ACUTE TUMOUR RESPONSE TO ZD6126 ASSESSED BY INTRINSIC-SUSCEPTIBILITY MAGNETIC RESONANCE IMAGING

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Introduction ZD6126 is a novel vascular-targeting agent that causes the selective destruction of tumour blood vessels, cessation of tumour blood flow and death of tumour cells due to nutrient starvation, resulting in massive tumour necrosis[1]. We have previously shown the antivascular effect of 50mg/kg ZD6126 on rat GH3 prolactinomas to be profound 24 hours after administration[2]. We are currently further investigating the use of intrinsic-susceptibility magnetic resonance imaging (MRI) to provide an early, acute marker of tumour response to ZD6126. Paramagnetic deoxyhaemoglobin creates magnetic susceptibility perturbations around blood vessels, increasing the effective transverse relaxation rate R_2* which, in the absence of other changes, depends on tissue deoxyhaemoglobin levels and hence may provide an acute index of changes in tissue oxygenation. We hypothesised that following treatment with ZD6126, haemoglobin within erythrocytes would deoxygenate, resulting in an increase in tumour R_2*.

Methods Female Wistar rats bearing GH3 prolactinomas were used. Multi-gradient echo (MGRE) images were acquired at 4.7T from up to five contiguous 1mm thick transverse slices through each tumour, using a train of 8 echoes with TR=80ms, initial TE=5ms and TESPACE=5ms. Tumour R_2* maps for each slice were generated using all 8 gradient-echo image sets by fitting a single exponential to the signal intensity pixel-by-pixel. The average R_2* for each slice from each individual tumour was determined from a region-of-interest encompassing the whole tumour but excluding the surrounding skin/muscle. Tumour R_2* was measured prior to and either a) for up to 35 minutes immediately following administration or b) 24 hours post-treatment with either 50mg/kg ZD6126 or saline i.v.

Immediately after MRI, the rats were injected with 15mg/kg of the perfusion marker Hoechst 33342 via a tail vein and, 1 minute later, the tumours rapidly excised and frozen. Sections from each tumour were subsequently cut and fluorescence signals of whole tumour sections recorded using a motorized scanning stage on a fluorescence microscope. The area of the tumour section with Hoechst 33342 fluorescence was determined and expressed as a percentage of the area of the whole tumour section (mean Hoechst perfused area, mHPA). These data were used to validate any changes in intrinsic R_2* contrast induced by ZD6126.

Results The figure shows representative R_2* maps acquired from one GH3 prolactinoma prior to and 35 minutes after administration of ZD6126, and another GH3 prolactinoma prior to and 24 hours post-treatment with ZD6126. The data are summarised in Table 1 (mean ± 1 s.e.m., *p<0.02, **p<0.01, Student's t-test).

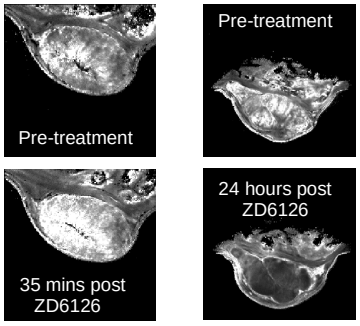

Table 1	Pre-treatment	Saline (n=6)	Pre-treatment	35min post ZD6126 (n=7)
R_2* (s^{-1})	110 ± 6	108.7 ± 6	106.4 ± 6	123.2 ± 10*
mHPA (%)		13.6 ± 2		9.1 ± 1
	Pre-treatment	Saline (n=5)	Pre-treatment	24hrs post ZD6126 (n=6)
R_2* (s^{-1})	109.2 ± 14	123.6 ± 17	114.6 ± 10	63 ± 8**
mHPA (%)		17.4 ± 3		5.3 ± 1**

Discussion

Tumour R_2* significantly increased over the first 35 minutes of challenge with ZD6126, supporting our original hypothesis, and was also consistent with a decrease in tumour perfusion as indicated by uptake of Hoechst 33342. Furthermore, localised increases in R_2* could be observed as early as 7 minutes after administration of ZD6126. Histogram analysis revealed that pixels with a relatively fast basal R_2* showed the greatest increase in R_2* in response to ZD6126. Interestingly, tumour R_2* significantly decreased 24 hours post-treatment with ZD6126, which correlated with a significant decrease in tumour perfusion measured by Hoechst 33342 uptake. Both effects were primarily associated with the tumour core. The decrease in tumour R_2* observed at this time point could be due to i) the agglomeration of deoxygenated erythrocytes into localised tumour regions decreasing the magnetic field inhomogeneity elsewhere, ii) vessel collapse prior to necrosis decreasing the blood volume, and iii) development of oedema. A change in tumour R_2* may provide a simple, convenient and early imaging marker for detecting acute changes induced by vascular targeting agents.

1) Blakey DC *et al*. Clin. Cancer Res. 2002;8:1974-83. 2) Robinson SP *et al*. Br. J. Cancer 2003;88:1592-1597.
Supported by The Royal Society, AstraZeneca, BBSRC and Cancer Research UK, [CRC] grant SP 1971/0701.