Measurement of the increase in vessel size induced by a vascular disrupting agent in orthotopic prostate tumours using vessel size imaging

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Introduction
Vascular disrupting agents (VDAs) have been developed to exploit differences between normal and tumour vasculature, with the aim of selectively disrupting and destroying the tumour endothelium, whilst leaving normal blood vessels relatively unaffected [1]. ZD6126 (N-acetylcysteinyl-O-phosphate) is a VDA shown to have significant anti-tumour activity against a broad range of human xenografts in rodent model systems and human tumour vasculature [2,3]. This class of agents typically does not induce tumour regression, so imaging biomarkers of functional vasculature are required to evaluate tumour response. The aim of this study was to assess tumour vessel size index (VSI, a weighted average measure of vessel diameter), fractional blood volume (**μ**VSI) (determined in vivo by susceptibility contrast MRI), and the apparent diffusion coefficient (ADC), to provide non-invasive imaging biomarkers of response to ZD6126 in orthotopic PC3 prostate tumours. Use of the fluorescent endothelial stain Hoechst 33342 also enabled the qualification of VSI and blood volume measurements. These parameters were estimated using a novel, robust Bayesian maximum a posteriori approach [4].

Methods and Materials

**Study design:** Orthotopic PC3 prostate tumours were propagated in 6 nude NCr mice. At 20 days following inoculation, VSI, **μ**VSI and ADC of each of the tumours was estimated according to the acquisition and analysis protocol described below. Immediately following this scan, ZD6126 was administered (200mg/kg i.p.). At 24 hours after this dosing, each mouse underwent a second MR scan, after which Hoechst 33342 (a fluorescent endothelial stain) was administered as a terminal experiment. A second cohort of 6 nude mice was solely administered Hoechst 33342 at 20 days in order to provide a pre-treatment control.

**Acquisition and analysis:** All measurements were undertaken on a 7T Bruker horizontal bore scanner. The change in R\* and R2 induced by USPIO (ferumoxtran-10, Sirenem, Guerbet) and the ADC were used to estimate VSI, according to the approach described by Troprès et al [5]. R\* and R2 were estimated from data acquired using a multi-gradient echo sequence (TR=200ms, 8 echoes ranging from 6 to 28ms) and multi-spin echo sequence (TR=3000ms, 12 echoes ranging from 12 to 144 ms), respectively. ADC values were estimated from a diffusion-weighted spin-echo sequence (6 b-values from 6 to 500 s/mm², TR=1000ms). Parameter estimation was performed using a novel Bayesian maximum a posteriori algorithm which took into account the Rician distribution of noise in magnitude MR data in order to provide unbiased parameter estimates [4]. It also provided the ability to identify non-enhancing (non-perfused) pixels or parameter estimates with a large associated uncertainty, thereby facilitating their removal from the VSI calculation. Furthermore, the tumour enhancing fraction (TEF, the fraction of tumour pixels that significantly enhanced due to USPIO, with p<0.1) was determined.

**Results and Discussion**
Mean tumour volume was 581±116 mm³ pre-ZD6126 and 644±141 mm³ at 24 hours after treatment. The change in tumour volume was not significant (p<0.1).

**Figure 1:** Percentage changes in each MR biomarker at 24 hours following administration of ZD6126 (**p**<0.05).

<table>
<thead>
<tr>
<th>VSI</th>
<th>Blood volume</th>
<th>TEF</th>
<th>ADC</th>
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<tbody>
<tr>
<td>Pre: (5.6±1.2) %</td>
<td>Post: (5.0±0.3)</td>
<td>Pre: (14.5±1.9) %</td>
<td>Post: (5.9±0.9) %</td>
</tr>
<tr>
<td>Pre: (10.9±0.3) %</td>
<td>Post: (4.1±0.1) %</td>
<td>Pre: (650±36)×10⁻⁶ mm²/s</td>
<td>Post: (574±35)×10⁻⁶ mm²/s</td>
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Measurements of vessel diameter were performed on Hoechst-stained whole-tumour sections. These were converted into VSI values (VSicalc) using an approach described by Troprès et al. [6]. TEF and **μ**VSI estimates were compared with measurements of Hoechst perfused area (HPA).

**Conclusions**
Non-invasive VSI MRI using a novel and robust parameter estimation procedure therefore revealed:

- i) the presence of large vessels in orthotopic PC3 prostate tumours, relative to those typically found in ectopic tumours;
- ii) a significant increase in vessel calibre and overall reduction in blood volume at 24 hours after administration of the vascular disrupting agent ZD6126. These estimates agreed well with equivalent histological measures.

**Acknowledgements:** This work was supported by Cancer Research UK (C1060/A808/G7643 and C16412/A6269), NHS funding to the NIHR Biomedical Research Centre and The Royal Society.

**References:**

**Figure 1:** Percentage changes in each MR biomarker at 24 hours following administration of ZD6126 (**p**<0.05).

**Figure 2:** False-colour images of Hoechst stained tumour sections in a control (left) and treated tumour (right), with vessel diameter measurements overlaid. Dark regions correspond to fluorescing endothelial cells lining perfused blood vessels.

**Figure 3:** Top: Example blood volume (**μ**VSI) maps pre- and post-ZD6126 (left and right, respectively). Bottom: Representative whole-tumour Hoechst-stained sections. Both sets of images illustrate the acute reduction in blood volume induced by ZD6126.