Intracellular Selective Acidification of Human Melanoma Xenografts by Lonidamine: A 31P Magnetic Resonance Spectroscopy Study

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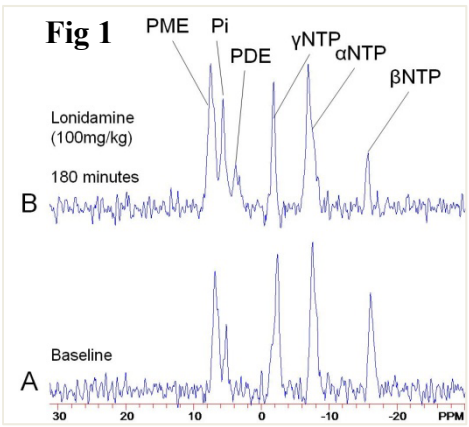
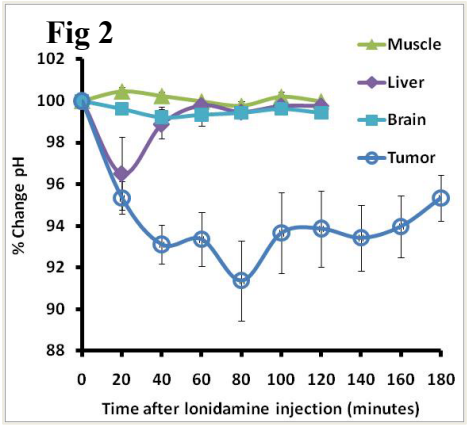
Introduction: Melanoma is an increasingly common, potentially fatal form of skin cancer; it accounts for approximately 4% of skin cancer cases but for 80% of all skin cancer deaths (1). In general, this disease is not responsive to radiation therapy or most forms of chemotherapy. Surgical excision is the only proven therapy that leads to cureif the cancer is detected early (2). However, if recurrence occurs with metastasis, the prognosis is very poor since effective methods for treating the systemic disease are not available. The goal of this project is to develop a method for systemic therapy of melanoma.Selective acidification could provide a basis for sensitization of melanoma to hyperthermia and to alkylating agents that exhibit large increases in activity in an acidic environment (3). This study monitors intracellular pH (pHi) and bioenergetics in tumors and normal tissues (brain, muscle, and liver) using P-31 MR spectroscopy after the treatment with lonidamine, an agent that inhibits transmembrane monocarboxylate transporters (MCT) (4,5).

Material and Methods: : 4-6 week old male Balb/c athymic nude mice (n=9) were used in the tumor study. DB-1 human melanoma cells (10^6) in 0.1 ml of HBSS were inoculated subcutaneously into the right thigh of each animal. Melanoma xenografts were allowed to grow until they reached 10-13 mm in diameter along the longest axis of the tumor. Mice were anesthetized using 1% isoflurane in oxygen, and the MR studies were performed on a 9.4 T/31 cm horizontal bore Varian system. In-vivo P-31 spectra were acquired with a homemade resonator (13mm in diameter). The animal was mounted in the coil such that the subcutaneous tumor projected into the resonator. The oxygen was delivered through a custom-built nose cone. Sub-dermal needle electrodes and a rectal thermistor were placed and connected to the mousefor electrocardiogram and core body temperature monitoring. The animal's core temperature was maintained at $37(\pm1)^0$C during the scan. A respiration pillow was placed over the thorax and a pulseoximeter over the tail to monitor respiration and oxygen saturation, respectively. Lonidamine (100 mg/kg) was injected intraperitoneally after removing the animal from the magnet following acquisition of baseline spectra. Brain (n=3), surgically exposed liver (n=3) and muscle (n=3) served as representative normal tissues to study the effects of the drug. Data were processed offline by using NUTS (Livermore, CA, USA) and MestRec (Mestrelab Research, Spain) software. The pHi was determined from the Henderson-Hasselbach equation using the chemical shifts of Pi, referenced to the α-NTP resonance.

Results: In 9 animals (nonlocalized=7, localized=2) a consistent decrease in pHi was observed after the injection of lonidamine (Fig 1). A maximum decrease in pHi (0.6 unit, p = 0.003) was observed after 80 minutes (6.3±0.13). No change in pHiwas observed in the brain (n=3) and leg muscle (n=3), whereas in surgically exposed liver, a transient decrease in pHi (~0.2 unit, p= 0.19) was observed after 20 minutes(Fig 2). The bioenergetics (βNTP/Pi ratio) of the tumor remained low (~56%, p = 0.004) from baseline to 180 min. We didn't observe a decrease in bioenergetics in the brain or hind muscle. However, the liver ratio decreased from baseline (1.61±0.14)till 120 minutes (1.15±1.0), and a maximum decrease (~21%, p = 0.04) was observed after 40 minutes (1.12±0.07) (Fig 3).





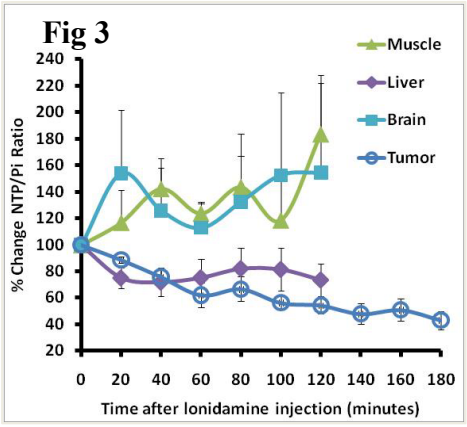


Fig. 1 Shows localized (ISIS) in vivo P-31 MR spectra of the melanoma xenograft (A) pre- and (B) 180 minutes post-administration of lonidamine (100 mg/kg i.p.). **Fig. 2** pHi change as a function of time. Initial values (mean pHi±SEM) for hind leg muscle, liver, brain, and tumor were 6.79±0.05, 7.11±0.08, 7.11±0.07 and 6.87±0.09, respectively. **Fig. 3** Change in bioenergetics (βNTP/Pi ratio) in tumors and normal tissues as a function of time. Initial values (mean±SEM) for hind leg muscle, liver, brain, and tumor were 1.82±2.0, 1.61±0.14, 1.95±0.66 and 2.00±0.27, respectively.When not displayed, SEM values were smaller than the symbol size.

Discussion: The P-31 MR spectra clearly show that lonidamine (100mg/kg) induces intracellular acidification and decreases the bioenergetics of the melanoma xenograft in vivo, a critical parameter for thermosensitizationand/or improving tumor response to alkylating agents. The concept of manipulating tumor pHi with metaiodobenzylguanidine (MIBG) and α-cyano-4-hydroxycinnamate (CNCn) has been advocated by our group in previous studies (3,6). Lonidamine acts as an MCTinhibitor on the cell membrane (preventing the release of lactate and protons) (5) and has a sustained acidification effect for more than three hours compared to the shorter durational effects of MIBGand CNCn. Tumor acidification correlated with a decrease in the bioenergetics of the tissue as measured by the βNTP/Pi ratio (Fig. 3). This may reflect the ability of lonidamine to act similarly to CNCn by blocking both plasma membrane MCTs and mitochondrial pyruvate transporters. There was a transient acidification and a slight decrease in the energy metabolism of surgically exposed liver, although the dose of lonidamine was well tolerated. The liver, as a detoxification organ, likely receives a significant dose of lonidamine, but the drug effects are only transient due to the organ's high metabolic activity and high rate of perfusion. Our future plans include the pharmacokinetic study of lonidamine using high performance liquid chromatography, biochemical studies to examine toxicity of the drug and the selection of appropriate chemotherapeutic agents to maximize tumor growth delay. Ultimately, we hope to translate this method into the clinic by utilizing ^{1}H MRS methods to monitor lactate in the tumors with the Had/Sel-MQC pulse sequence (7).

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