Analysis of vascular function by DCE-MRI in a human endothelial cell derived angiogenesis model in mice

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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 three-dimensional network of human neo-vessels connected to the mouse vascular system when implanted subcutaneously in immune deficient mice (1). This assay provides the opportunity to study the function of newly formed human vasculature and the effect of anti-angiogenic therapies in an animal model *in vivo* using dynamic contrast enhanced MRI (DCE-MRI).

Methods

HUVEC spheroids were suspended in Matrigel/fibrin (0.5 ml) with growth factors and subcutaneously injected into the groin of SCID (Severe Combined Immunodeficiency Disorder) mice (n=11). The vascular network was allowed to form in the Matrigel plugs and to connect with the mouse circulation. MRI measurements were performed on a 9.4 T animal scanner (BioSpec 94/20, Bruker, Ettlingen, Germany) 5 and 13 weeks (n=3) or 3.5 weeks (n=4 treated with an anti-angiogenic substance, n=4 controls) after implementation of HUVECs. T_1-weighted and T_2-weighted multislice RARE scans (TE/TR= 1.5 s/7.5 ms or 2.5 s/36 ms, slice thickness 1 mm, in-plane resolution 0.12 mm) were acquired to localize the Matrigel plugs. A series of DCE-MR images was acquired pre- and post-injection (i.v.) of the contrast agent (CA) Gd-DTPA (Magnevist, Bayer-Schering, Berlin, Germany; dose 0.1 mmol Gd/kg) with an inversion recovery (IR) TrueFISP sequence (2) to measure the perfusion and the permeability of the blood vessels in the Matrigel plugs (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). DCE-MRI data were analysed using two different 2-compartment pharmacokinetic models: I. Tofts model (3) yielding the transfer constant K^{trans} and the extracellular-extravascular leakage space v_e; II. modified Brix 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 the dorsal muscle (reference region). After the last MRI exam biotinylated Ulex Europaeus Agglutinin was applied i.v. to allow the analysis of the perfusion status of the human vessels. Finally, plugs were removed, and the neovasculature was investigated by immunohistochemical staining.

Results

The Matrigel plugs were easily detectable in T_1- and T_2-weighted scans. DCE-MRI analysis showed that the plugs were perfused. The modified Brix model fitted the DCE-MRI data in most Matrigel plugs better (smaller residual error) than the Tofts model. v_e was close to 1 in most plugs. K^{trans}, k_{ep}, and iAUC in treated Matrigel plugs were always lower than in control plugs indicating a decrease in perfusion. The onset of the enhancement in treated plugs was delayed compared to dorsal muscle. Immunohistochemistry showed a distinct human vascular network, most of the vessels were perfused (61 % +/-12.5 at time point 13 weeks).

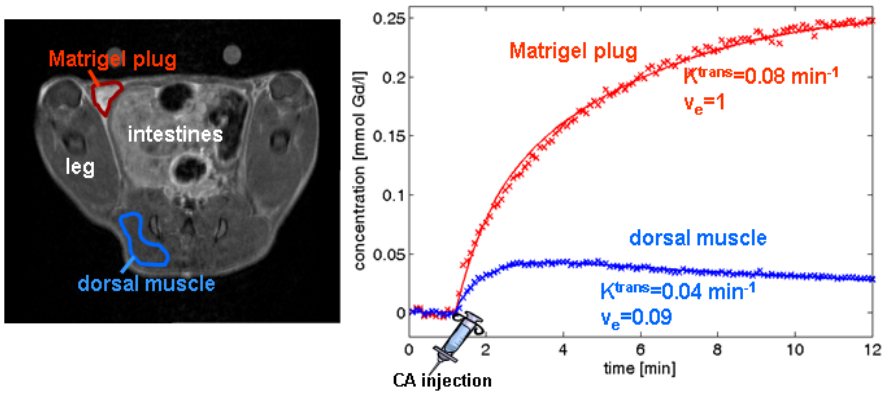

Fig.: left: T_1w RARE image post-CA showing the Matrigel plug (red) and the reference region dorsal muscle (blue). Right: CA concentration curve acquired with DCE-MRI and fitted curve (Tofts model, solid line) in the Matrigel plug (red) and dorsal muscle (blue) at time point 5 weeks.

Discussion

DCE-MRI allows the analysis of the perfusion state in this angiogenesis model *in vivo*. CA uptake in the Matrigel plug was higher compared to muscle due to the large leakage space (Matrigel consists almost entirely of extracellular-extravascular space). K^{trans}, k_{ep}, and iAUC depend on blood flow and vessel permeability. A decrease in these calculated vascular parameters in the treated group may be explained by a decrease in vessel density and vessel size (leading to decreased blood flow) as shown by histology. These first results in treated Matrigel plugs showed a decreased perfusion due to the anti-angiogenic effect.

References

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