while LND had no significant effect on the activity of CPT, it substantially enhanced the activity of LPAM (8). 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, intracellular pH (pHi) (n=3), extracellular pH (pHi) (n=3) and bioenergetics (βNTP/Pi) (n=3) estimation were performed as described in our recent publication (8). LPAM was injected i.v. after 40 min following LND administration without removing the animal from the magnet to monitor the additive effect of LND on tumor pH and bioenergetics. Four cohorts of five age- and weight-matched animals were randomized to the following treatment groups: cohort 1 (sham treated control) was infused intravenously (i.v.) with PBS and given appropriate sham intraperitoneal (i.p.) injections of tris/glycine buffer; cohort 2 was infused i.v. with PBS 40 minutes after LND administration i.p. (100 mg/kg); cohort 3 was injected i.p. with tris/glycine buffer and infused i.v. with CPT (7.5 mg/kg delivered in ~10 sec) in PBS; cohort 4 was injected i.p. with LND (100 mg/kg) and after 40 minutes (determined by ^{31}P MRS) (8), CPT (7.5 mg/kg) was infused i.v. The same procedures and sham-treatment groups were included for the LPAM study, with CPT being replaced by LPAM (8). CPT and LPAM were freshly prepared prior to injection. Depending on the treatment group, either tris/glycine or LND (4.5 ul/g) and CPT, LPAM or PBS (5.0 ul/g) were injected. Analysis of variance with Tukey multiple comparisons was used for statistical analysis (SPSS 16). The data of intracellular pH (pHi), extracellular pH (pHe) and βNTP/Pi at time points following LND administration were compared. Measured tumor volumes were graphed on a semi-log scale against time in days. Comparisons were made between parallel logarithmic regions of tumor growth of the treated animals and saline treated controls. This value was in excellent agreement with the equation, $\log_{10}$ cell kill = (T-C)/3.32Td, where the numerator denotes the tumor growth delay (T being treated cells and C controls), and Td is the tumor doubling time after treatment. To assess the significance of treatment effects, we fit spline models to the longitudinal tumor growth data. We conducted the tumor growth modeling in SAS Proc Mixed (SAS Version 9.2; SAS Institute; Cary, NC). Other analyses were performed in Microsoft Excel 2010.

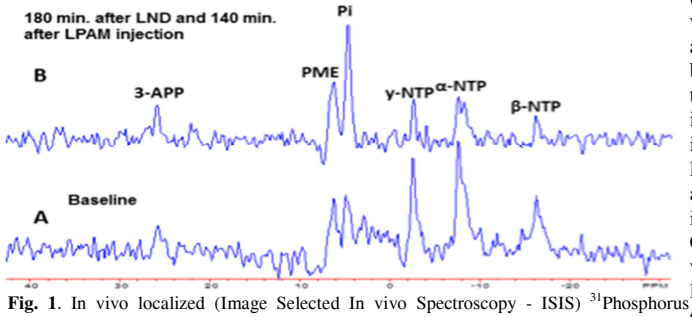

Fig. 1. In vivo localized (Image Selected In vivo Spectroscopy - ISIS) ^{31}Phosphorus magnetic resonance spectroscopy spectra of human melanoma xenograft grown subcutaneously in nude mice (A) pre- and (B) 180 min and 140 min. post administration of LND (100 mg/kg, i.p.) and Melphalan (7.5 mg/kg; i.v.), respectively.

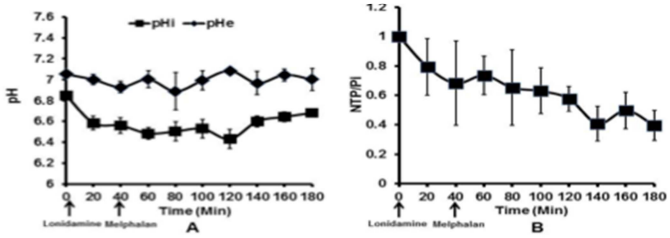

Fig. 2. (A) The intracellular pH (pHi) and extracellular pH (pHe) profile (B) the changes of βNTP/Pi (ratio of peak area) relative to baseline as a function of time of human melanoma xenografts in response to LND (100 mg/kg; i.p.) administration at time zero and melphalan (7.5 mg/kg; i.v.) at time 40 min. The values are presented as mean ± S.E.M. When not displayed, S.E.M. values were smaller than the symbol size.

Results: *In vivo* ^{31}P MRS (Fig. 1) demonstrates that human melanoma xenografts in immunosuppressed mice treated with the monocarboxylate transport (MCT) inhibitor (LND, 100 mg/kg, i.p.) exhibit a sustained and tumor-selective decrease in pHi from 6.85 ± 0.02 to 6.43 ± 0.09 ($p = 0.004$) after 120 min following LND and LPAM administration. pHe exhibited a smaller non-significant decrease from 7.05 ± 0.02 to 6.89 ± 0.18 ($p = 0.94$) (Fig. 2). Tumor bioenergetics (NTP/Pi) also decreased 60.5 ± 0.23% ($p = 0.27$) relative to the baseline level and normalized with baseline values (Fig. 2). LPAM did not produce any significant effect on pHi, pHe and both bioenergetics and pHi decreases were maintained for at least 3 hr following LND treatment. The effects of treatment with LND + CPT or LND + LPAM were evaluated by tumor growth delay experiments (Fig. 3). CPT + LND produced a very slight growth delay similar to the effect of LND alone, whereas CPT given as a single agent had essentially no effect on tumor growth. LND + LPAM proved much more effective producing a growth delay of 19.9 ± 2.0 d (tumor doubling time = 6.15 ± 0.31d, log10 cell-kill = 0.975 ± 0.110, cell-kill = 89.4 ± 2.2%) compared to LND alone of 1.1 ± 0.1 d and LPAM alone of 4.0 ± 0.0 d. **Discussion:** As noted above, the activity of platinum based (3) and N-mustard alkylating agents (3-8) increases with increasing acidification of tumors. In the case of N-mustards, this appears to be due to three effects: 1) increased concentrations of the active intermediate cyclic aziridinium ion intermediate, 2) decreased concentrations of competing nucleophiles such as hydroxide and glutathione, whose production is diminished by decreased activity of gluthathione-S-transferase under acidic conditions, and 3) decreased DNA repair due to acid inhibition of O^6-alkyltransferase (6, 10). This is probably largely because acid shifts the equilibrium between the various forms of these agents towards the more active forms of these agents. In the case of N-mustards, the active species is the cyclic aziridinium ion. CPT alkylation involves a complex equilibrium with replacement of the chloride ion with water or hydroxyl groups; the active agent for cross-linking being the diaquo species (10). Monoacquo species can react with guanine, but also interact with phosphate and carbonate as well as glutathione. Acid may favor aquation and thus facilitate DNA substitution. Hence, the three mechanisms outlined above for N-mustards could also apply to CPT. CPT produced no significant growth delay, and the small growth delay noted in combination with LND can be attributed completely to the action of LND. It is hard to explain the lack of enhanced CPT activity in the presence of LND. Perhaps, acidification leads to retention of CPT in the cytosol because of the production of charged anionic CPT adducts with water. However, it was gratifying to find that LPAM had substantial activity against DB-1 melanoma when administered alone, and this activity was substantially enhanced following the addition of LND, yielding approximately one log order of tumor cell-kill with one treatment. No changes were seen in the NMR measured parameters with the addition of LPAM when compared to animals given LND only. **Acknowledgements:** This study is supported by grant 1-R01-CA-129544.

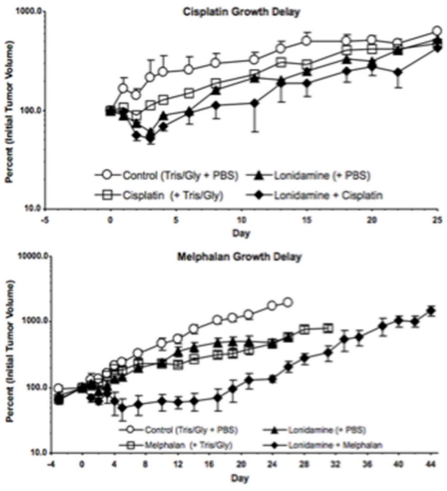

Fig. 3. Growth delay experiments performed on DB-1 human melanoma xenografts in nude mice treated with 7.5 mg/kg cisplatin (upper) and 7.5 mg/kg melphalan (lower). When not shown, error bars are less than symbol size. Note difference in time scales for cisplatin and melphalan graphs.

References: (1) Cancer facts and figures, 1994. (2) MacLennan R, et al. J Natl Cancer Inst 84, 1992, 1427-1432. (3) Atema A, et al. Int J Cancer 54, 1993, 166-172. (4) Canter RJ, et al. Ann Surg Oncol 11, 2004, 265-273. (5) Jahde E, et al. Cancer Res 49, 1989, 2965-2972. (6) Kuin A, et al. Br J Cancer 79, 1999, 793-801. (7) Wong P, et al. Clin Cancer Res 11, 3553-3557, 2005. (8) Nath K, et al NMR Biomed 2012 (Epub ahead of Print). (9). Corbett THaV F. A (Pergamon Press, New York), 1987. (10) Todd RC, et al. J Am Chem Soc 129, 2007, 6370-637.