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Magnetic Resonance Imaging Identifies Differential Response to Pro-Oxidant Chemotherapy in a Xenograft Model[1]

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Abstract

Induction of oxidative stress is a key component of cancer therapy. Pro-oxidant drugs have been demonstrated to enhance the efficacy of radiotherapy and chemotherapy. An emerging concept is that therapeutic outcomes are dictated by the differential redox buffering reserve in subpopulations of malignant cells, indicating the need for noninvasive biomarkers of tumor redox that can be used for dose identification and response assessment in a longitudinal setting. Magnetic resonance imaging (MRI) enhanced with the thiol-binding contrast agent Gd-LC6-SH, and hemodynamic response imaging (HRI) in combination with hypercapnia and hyperoxia were investigated as biomarkers of the pharmacodynamics of the small molecule pro-oxidant imexon (IMX). Human multiple myeloma cell lines 8226/S and an IMX-resistant variant, 8226/IM10, were established as contralateral tumors in SCID mice. T1slope, an MRI measure of the washout rate of Gd-LC6-SH, was significantly lower post-IMX therapy in 8226/S tumors compared with vehicle controls, indicating treatment-related oxidization of the tumor microenvironment, which was confirmed by analysis of tumor tissue for thiols. T1slope and *ex vivo* assays for thiols both indicated a more reduced microenvironment in 8226/IM10 tumors following IMX therapy. HRI with hypercapnia challenge revealed IMX inhibition of vascular dilation in 8226/S tumors but not 8226/IM10 tumors, consistent with decreased immunohistochemical staining for smooth muscle actin in treated 8226/S tumors. MRI enhanced with Gd-LC6-SH, and HRI coupled with a hypercapnic challenge provide noninvasive biomarkers of tumor response to the redox modulator imexon.

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Introduction

Oxygen free radicals, or reactive oxygen species (ROS), are by-products of normal mitochondrial metabolism [1]. Historically, ROS, such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\cdot}$), have been recognized for their role in age-related damage of proteins, lipids, and DNA. More recently, studies have identified ROS as key second messengers that can activate signaling pathways to drive essential physiological functions, including cell division, migration, metabolic activity, and programmed cell death. The intracellular activity of ROS in signal transduction and the overall redox status of the cell are tightly regulated by endogenous antioxidant systems. An imbalance of the redox system in tumor cells has been identified as a potential therapeutic target for anticancer drugs [2,3].

Imexon (IMX) is an aziridine-containing iminopyrrolidone that is in phase II clinical trials for various malignancies [4–8]. Previous studies have demonstrated that IMX reacts with sulfhydryl groups

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resulting in a decrease of intracellular thiols, an accumulation of ROS, loss of mitochondrial membrane potential, and apoptosis [9–11]. Biochemical analyses have shown that this interaction occurs via classical aziridine ring opening to bind glutathione (GSH) or via nucleophilic attack at the cyclic amidine moiety by the sulfur atom in cysteine [12].

Inactivation of common chemotherapeutic agents, such as platinum drugs, nitrogen mustards, doxorubicin, and nitrosoureas, can occur via conjugation of reduced glutathione with electrophilic centers of these molecules. Through hydrogen donation, sulfhydryl-containing species such as cysteine and GSH can also effect repair of DNA damage produced by ionizing radiation [13]. Therapeutic agents such as IMX that deplete tumor thiol reserves may therefore enhance the anticancer efficacies of both chemotherapy and radiotherapy. In a recently concluded phase 2 study, patients with relapsed/refractory B-cell non-Hodgkin lymphoma were treated with IMX as a single agent. Progression-free survival was calculated, and clinical response was correlated with a redox signature score that was generated from gene expression analyses of both pretreatment tumor biopsies and pre- and posttreatment peripheral blood mononuclear cells [4]. These laboratory correlates demonstrated that patients with higher redox scores were more likely to respond to the pro-oxidant IMX. However, no reliable method currently exists for measuring intratumoral oxidative stress directly and accurately that would allow *a priori* prediction of patient response. There is a clinical need for a noninvasive correlate of oxidative stress to inform treatment selection, monitor tumor response longitudinally, and assess spatial heterogeneity in solid tumors. The objectives of this study were therefore to develop imaging biomarkers of the pharmacodynamics of IMX and to relate our findings to differences in the responses of drug-sensitive and drug-resistant tumors to IMX.

Gd-LC6-SH is a thiol-bearing DOTA complex of gadolinium, and we have previously demonstrated that the decrease in tumor longitudinal relaxation time produced on magnetic resonance imaging (MRI) by Gd-LC6-SH at 60 minutes postinjection (= ΔT1) was correlated with tumor thiol content in Mia-PaCa-2 pancreatic and NCI-N87 gastric cancer xenografts [14]. A limitation to the interpretation of tumor ΔT1 in terms of tumor redox status is that it would also be a function of tumor perfusion that delivers the agent and its extracellular volume of distribution within the tumor. Gd-LC6-SH binds to plasma albumin at the conserved Cys^{34} site, which prolongs its retention in the tumor microenvironment. The washout rate of Gd-LC6-SH from the tumor will be a function of the local concentration of small molecule thiols that compete for binding to albumin at the same site. We hypothesize that the rate of change of tumor T1 during washout of Gd-LC6-SH (= T1slope) is a biomarker of tumor redox status that is independent of tumor perfusion and extracellular distribution volume fraction; faster washout rates will be expected in more reducing microenvironments.

In this study, we report that the T1slopes of IMX-sensitive 8226/S and IMX-resistant 8226/IM10 multiple myeloma tumor xenografts measured *in vivo* in control and IMX-treated mice correlate with tumor thiol content measured *ex vivo* using histologic and enzymatic assays. Treatment of 8226/IM10 tumors with the pro-oxidant IMX produced a trend toward a more reduced tumor microenvironment, suggesting a compensatory response in these IMX-resistant tumors. We also report that hemodynamic response imaging (HRI) by MRI in combination with hypercapnia provides a noninvasive biomarker for the effect of IMX on tumor vascular maturation. We discuss the potential for Gd-LC6-SH MRI and HRI in pretherapy prognostication and posttherapy response assessment.

Materials and Methods

Mice, Tumors, and Drug

All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of Arizona. The 8226/S human myeloma cells were originally obtained from ATCC, and the IMX-resistant 8226/IM10 cell line was created by continuous exposure to IMX as previously described [15,16]. Dual xenografts on contralateral flanks of 6-week-old female SCID mice were established by subcutaneous injection of 10×10^6 cells. Animals were monitored every 3 days for general health, body weight, and tumor volume by caliper measure. Mean tumor volume was used to stratify animals into equal treatment groups when the tumors were approximately 100 to 250 mm^3. IMX was generously provided by AmpliMed Corp, Tucson, AZ, and was prepared as a 15-mg/ml solution in 0.9% NaCl. Animals were administered either IMX (150 mg/kg, intraperitoneally [i.p.]) or saline (0.25 ml per 25 g, i.p.) on a day 1-5-9 schedule. MRI was done on day 0 (pretreatment) and 2 hours post drug administration on day 9.

Hemodynamic Response Imaging

All MRI measurements were made on a Bruker Biospec MR imager with a 7-T horizontal bore magnet equipped with 600-mT/m self-shielded gradients (Bruker Biospin, Billerica, MA). Mice were anesthetized using isoflurane (1-2%, rest air) and cannulated at the tail vein for injections. T2*-weighted gradient echo (GRE) images were collected using the following parameters: repetition time (TR) = 200 milliseconds, echo time (TE) = 15 milliseconds, flip angle (FA) = 45°, matrix = 128 × 128, number of averages (NA) = 8. Six consecutive images were acquired with the mouse breathing air followed by 7 consecutive images after the breathing gas was changed to air-CO_2 (95% air, 5% CO_2) and a final 7 consecutive images acquired as the animal breathed carbogen (95% O_2, 5% CO_2). The first image acquired following each change in breathing mix was left out during data analysis to account for gas exchange and physiological equilibration. Indices of vasodilation (VD) and vascular function (VF) were calculated by the method of Abramovitch and colleagues [17,18], as follows:

$$\mathrm{VF} = \ln\left(\frac{I_{\mathrm{O2\text{-}CO2}}}{I_{\mathrm{air\text{-}CO2}}}\right)/(\mathrm{TE}\bullet C_{\mathrm{MRI}}) \quad (1)$$

$$\mathrm{VD} = \ln\left(\frac{I_{\mathrm{air\text{-}CO2}}}{I_{\mathrm{air}}}\right)/(\mathrm{TE}\bullet C_{\mathrm{MRI}}) \quad (2)$$

Here I_{air}, $I_{\mathrm{air\text{-}CO2}}$, and $I_{\mathrm{O2\text{-}CO2}}$ are the T2*-weighted MRI signal intensities with the mouse breathing the respective gas mixes. The constant C_{MRI} incorporates the effects of the magnetic field strength and the susceptibility of deoxyhemoglobin and was scaled linearly from the value calculated by Ogawa and colleagues [19] to 892 s^{-1} at 7 T.

Gd-LC6-SH MRI

{1,4,7-Tris(carboxymethyl)-10-[N-(6-mercaptohexyl)carbamoyl]-1,4,7,10-tetraazacyclododecanato}gadolinium (Gd-LC6-SH)