Metabolite changes in HT-29 xenograft tumors following HIF-1α inhibition with PX-478 as studied by MR spectroscopy in vivo and ex vivo.

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Introduction: **PX-478** is a novel agent that suppresses both constitutive and hypoxia-induced levels of the hypoxia-inducible transcription factor, HIF-1α, in cancer cells [1]. The inhibition of tumor growth by **PX-478** is positively associated with HIF-1α levels in a variety of human tumor xenografts in *SCID* mice. The aim of this study was to identify metabolic markers for HIF-1α inhibition and tumor response to PX-478 treatment to identify possible biomarkers prior to phase I/II clinical trials. Single voxel in vivo localized ^{1}H spectra were obtained from HT-29 tumor xenografts before and until 24h after treatment with PX-478. The profiles of water soluble and lipophilic metabolites with both ^{1}H and ^{31}P in vitro spectroscopy were studied on control and treated HT-29 tumor extracts.

Methods: SCID Mice bearing HT-29 colon xenografts were treated with either vehicle or with 125 mg/kg PX-478 (Prolx Pharmaceuticals) and studied over time with single voxel *in vivo* ^{1}H MRS. This was performed on a 4.7 T 40-cm horizontal bore MR imager (Bruker, Billerica, MA) using a PRESS technique. For ex-vivo high resolution spectroscopy, mice were treated with either vehicle or with increasing doses of PX-478 (50-200 mg/kg) and sacrificed 24 hours later. Extraction of the water-soluble metabolites and of the lipids was performed by a dual phase extraction (DPE) method [2]. All *ex vivo* spectra were recorded on a DRX-500 NMR spectrometer (11.7 Tesla, Bruker, Rheinstetten, Germany). 3-(Trimethylsilyl)propionic acid (TSP) was used as an external standard for quantification with ^{1}H spectroscopy (128 acquisitions, 65Kdata points, 10 KHz sweep width, 277K). 1-APP (1-aminopropylphosphonate) was used as an external standard for ^{31}P spectroscopy (5000 acquisitions for aqueous extracts and 1200 acquisitions for lipid extracts, 65Kdata points, 10 KHz sweep width, 280K). All animal protocols were approved by the University of Arizona Institutional Animal Care and Use Committee.

Results: In vivo, a significant reduction in the total choline (tCho) signal of the PX-478-treated group relative to pre-treatment values was observed 12 and 24h after treatment. tCho may comprise signals from choline-containing compounds, such as glycerolphosphocholine (GPC), phosphocholine (PC), and choline itself, together with contributions from other metabolites, such as myoinositol (mI), taurine (Tau), and phosphoethanolamine (PE). Although the lactate and lipid (lac+lip) peak shows a trend to decrease, no significant change was observed at any time point. This was also confirmed in extracts which showed trends towards decreased lactate, yet which were also insignificant. In vitro studies and ex vivo immunocytochemistry show that PX-478 causes a significant reduction in glucose consumption, lactate production and expression of the HIF-induced glucose transporter, GLUT-1. Ex vivo, the level of PC (P=.02), GPC (P=.02), and mI (P=.05) were significantly decreased in the PX-478-treated tumor extracts in comparison with vehicle-treated tumor extracts, while changes in the other metabolites were insignificant. In vitro ^{31}P-MRS of the aqueous extracts showed significantly decreased levels of PE (P=.04), PC (P=.02), glycerophosphoethanolamine (GPE) (P=.01), and GPC (P=.03) in the PX-478 treated tumors.

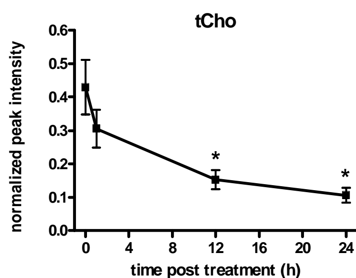

Figure 1

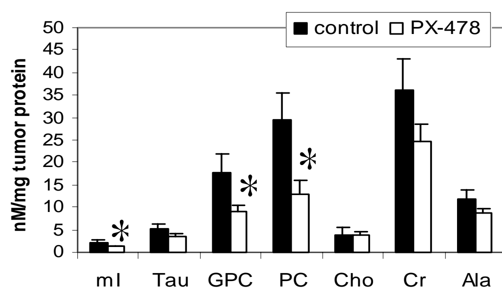

Figure 2

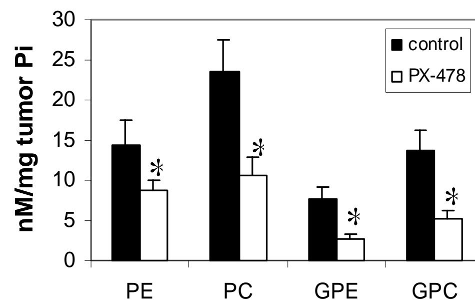

Figure 3

Figures. <1>. *In vivo* total choline following PX-478. (* P< 0.05). **<2>** *Ex vivo* aqueous metabolites from ^{1}H and **<3>** ^{31}P MRS (B) in treated and controls. (* = p<0.05).

Conclusions: Elevated concentrations of choline metabolites have been observed by MRS in a variety of malignancies, and progression of tumors to a malignant phenotype is associated with an overall increase in the content of PC and GPC. In vivo as well as ex vivo results in this study showed a decrease in those metabolites after treatment with PX-478. Both PMEs and PDEs have been proposed as possible indicators of malignancy, tumor response to therapy, and even predictors of long-term response. Our results point out an elevated basal level of both PME and PDE in aqueous extracts of HT-29 tumors that are significantly decreased after anti-tumor treatment with PX-478. Although there was a tendency for lactate to decrease in response to this drug, the changes were not significant. This is interesting since this drug caused significant decreases in both glucose consumption and lactate production rates in vitro. We suspect that the lack of consistent effect on in vivo lactate levels was due to the combine effect of these changes with the significant reductions in perfusion also observed in response to this drug [3]. Nonetheless, the significant and robust change in tCho has identified this as a potential ^{1}H MRS-visible biomarker for drug response in vivo.

References:

[1] Welsh S, et al., (2004). Mol Cancer Ther 3, 233-44. **[2]** Katz-Brull R et al. (2002), Cancer Res. 62, 1966-70 **[3]** Jordan et al. Neoplasia, 2005 (in press)