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 (pHe) (n=3) and bioenergetics (βNTP/βPi) (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.v. 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.v. with LND (100 mg/kg) and after 40 minutes (determined by 31P 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 μl/g) and CPT, LPAM or PBS (5.0 μl/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/βPi 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, log2 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.

Results: In vivo 31P MRS (Fig. 1) demonstrates that human melanoma xenografts in immunosuppressed mice treated with the monocarboxylate transport (MCT) inhibitor lonidamine show significant decreases in pH and βNTP/βPi (8), compared to the following treatment groups: cohort 1 (sham treated control) was infused i.v. with PBS 40 minutes after LND administration i.p. (100 mg/kg); cohort 2 was injected i.v. with tris/glycine buffer and infused i.v. with CPT (7.5 mg/kg delivered in ~10 sec) in PBS; cohort 3 was injected i.v. with LND (100 mg/kg) and after 40 minutes (determined by 31P 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 μl/g) and CPT, LPAM or PBS (5.0 μl/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/βPi 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, log2 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.

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 glutathione-S-transferase under acidic conditions, and 3) decreased DNA repair due to acid inhibition of O’-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 diaque species (10). Monoaqua species can react with guanine, but also interact with phosphate and carbonate as well as glutathione. Acid may favor aquration 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.

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