Intracellular acidification of human melanoma xenografts by the respiratory inhibitor lonidamine plus hyperglycemia: A 31P magnetic resonance spectroscopy study

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Introduction: Melanoma is the most aggressive form of skin cancer; it accounts for approximately 4% of skin cancer cases but for 80% of all skin cancer deaths (1). Melanoma is primarily treated by surgical excision, which is often curative if the tumor is detected in its early stages. However, if recurrence occurs with metastasis, the prognosis is very poor since effective methods for treating the systemic disease are not available. Selective acidification could provide a basis for selective sensitization of melanoma to alkylating agents (cisplatin, cyclophosphamide and melphalan) that exhibit large increase in activity in acidic environment (2). So, our aim is that melanomas, like other tumors, exhibit high levels of glycolytic metabolism, which could provide a basis for their selective acidification through accumulation of lactate by blocking mitochondrial metabolism with lonidamine which inhibits oxidative phosphorylation in tumor under hyperglycemic conditions.

Material and Methods: Male athymic nude mice (01B74) of 4-6 weeks of age were included in the study. Cell lines (DB-1) were obtained from Dr. David Berd laboratory, Thomas Jefferson University. One million melanoma cells in 0.1 ml of Hanke's balanced salt solution (Invitrogen/Gibco, Carlsbad, CA, USA) were inoculated subcutaneously in to the right thigh of each animal. Melanoma xenografts were allowed to grow until they reached 10-13 mm in diameter along the long axis of the tumor. Tumor bearing mice were anesthetized with cocktail of ketamine hydrochloride (10mg/ml) and acepromazine (1mg/ml). A tail vein catheter was placed for i.v. infusion of glucose. Intramuscular catheter was placed for delivery of additional anesthetics. A stock solution of D-glucose (2.5 M) diluted to 0.6 M and then delivered through a tail vein catheter with a syringe pump (Harvard Apparatus, Holliston, Mass). The MR studies were performed on a 9.4 T/8.9 cm vertical bore Varian system. In-vivo ^{31}P spectra were acquired with a home made surface coil (13mm in diameter). The animal was mounted in the coil such that the subcutaneous tumor projected in to the resonator. The oxygen was delivered through a custom-built nose cone. An optical temperature probe (Luxtron, Mountain View, CA, USA) was inserted rectally and provided thermostatic control of an in-house built warm air source. The output of the device was directed over the animal, which maintained the animal's core body temperature at $37^0 \pm 2^0$. Lonidamine (50 mg/kg) was injected intraperitonially by removing the animal from the magnet during the acquisition. Data were processed off line by using NUTS software (Livermore, CA, USA). The intracellular pH (pHi) was determined from the Henderson Hasselbach equation using the chemical shifts of Pi, referenced to α-NTP resonance.

Results: In preliminary MR experiment after the glucose infusion of one animal showed that pHi of the tumor was altered by ~ 0.7 unit during 120 min of glucose infusion and administration of lonidamine 20 min later (fig. 1)

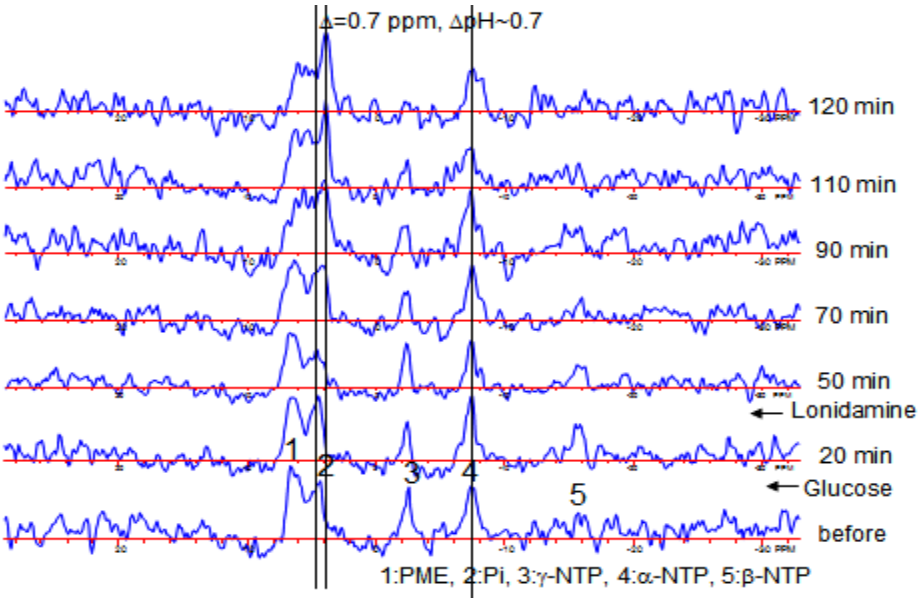

Fig1: Representative ^{31}P MR spectra of melanoma xenografts developed in athymic nude mice showing change by ~ 0.7 unit during 120 min of glucose infusion and administration of lonidamine 20 min later.

Discussion: Preliminary result of this study demonstrates that lonidamine combined with hyperglycemia acidified human melanoma xenografts by reducing pHi. Here, we report for the first time that lonidamine decreases pHi *in vivo*, a more critical parameter for thermosensitization to improve tumor response to alkylating agents. While the concept of manipulating tumor pH with m-iodobenzylguanidine (MIBG) has been advocated by our group earlier (3, 4). Lonidamine is widely used in Europe and Canada to modulate the effects of various anti-neoplastic agents. Our future plan is to optimize the lonidamine dose, to monitor physiological changes, to examine potential functional toxicity of these procedures to critical tissues such as the brain, skeletal muscle, heart, kidneys, liver and ultimately translation of this method in to the clinic by utilizing ^{1}H MRS methods to monitor lactate in the tumors by the Had/Sel-MQC pulse sequence to edit the lactate peak during data acquisition (5).

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