AZD2171 and ZD6474, VEGF signalling inhibitors, significantly reduce tumour vascular permeability in a human SW620 xenograft as detected by macro-molecular dynamic contrast enhanced MRI

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Introduction: Tumours rely on a vascular network to obtain oxygen and nutrients. The secretion of VEGF by tumours both stimulates neovascularisation and increases the permeability of existing vasculature to plasma macromolecules and nutrients[1]. Consequently, inhibition of VEGF signalling represents a good therapeutic strategy. ZD6474 (a heteroaromatic substituted anilinoquinazoline) is a potent p.o active (IC_{50} 40nm), low molecular weight inhibitor of KDR (VEGFR-2) tyrosine kinase activity with additional activity against EGFR tyrosine kinase[2]. AZD2171 is a highly potent (IC_{50} <1nM) inhibitor of KDR tyrosine kinase. Both compounds have broad spectrum preclinical activity in tumour xenograft models. DCEMRI provides the opportunity to non-invasively assess treatments targeting the vasculature by resolving haemodynamic parameters. Thus, by administering contrast media vascular parameters associated with tumour blood flow per unit of volume of tissue F/V_t (ml. sec^{-1}. ml^{-1}), fractional plasma volume νp (%) and permeability surface area product PSρ (sec^{-1}) can be assessed. However, microvascular characteristics estimated from macro-molecular DCEMRI have reportedly been significantly superior to those derived form small agents[3]. P792 ('Vistarem',Guerbet, Paris)[4] is a gadolinium chelated macromolecular rapid clearance blood pool agent (MMRCBPA) of 6.47 kDa, currently in Phase III trials, which is used here to simultaneously resolve multiple haemodynamic constituents. In the present investigation the vascular input function (VIF) and tumour contrast uptake data were acquired simultaneously using a modified keyhole technique before and 24 hours after treatment.

Methods: Athymic rats were injected subcutaneously at a site dorsal to the heart with the human colorectal carcinoma SW620. After ten days tumour growth the animals were randomised into three treatment groups 3 days prior to their first imaging session (n=6 ZD6474 50mg kg^{-1}, n=8 AZD2171 3 mg kg^{-1}, n=3 vehicle polysorbate). For imaging, rats were anaesthetised with 1.5% isoflurane and placed in a purpose built Perspex lidded bed. A tail vein was catheterised for i.v. administration of P792 (0.0045mmol Gd/kg) and the lid placed over the animal accompanied by a carriage of P792 calibration agar gels in-situ. Imaging was performed at 4.7T (Varian, Palo Alto, CA) using a 6 cm diameter birdcage resonator. A single-slice modified keyhole sequence (TR /TE /α° 0.013 /2.3 /15, FoV 6cm, SlThk 5mm, sagittal saturation band ~ 20mm across left ventricle) with a temporal resolution of 0.5s/image was acquired through the tumour and left ventricle before and 24 hours after treatment. The SI obtained in the regions of interest (ROI) in the left ventricle and tumour was measured for each time point and was converted to concentration using the in-situ P792 calibration agar gels. F/V_T, ν_p, and PSρ were resolved by inserting the P792 VIF and tumour changes into a bi-compartmental mono directional model. The tumour was excised for Haematoxylin & Eosin staining to assess cellular viability and necrosis.

Results: Tumour P792 uptake was significantly different between vehicle and treatment groups, pre and post compound administration. Thus, after treatment, up to 70% less P792 remained in the tumour ($p<0.00005$) at the end of the DCEMRI acquisition (5 min). (Fig.1). Simultaneously measuring the VIF and tumour contrast agent uptake for each animal, before and after treatment, permitted precise compartmental modelling. No significant differences were observed in F/V_T, ν_p, and PSρ between pre and post vehicle treatment. AZD2171 significantly reduced both PSρ (80%, $p<0.005$) and ν_p (68% $p<0.05$). ZD6474 significantly reduced PSρ (71%, $p<0.005$) (Fig. 2). HE histopathology showed no difference between vehicle and compound treated groups in tumour volume or viable:necrosis percentage suggesting this to be insensitive to early treatment changes induced by anti-VEGF therapy.

Figure 1

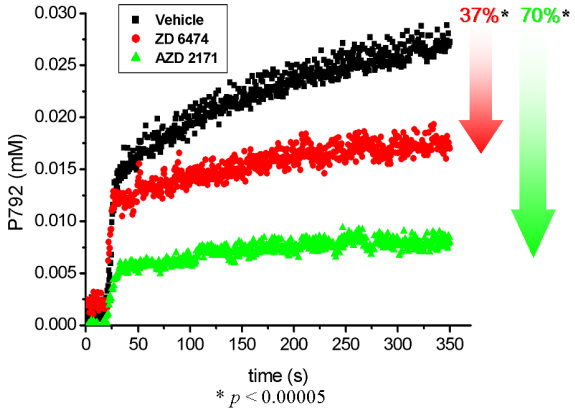
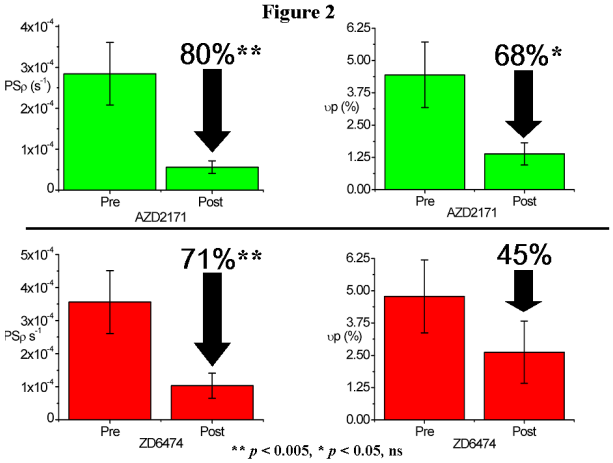


Figure 2



Discussion: Combining a fast imaging sequence and the administration of a MMRCBPA allowed the simultaneous resolution of haemodynamic constituents prior to therapy, and following treatment with AZD2171 or ZD6474. Treatment with either compound greatly reduced P792 uptake in the tumour. Furthermore, concurrent measurement of the VIF allowed a significantly reduced PSρ to be accurately determined. After chronic treatment with anti-VEGF therapy, compensatory mechanisms have been suggested to account for an apparent increase in νp[5]. However, acute AZD2171 administration significantly reduced νp. This investigation successfully demonstrated for the first time the anti-VEGF effects of ZD6474 (in Phase II) and AZD2171 (in Phase I) in a xenograft tumour model using a RCBPA currently in Phase III trials. These two antiangiogenic agents differ in their receptor selectivity profiles and potency of VEGFR inhibition. In the future P792 may enable multiple haemodynamic parameters to be resolved with high sensitivity following treatment with agents that compromise tumour vasculature.

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[5] Drevs J *et al*., PTK787/ZK222584, a specific vascular endothelial growth factor receptor tyrosine kinase inhibitor, affects the anatomy of the tumour vascular bed and the functional vascular properties as detected by dynamic enhanced magnetic resonance imaging. *Cancer Research* 62: 4015-4022 2002