Using dynamic contrast enhanced MRI and immunohistochemistry to monitor tumour response to tirapazamine, a hypoxia-targeting chemotherapeutic agent

L. J. Bains[1], J. H. Baker[2], A. I. Minchinton[2], and S. A. Reinsberg[1]

[1]Physics and Astronomy, University of British Columbia, Vancouver, British Columbia, Canada, [2]Medical Biophysics, British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada

Introduction

Tirapazamine is a hypoxia-activated prodrug which has shown promise in treating hypoxic tumours, and is in phase III clinical trials. Recent immunohistological studies by Minchinton *et al.* uncovered anti-vascular effects of this drug [1]. We quantified this effect in xenograft tumours using dynamic contrast enhanced (DCE) MRI in combination with immunohistochemistry. Implanted fiducial markers were used for accurate coregistration of pre- and post-treatment MR images with histological sections. Dramatic decreases in perfusion were seen in the 24 hours after treatment with tirapazamine. Furthermore, analysis of DCE data revealed that the initial area under the contrast-time curve (IAUC) may be a predictor of treatment response for tirapazamine, since tumours with higher overall pre-treatment IAUC showed a diminished response to the drug. This study is the first use of MRI to monitor the *in vivo* effects of tirapazamine.

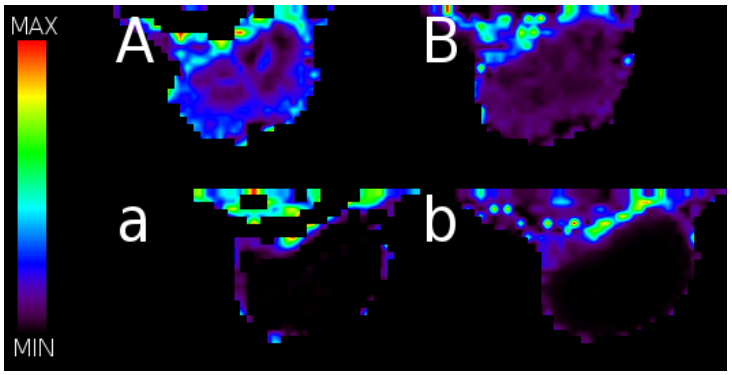

Figure 1: A) IAUC and B) K^{trans} before treatment. a) IAUC and b) K^{trans} after treatment with tirapazamine, showing a dramatic vascular shutdown

Materials and Methods

Mice: Fourteen SCID mice with subcutaneous HCT-116 (human colorectal cancer) xenografts were implanted with wax/saline fiducial markers to allow registration of pre/post treatment MR scans with histological sections. Mice received one MRI scan immediately prior to tirapazamine treatment and a second scan at 24 hours after treatment, with tumour excision occurring after the second scan.
MRI: Imaging was performed on a 7 T Bruker Biospec 70/30 using a custom-built 4-turn distributed-capacitor solenoid. A two-TR FLASH protocol was used for the calculation of concentration of contrast agent with six 1mm slices (1.5 mm interslice distance) and in-plane resolution of 0.3 mm. Prior to contrast agent injection, one FLASH with TR/TE = 226ms/6ms and ten FLASH with TR/TE = 113 ms/6ms were used to calculate baseline T1. After the bolus iv injection of 10µl/g Gd-DTPA (0.3 mmol/kg), the TR=113ms FLASH was run continuously for 20 minutes with a time resolution of 14.5 s. A standard Kety model was used to find the transfer constant (K^{trans}) [2].
Immunohistochemistry: A robotic microscope was used to acquire whole-section carbocyanine fluorescence and haematoxylin/BrdU images from tissue slices corresponding to MRI imaging slices. An NIH-Image macro was used to find the number of carbocyanine-positive vessels per area as a measure of perfusion; haematoxylin/BrdU images were used to identify areas of necrosis.

Results and Discussion

Histology of treated tumours showed significantly more necrosis and less carbocyanine as compared to controls. MR analysis showed a significant decrease in whole-tumour IAUC (figure 2) and K^{trans} at 24 hours. As well, a significant increase was seen in the contrast between the tumour rim and tumour centre after treatment for both IAUC and K^{trans}, indicating an increase in perfusion heterogeneity. Significant correlations between IAUC and carbocyanine-positive vessels per pixel were seen, confirming that implanted fiducial markers are an effective means of coregistering MRI and histological data.

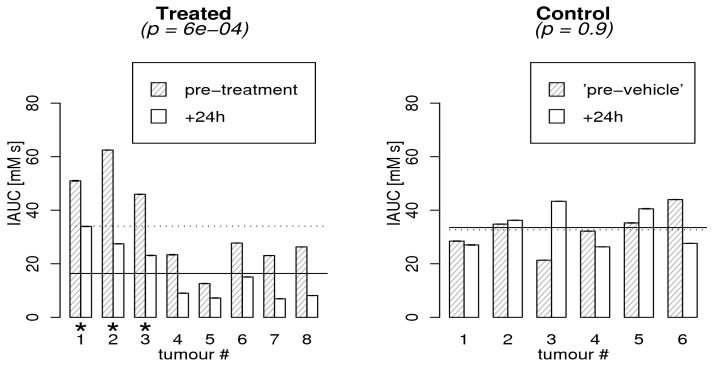

Figure 2: Whole-tumour mean IAUC before (hashed) and after (white) treatment. A significant difference was observed between the population mean IAUC before (dashed) and after (solid) treatment. Partial responders to treatment are starred.

A subset of three tumours had significantly higher post-treatment IAUC and more carbocyanine-positive vessels per area than the other treated tumours, indicating only partial response to treatment. An examination of the pre-treatment data for these three tumours showed that they had higher and more homogeneous pre-treatment IAUC, suggesting that elevated pre-treatment IAUC may predict poor response to tirapazamine.

Conclusions

Widespread vascular shutdown 24 hours after treatment with tirapazamine was demonstrated for the first time using in-vivo DCE MRI and validated with immunohistochemistry. Implanted fiducial markers were shown to be an effective means of co-registering multiple MR scans with histology. IAUC values which are low overall or low in the centre of the tumour were identified as potential predictors of tumour response to tirapazamine.

[1] LA Huxham et al. Radiother and Oncol, 78:138–145, 2006.
[2] PS Tofts et al. JMagn Reson Imaging, 10:223-32, 1999.