The Effect of cediranib on the Vascular Structure and Function of C6 Rat Xenografts with Combined Carbogen USPIO (CUSPIO) Imaging

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Introduction: The combined carbogen USPIO (CUSPIO) imaging protocol combines two MRI biomarkers, $\Delta R_2^*_{carbogen}$ and $\Delta R_2^*_{USPIO}$, with a novel segmentation scheme which allows comparison of their spatial distribution [1]. The CUSPIO imaging method has been shown to yield deeper information about tumour vasculature compared to using the two ΔR_2^* biomarkers in isolation. In particular, the CUSPIO method provides a novel method to assess the degree of co-localisation of spatial distribution of plasma and erythrocyte perfusion in tumour tissue, and visualise vascular functionality. Cediranib is an inhibitor of VEGFR-1, 2 and 3, and has been shown to cause a significant decrease in blood volume and vascular permeability in a number of subcutaneous tumour models [2, 3]. **The aim of this study was to apply the CUSPIO imaging protocol to a C6 rat xenograft tumour model treated with cediranib in order to interrogate any subtle effects this therapeutic may have on tumour vascular architecture and function, which would be undetectable with conventional susceptibility weighted MRI.**

Methods: *Data Acquisition:* Male nude rats were injected subcutaneously on the right flank with 1×10^5 C6 glioma cells. Size matched tumours underwent a baseline imaging session, followed by dosing with either 3mg/kg cediranib or vehicle at 0hrs, 24hrs and 48hrs. Two hours after the final dosing, the rats underwent a second imaging session. All images were acquired on a 4.7T horizontal bore Bruker system using a 72mm birdcage coil. T_2w morphological (RARE) images were acquired for tumour delineation, followed by two baseline multi gradient echo MGE acquisitions (3 contiguous 1mm slices, TR=200msec, TE=6–28ms, 4ms echo spacing, 8 averages) acquired during air breathing. The gas supply was then switched to carbogen (95% O_2, 5% CO_2) delivered via a nosepiece. Following a 10 minute transition time, a further identical MGE image set was acquired. The gas supply was reverted to air and, after a 10 minute transition time to clear residual carbogen, a second baseline MGE image set was acquired. A final MGE image set was acquired one minute after i.v injection of 200μmol/kg USPIO (ferumoxtran-10, Sinerem, Guerbet). *Data Analysis:* MGE data were fitted using a Bayesian approach which took into account the Rician data distribution [4]. This method enabled calculation of the probability that a given estimate of ΔR_2^* in each pixel was significantly greater than zero. This allows the exclusion of voxels where there was non-significant change in R_2^*. RGB maps were generated with a red channel designated to pixels with a positive $\Delta R_2^*_{carbo}$, the blue channel to pixels with negative $\Delta R_2^*_{carbo}$ and the green channel to positive $\Delta R_2^*_{USPIO}$. Regions with both negative $\Delta R_2^*_{carbo}$ and positive $\Delta R_2^*_{USPIO}$ therefore appeared cyan (blue + green) and regions with both positive $\Delta R_2^*_{carbo}$ and positive $\Delta R_2^*_{USPIO}$ appeared yellow (red + green). After the final MRI session, tumour perfusion was evaluated by Hoechst 33342 fluorescence microscopy.

Results: Mean values of baseline R_2^*, $\Delta R_2^*_{carbogen}$, fractional blood volume (fBV, %), and Hoechst 33342 perfused area (HPA, %) are shown in Figure 1. Mean baseline R_2^* increased significantly after treatment with cediranib, however there was no significant difference in the vehicle group. $\Delta R_2^*_{carbogen}$ was negative only in the tumours from cediranib treated animals. The positive $\Delta R_2^*_{carbogen}$ measured in both baseline and the vehicle treated cohorts may be explained by a vascular steal effect, whereas negative $\Delta R_2^*_{carbogen}$ in the cediranib treated cohort is evidence that inhibition of VEGF signalling has resulted in a more functional vasculature, perhaps caused by vascular normalisation. The tumours from the cediranib treated animals exhibited a significant decrease in mean fBV, compared to a non significant change in the vehicle treated group, which was corroborated by reduced Hoechst 33342 uptake. Baseline CUSPIO RGB maps of C6 tumours exhibited large regions of cyan voxels, which represent tumour tissue that was hypoxic at baseline, became more oxygenated during carbogen breathing, and remained perfused after injection of USPIO particles (Figure 2). This suggests the C6 tumours are characterised by well perfused tumour tissue possessing functional vasculature. The high spatial frequency of green voxels in the baseline RGB maps also suggests well perfused tissue. RGB maps from tumours from cediranib treated animals showed an increased number of black voxels, as well as a decrease in the density of green voxels in comparison to vehicle treated animals, consistent with a reduction in vascular density and blood volume. Compared to vehicle, the tumours from cediranib treated animals showed no significant difference in the percentage of green or cyan voxels, and a significantly higher percentage of red and blue voxels, which represent vascular shutdown. This is consistent with cediranib targeting immature blood vessels, and leaving mature blood vessels that were more able to innervate [5]. **Conclusions:** Treatment of C6 rat xenografts with cediranib caused a significant decrease in fractional blood volume, which was associated with a decrease in HPA. Novel CUSPIO imaging data suggested that treatment with cediranib targeted immature blood vessels, resulting in a more normalised mature vasculature with the ability to innervate in response to carbogen breathing. This interpretation of susceptibility weighted MRI data would not be possible without using the CUSPIO method to evaluate spatial distribution of ΔR_2^* responses.

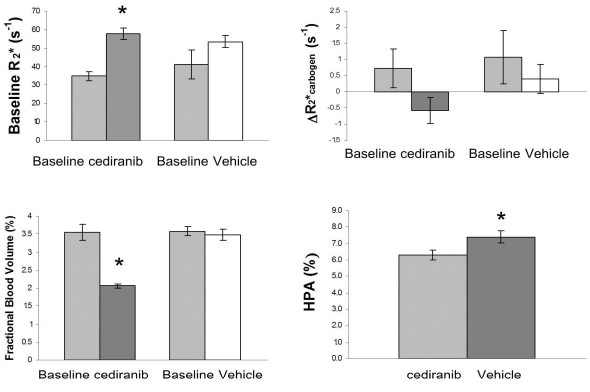

Figure 1 – Mean values of baseline R_2^*, ΔR_2^*carbogen, fractional blood volume, and Hoechst 33342 perfused area for C6 rat xenografts at baseline and post treatment with either cediranib or vehicle (* p <0.05)

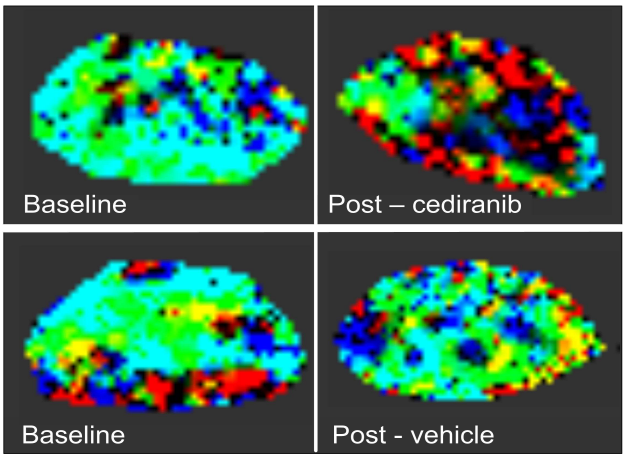

Figure 2 – Representative CUSPIO RGB maps showing the spatial co-localisation of $\Delta R_2^*_{carbogen}$ and $\Delta R_2^*_{USPIO}$ in C6 rat xenografts at baseline, and 48hrs post treatment with 3mg/kg cediranib (top) or vehicle (bottom).

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