Analysis of vascular function by DCE-MRI in a human endothelial cell derived angiogenesis model in mice under anti- and pro-angiogenic treatment

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Introduction

It was recently demonstrated that human umbilical vein endothelial cells (HUVECs) embedded in a Matrigel-fibrin matrix develop a functional network of human neo-vessels connected to the mouse vascular system when implanted subcutaneously in mice (1). This assay provides the opportunity to study the function of newly formed human vasculature and the effect of anti-angiogenic therapies including antibodies specific for human targets in an animal model in vivo using dynamic contrast enhanced MRI (DCE-MRI), bioluminescence imaging, and immunohistochemistry.

Methods

Transduced HUVECs showing luciferase activity were suspended in Matrigel/fibrin (0.5 ml) and subcutaneously injected into the groin of immune deficient mice. The mice were assigned into five treatment groups (10 mice each of which 4 were analyzed with MRI) and treatment started on day 1 after implantation: 1. control (saline); 2. sunitinib (Sutent, Pfizer) 3. PTK787 (Novartis/Bayer Healthcare); 4. human antibody (AB) stimulating angiogenesis; 5. human AB inhibiting angiogenesis. After 3 weeks of treatment MRI measurements were performed on a 9.4 T animal scanner (Bruker BioSpec 94/20) to study the function of the developed neovasculature. T_1-weighted and T_2-weighted multislice RARE scans were used to localize the Matrigel plugs. Series of DCE-MR images were acquired pre- and post-injection (i.v.) of the contrast agent (CA) Gd-DTPA (Magnevist, Bayer-Schering; dose 0.1 mmol Gd/kg) with an inversion recovery (IR) TrueFISP sequence (2) (1 slice of 2 mm thickness, in-plane resolution 0.20x0.26 mm, TE/TR 1.45/2.91 ms, 10 TIs: 110…1918 ms, temporal resolution 6 s, 120 scans). Quantitative T_1 values and CA concentrations were calculated from the DCE-MRI data. The curves were analysed using two different 2-compartment pharmacokinetic models: I. Tofts model (3) yielding the transfer constant K^{trans}; II. Hoffmann model (4,5) yielding the rate constants k_{ep} and k_{el}. Additionally, the initial area under the curve iAUC in the Matrigel plug was calculated and normalized to iAUC in dorsal muscle (reference region).

In vivo bioluminescence imaging in mice was performed twice weekly to measure luciferase activity. Ex vivo bioluminescence imaging was performed after removal of the Matrigel plugs. After the MRI exam biotinylated Ulex Europaeus Agglutinin was applied i.v. before necropsy to allow the analysis of the perfusion status of the human vessels. The plugs were removed and ex vivo bioluminescence imaging and immunohistochemical stainings were performed.

Results

DCE-MRI analysis showed differences in the CA concentration time courses depending on the treatment (Fig. 1). Sunitinib or PTK significantly decreased normalized iAUC as compared to control while stimulating AB increased it (Fig. 2 left). K^{trans} was significantly lower under PTK and sunitinib treatment (trend for decrease with inhibiting AB). In some Matrigel plugs the curves could be fitted more accurately with the Hoffmann model. k_{ep} showed a trend for decrease in the PTK and sunitinib groups. k_{el} was significantly lower for inhibiting AB.

Bioluminescence imaging showed a significant decrease in HUVEC luciferase activity during sunitinib and inhibiting AB treatment and a significant increase during stimulating AB treatment (Fig. 2 right). Vessel counting on sections revealed similar results: less vessels for sunitinib and inhibiting AB, more vessels for stimulating AB; less perfused vessels for sunitinib and more perfused vessels for stimulating AB as compared to control. Bioluminescence and vessel counts for PTK were not different to control.

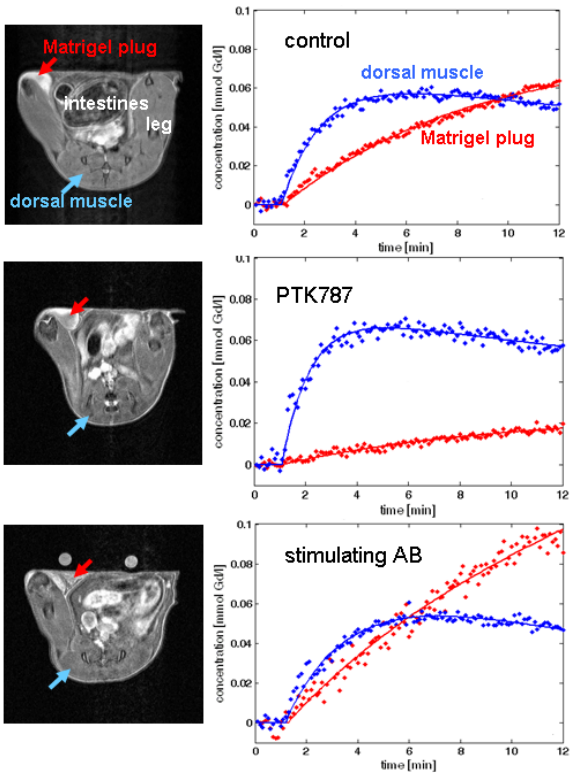

Fig 1.: left: T_1w RARE image post-CA showing the Matrigel plug (red) in the groin Right: CA concentration curve acquired with DCE-MRI in the Matrigel plug (red) and dorsal muscle (blue) in control (top), after treatment with anti-angiogenic PTK787 (middle) and pro-angiogenic antibody (bottom).

Discussion

The decreased perfusion after anti-angiogenic treatment with sunitinib can be explained by decreased number of vessels. Pro-angiogenic treatment had the opposite effect. Treatment with the less potent PTK did not decrease the number of vessel so the decrease in perfusion as detected by DCE-MRI was presumably caused by a decreased blood flow and/or a decreased permeability, i.e. changed vascular function. The inhibiting AB decreased the number of vessels but vascular function was not significantly different from control. DCE-MRI in our angiogenesis model can show differences in vascular function of human neovasculature and thus yields additional information to bioluminescence and immunohistochemistry which assess vascular morphology. This method can be applied for drug testing.

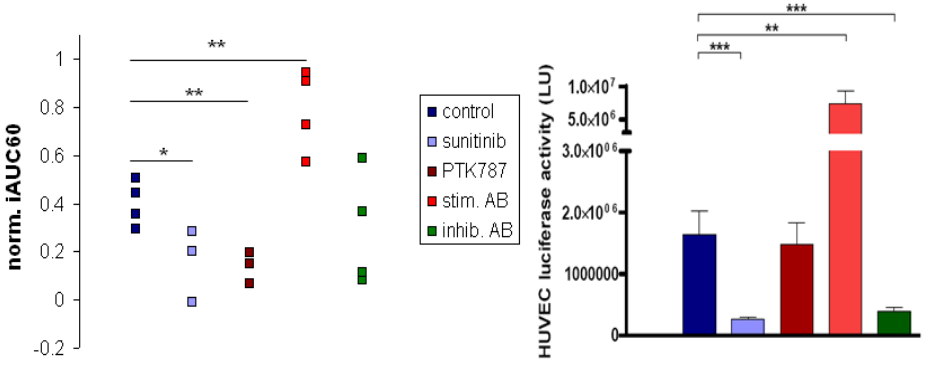

Fig. 2: Initial area under the CA concentration curve (left) acquired with DCE-MRI and luciferase activity (right) acquired with bioluminescence imaging in treated Matrigel plugs

References

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