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, ΔR* carbogen and ΔR* 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* 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 1×10^3 mg/kg cediranib or vehicle at 0hrs, 24hrs and 48hrs. Two cohorts were treated, which was corroborated by any subtle effects this therapeutic may have on tumour vasculature. The high spatial frequency of growth cediranib, however there was no significant difference in the percentage of cyan voxels, which regions in the vehicle treated group, which was reverted to air and, after a 10 minute transition time, a further identical MGE image set was acquired. The gas supply was then switched to carbogen (95% O2, 5% CO2) 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* in each pixel was significantly greater than zero. This allows the exclusion of voxels where there was non-significant change in R*. RGB maps were generated with a red channel designated to pixels with a positive ΔR*carbogen, the blue channel to pixels with negative ΔR*carbogen and the green channel to positive ΔR*USPIO. Regions with both negative ΔR*carbogen and positive ΔR*USPIO therefore appeared cyan (blue + green) and regions with both positive ΔR*carbogen and positive ΔR*USPIO appeared yellow (red + green). After the final MRI session, tumour perfusion was evaluated by Hoechst 33342 fluorescence microscopy.

Results: Mean values of baseline R2*, ΔR* carbogen, fractional blood volume (IBV, %), and Hoechst 33342 perfused area (HPA, %) are shown in Figure 1. Mean baseline R2* increased significantly after treatment with cediranib, however there was no significant difference in the vehicle group. ΔR* carbogen was negative only in the tumours from cediranib treated animals. The positive ΔR* USPIO measured in both baseline and the vehicle treated cohorts may be explained by a vascular steal effect, whereas negative ΔR* 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 IBV, 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* responses.

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