# Selective acidification and de-energization of A2780 ovarian cancer xenografts using lonidamine: A preliminary 1H and 31P MRS study

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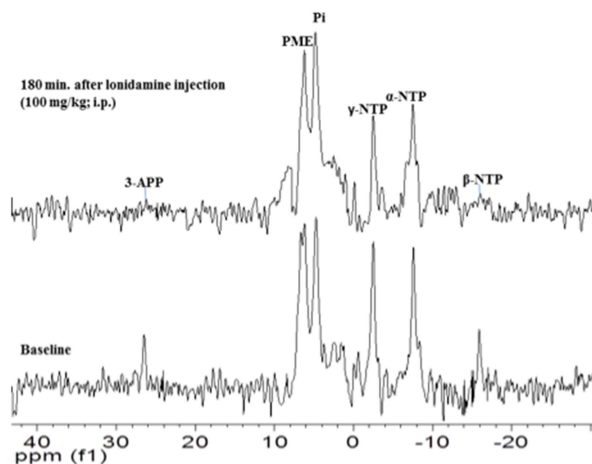
Fig. 1. *In vivo* localized (Image Selected In vivo Spectroscopy - ISIS) [31]Phosphorus magnetic resonance spectroscopy spectra of ovarian cancer xenografts grown subcutaneously in nude mice (lower) pre- and (upper) 180 min. post LND administration (100 mg/kg, i.p.).

Introduction: Ovarian cancer, if detected early, is highly curable by surgery. However, 70% of initial cases are diagnosed at advanced stages when peritoneal spreading is present. At such stages, first-line chemotherapy following surgical debulking of tumor usually induces cancer remission but does not eradicate it. In the majority of the patients, the cancer will relapse, and when given chemotherapy develop drug resistance and become unresponsive to subsequent chemo-treatment. Consequently, eradicating cancer cells during the first-line therapy and overcoming drug resistance would be critical to prolong the remission and survival of patients. To achieve this goal, we propose to exploit the metabolism and microenvironment of the tumor. Specifically, we propose to test a metabolic modulator that traps lactic acid inside cancer cells and remarkably reduces their energy production. Such manipulations would sensitize the cancer cell to thermo- and specific chemotherapy (e.g., doxorubicin) and suppress drug efflux pump activity. Importantly, the distinctive features of cancer metabolism may allow metabolic modulation to be cancer "specific" with no or highly reduced collateral damage to normal tissues (e.g., bone marrow & heart).
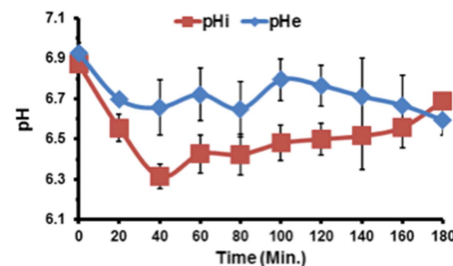
Fig. 2. The intracellular pH (pHi) and extracellular pH (pHe) profile relative to baseline as a function of time of ovarian cancer xenografts in response to LND (100 mg/kg; i.p.) administration at time zero. The values are presented as mean ± S.E.M. When not displayed, S.E.M. values were smaller than the symbol size.
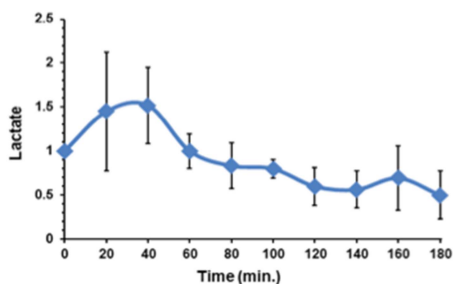
Material and Methods: 4-6 weeks old female athymic nude (NCI Production) (n=3) were used in the study. A2780 ovarian cancer cells (5×10^6) in 0.1 ml of RPMI 1640 medium were inoculated subcutaneously into the right thigh of each animal. Ovarian cancer xenografts were allowed to grow until they reached 7-10 mm in diameter along the longest axis of the tumor. Mice were anesthetized using 1% isoflurane in oxygen delivered through a custom-built nose cone, and the MR studies were performed on a 9.4 T/31 cm horizontal-bore Varian system. *In vivo* [31]P and ^{1}H spectra were acquired with a homemade 10 mm and 15 mm (inner diameter) resonator, respectively. The animal was mounted in the coil such that the subcutaneous tumor projected into the resonator. A rectal thermistor and respiration pillow were placed and connected to a device that monitors vital signs including ECG, core temperature and respiration in small animals. The animal's core temperature was maintained at $37(\pm1)$ ^{0}C during the scan. LND (100 mg/kg) was injected intraperitoneally through 26 ga × ¾ inch catheter without removing the animal from the magnet after acquisition of baseline spectra. Intracellular pH (pHi), extracellular pH (pHe), bioenergetics (βNTP/Pi) and lactate measurement were performed as described elsewhere (1). Nonparametric Mann-Whitney tests were used for statistical analysis (SPSS 16). The time courses of pHi, pHe, bioenergetics and lactate of the tumor before and following LND administration were determined.
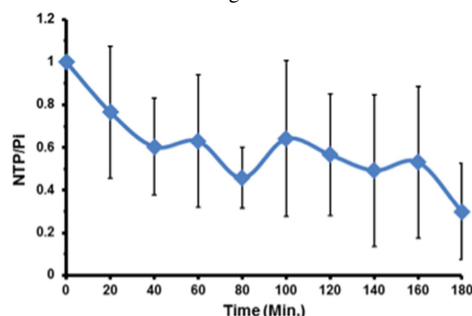
Fig. 3. Bioenergetics (βNTP/Pi; ratio of peak area) relative to baseline as a function of time of ovarian cancer xenografts in response to LND (100 mg/kg; i.p.) administration at time zero. The values are presented as mean ± S.E.M. When not displayed, S.E.M. values were smaller than the symbol size.

Results: *In vivo* ^{31}P MRS (**Fig. 1**) demonstrates that A2780 cancer xenografts in immunosuppressed mice treated with the MCT inhibitor LND exhibit a sustained and tumor-selective decrease in pHi and pHe of 0.56 ± 0.10 (p < 0.05) and 0.34 ± 0.23 (p = 0.05), respectively (**Fig. 2**). Tumor bioenergetics (βNTP/Pi) also decreased by 70.0 ± 22% (p < 0.05) following LND administration relative to the baseline level (**Fig. 3**).

The integrated intensity of the steady-state tumor lactate peaks was increased after 40 min. (p < 0.05) relative to the baseline level following LND administration as shown in **Fig. 4**

Discussion: The ^{31}P MR spectra clearly show that LND leads to intracellular acidification and depression of bioenergetics of the ovarian cancer xenograft *in vivo*; these are critical parameters for thermosensitization and/or improving tumor response to alkylating agents and anthracyclines. The concept of manipulating tumor pH and bioenergetics with metaiodobenzylguanidine (MIBG) and α-cyano-4-hydroxycinnamate (CHC) has been examined in our previous studies (2, 3). LND inhibits the MCT on the plasma membrane and may also block pyruvate entry into mitochondria for oxidation via the TCA (tricarboxylic acid) cycle. Thus, LND is a very attractive adjuvant for chemo- and thermo-therapy because, compared to CHC or MIBG. It has shown excellent safety profiles in clinical trials. While LND has been demonstrated to inhibit the export of lactate from the tumor cells of human DB-1 (1) and MCF-7 (4) tumors and glioma cells of rat origin (9L) (5), it is not clear if it inhibits transport of pyruvate into mitochondria as CHC does (4-6). However, the similar effect of CHC and LND on the bioenergetics of DB-1 melanomas (1, 3) and ovarian cancer here strongly suggest that it does. Therefore, the decline of bioenergetics monitored by ^{31}P MRS could be explained by a profound decrease in mitochondrial metabolism following LND administration.

References: (1). Nath K, et al NMR Biomed 26(1):98-105, 2013. (2). Zhou, R., et al. Cancer Research 3532-3536, 2000. (3). Zhou, R., et al. Academic Radiology 571-582, 2001. (4). Ben-Horin H, et al. Cancer Res 2814-2821, 1995. (5). Ben-Yoseph O, et al. J Neurooncol 36:149-157, 1998. (6). Mardor Y, et al. Cancer Res 60:5179-5186, 2000.

Fig. 4. Change in tumor lactate as a function of time after administration of LND (100 mg/kg). The area under the curve was compared with baseline at each time point and was normalized to baseline levels. The values are presented as mean ± S.E.M. When not displayed, S.E.M. values were smaller than the symbol size.