Thioredoxin Inhibition Endpoints for PX-478, a Novel Cancer Therapeutic, by Pre-Clinical Dynamic Contrast Enhanced MRI and 1H MRS

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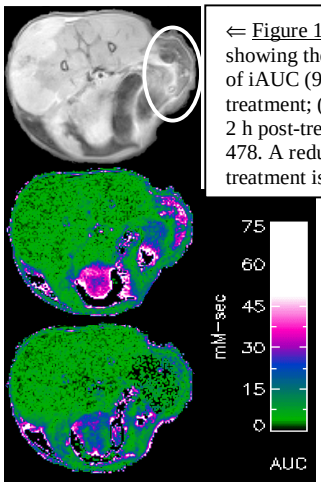
Introduction: The redox protein thioredoxin-1 is overexpressed in many cancers, and through stabilization of hypoxia-inducible factor 1α (HIF-1α), leads to increased production of Vascular Endothelial Growth Factor (VEGF) and tumor angiogenesis [1]. The experimental drug PX-478 is an inhibitor of thioredoxin reductase, and produces acute reduction in circulating levels of VEGF and tumor levels of HIF-1α in tumor-bearing mice. Magnetic resonance imaging (MRI) has the capability to provide early tumor response data in anti-angiogenic therapy. We have employed dynamic contrast-enhanced MRI (DCE-MRI) with both small (Magnevist®) and large (albumin-Gd-DTPA) MW contrast reagents to monitor response of HT-29 colon cancer xenografts to PX-478 in mice. The DCE-MRI data were analyzed using a modified Kety model [2] or on the basis of initial-area-under-the-curve (iAUC) and enhancement kinetics. We have also examined the usefulness of single voxel 1H spectroscopy in assessing tumor response to PX-478.

Methods: All animal protocols were approved by the University of Arizona Institutional Animal Care and Use Committee (IACUC). Severe Combined Immune Deficient (SCID) mice were implanted on the flank with HT-29 cells, a tumorigenic, non-metastatic colon carcinoma cell line. Tumors were allowed to grow to 100-1000 mm3 prior to imaging or spectroscopy. Mice were anesthetized using 1.0-1.5% isoflurane carried in 100% O2. The tail was cannulated 3-5 cm from the base of the tail to avoid compromising vessel integrity during injection. The mouse was placed in a holder and inserted into a 24 mm ID birdcage coil (Doty Scientific, Columbia, SC). Body temperature was maintained 37-38 °C with a circulating water blanket.

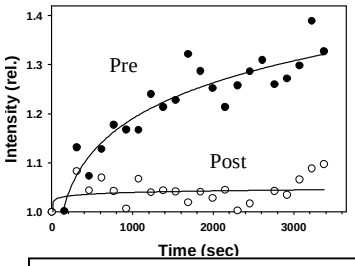
All imaging was performed on a 4.7 T horizontal bore MR imager (Bruker, Billerica, MA).. Following acquisition of proton density and high S/N spin echo images, a dynamic series of spin-echo images (TR=150-300 ms, TE=5.9-8 ms, NA=1-4, NR=23-60) were collected over 60 minutes, with the contrast agent solution (0.1 mmole/Kg Magnevist or 600 mg/kg Albumin-Gd-DTPA, in 0.05 mL) being injected during repetitions 2-5. Data analysis was performed using programs written in Interactive Data Language (Research Systems, Boulder, CO). In a separate cohort of 7 mice, single voxel 1H spectroscopy was performed using the VSEL implementation of the PRESS localization technique. Scans were performed at 0,1,2,4,12 and 24 hours post therapy.

Results: Initial results indicate that treatment with 125 mg/kg PX-478 (intraperitoneal) causes an acute reduction in iAUC of Magnevist 2-4 hours following treatment (figure 1). We also observed a decrease in the albumin-Gd-DTPA enhancement by 4 hours following drug treatment (figure 2). Changes in the total choline resonance with PX-478 treatment were visible by *in* vivo 1H MRS (figure 3):

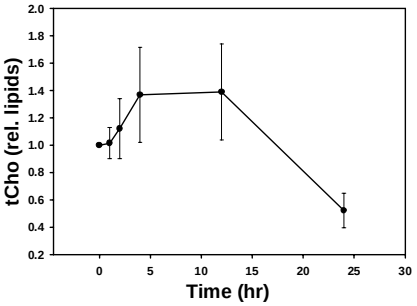
Conclusions: PX-478 shows promise in reducing VEGF and HIF-1α in animal models of cancer. DCE-MRI and 1H MRS are affected by PX-478 in HT-29 xenografts and may be useful biomarkers in human patients.



⇐ Figure 1. (Top) Axial anatomic image showing the tumor (circle); (Middle) map of iAUC (90 s post-contrast), pre-treatment; (Bottom) map of iAUC (90 s), 2 h post-treatment with 125 mg/Kg PX-478. A reduction in iAUC following treatment is apparent.



⇑ Figure 2. Enhancement following administration of albumin-Gd-DTPA. The slope 4 h following treatment with 125 mg/Kg PX-478 (hollow circles), was reduced compared to before treatment (filled circles).



⇑ Figure 3. Tumor response by ^{1}H MRS. Change in the ratio of 'total Choline' to lipids, with time following treatment with 125 mg/Kg PX-478.

References:

1. Welsh SJ, et al., Cancer Research 62:5089-5095, 2002.
2. Tofts et al., Journal of Magnetic Resonance Imaging 10:223-232, 1999.