The effects of Platelet-Derived Growth Factor on Vascular Permeability studied by MRI

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Introduction

Pericyte recruitment and vascular maturation is a crucial step in angiogenesis. The degree of mature versus immature vasculature is also a major determinant of tumour response to treatment. Mature, more patent vasculature is predicted to have more complete endothelial lining, basal membrane and smooth muscle and hence reduced permeability. The latter has not been yet unequivocally demonstrated *in vivo*. Tumours derived from B16 melanoma cells engineered to express platelet-derived growth factor PDGF-BB (B16/PDGF) have previously been shown to exhibit a significantly more mature vasculature, with higher pericyte coverage and increased degree of functional, more punctate blood vessels compared to wild type B16 melanomas (B16/ctr) (1,2). As a result, B16/PDGF and B16/ctr tumours provide an excellent comparative *in vivo* model system to investigate how PDGF signalling and the degree of vascular maturity influences vascular permeability. In this study we interrogated the difference in vascular permeability between B16/PDGF and B16/ctr tumours non-invasively *in vivo* using dynamic contrast-enhanced (DCE)-MRI.

Materials and Methods

Subcutaneous tumours were derived from B16/ctr or B16/PDGF cells and propagated in C57Bl6 mice. Size-matched tumours were investigated by DCE MRI on a 7T Bruker horizontal bore MicroImaging system, using a 3cm birdcage coil. Field homogeneity was optimised by shimming on the water signal for each tumour using the FASTmap algorithm. Native T_1 and T_2 were quantified from a single 1mm axial slice using an inversion recovery (IR) True-FISP sequence (baseline scan, FOV=3cm, matrix=128x96, TI=25-1450ms, 50 inversion times, TE=1.2ms, TR=2.5ms, scan TR=10s, 8 segments, 8 averages). DCE-MRI data were then acquired using the same geometry parameters and IR True-FISP sequence with 60 dynamic scans (8 inversion times, TI=130-1037ms, TR=4ms, TE=2ms, scan TR=10s, temporal resolution=20s, 2 averages), prior to and following i.v. injection of 0.1mmol/kg Gd-DTPA (Magnevist, Schering). IR true-FISP data were fitted using a robust Bayesian approach, which utilised the dual relaxation rate sensitivity (T_1 and T_2) of the IR-true-FISP sequence and incorporated the Tofts and Kermode pharmacokinetic model (3). Data were fitted pixelwise using in-house software, yielding parametric maps of native T_1 and T_2, the transfer constant K^{trans}, and the integrated area under the gadolinium enhancement curve, $IAUGC_{60}$. Native T_1 and T_2 were also quantified in triplicate from cell pellets derived from B16/PDGF and B16/ctr using the same IR True-FISP sequence.

Results and Discussion

Parametric $IAUCG_{60}$ maps revealed that parenchymal enhancement was predominantly associated with the periphery of both B16/PDGF and B16/ctr tumours, and appeared to be reduced in the B16/PDGF tumours. B16/PDGF tumours had a lower median K^{trans} and $IAUCG_{60}$ compared to control (Figure 1). We have previously shown no significant differences in the overall perfusion between B16/PDGF and B16/ctr tumours (2). Although $IAUCG_{60}$ and K^{trans} are compound imaging biomarkers of both vascular perfusion/permeability, together these data suggest that the differences in DCE MRI reflect differences predominantly in vascular permeability as a consequence of PDGF signalling.

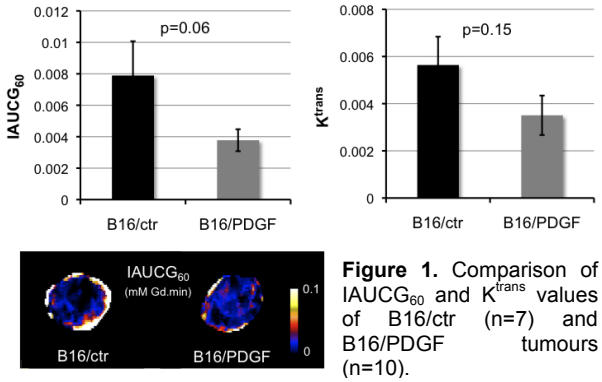

Figure 1. Comparison of $IAUCG_{60}$ and K^{trans} values of B16/ctr (n=7) and B16/PDGF tumours (n=10).

Interestingly, the B16/ctr tumours also revealed a significantly shorter T_1 value *in vivo* than B16/PDGF tumours (Figure 2). Short T_1 has been associated with high melanin content in melanoma (4). In the B16/PDGF model, elevation of PDGF-BB causes an increase in PDGFR expression, which in turn can stimulate the synthesis of melanin (5,6). B16/PDGF cells are thus predicted to have a lower T_1 than B16/ctr cells *in vitro,* as evidenced in Figure 2c. In contrast, B16/ctr tumours demonstrated a shorter T_1 *in vivo*, suggesting than the melanin content is not the main source of the T_1 contrast between B16/PDGF and B16/ctr tumours *in vivo*, although it might interfere with it. Increased tumour proliferation in melanoma has been associated with a concomitant increase in T_1 and T_2, a consequence of elevated interstitial and/or intravascular space (7). Whilst B16/PDGF tumours have a significantly faster growth rate (1,2), there was no significant difference in native T_2 between the B16/PDGF and B16/ctr tumours (62 ± 2 and 61 ± 2 ms respectively, p>0.9). Differences in necrosis could also be responsible for the difference in T_1. However, no differences in ADC, a proxy for necrosis, was previously reported between the two tumour types (2). The decrease in T_1 in B16/ctr tumours is thus most likely a consequence of increased macromolecule content within the extra-cellular space (ECS), consistent with increased extravasation of plasma proteins into the ECS through the more permeable vasculature of the B16/ctr tumours, as suggested by the DCE MRI data (8).

Conclusion

Collectively these data suggest that increased PDGF signalling and pericyte coverage results in tumours with lower vascular permeability *in vivo*.

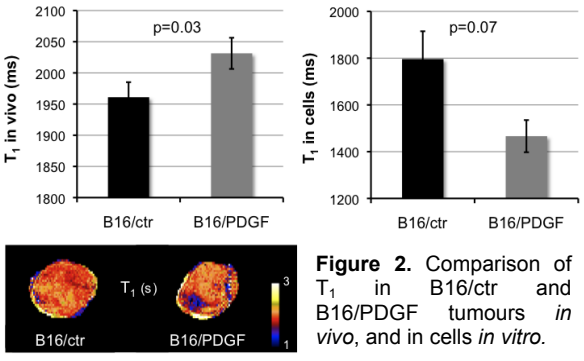

Figure 2. Comparison of T_1 in B16/ctr and B16/PDGF tumours *in vivo*, and in cells *in vitro.*

References. (1) Furuhashi *et al, Cancer Res* (2004), **(2)** Robinson *et al*, *Int. J. Cancer* (2008), **(3)** Walker-Samuel *et al, Proc ISMRM* (2009), **(4)** Enochs *et al*, *Radiology* (1997), **(5)** Graminski *et al*, *Nat. Biotechnol.* (1994), **(6)** Mahalincam *et al, J. Cell. Physiol.* (1996), **(7)** Olsen *et al*, *Magn. Reson. Imaging* (1999), **(8)** McSheehy *et al*, *Clin. Cancer Res.* (2010).

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