Assessment of vascular remodelling during antiangiogenic tumor therapy using DCE-MRI and vessel size imaging

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Introduction:

Purpose of our study was to assess vascular remodelling in tumors during antiangiogenic therapy with dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) in combination with vessel size imaging and to evaluate the vessel size index (*VSI*) as a novel biomarker of therapy response. Thus, nude mice bearing squamous cell carcinoma xenografts were treated with an antiangiogenic therapy and investigated by DCE-MRI and vessel size imaging over time.

Methods:

In total 12 nude mice bearing subcutaneous squamous cell carcinoma xenografts (A431) were investigated with DCE-MRI and vessel size imaging before and after 4 days of treatment. Six animals received antiangiogenic treatment with a selective multitargeted receptor tyrosine kinase inhibitor SU11248 (Sutent® (Sunitinib), Pfizer Inc., NY, USA: 60 mg/kg body weight) for 4 days. To exclude interactions between the different contrast agents DCE-MRI was done at the first day while vessel size imaging was performed at the second day. All MRI experiments were performed on a clinical 1.5 T whole body MR system (Siemens Symphony, Erlangen, Germany) using a custom-made small animal solenoid Tx/Rx radiofrequency coil. Animal and tumor morphology was assessed using a T1w spin echo sequence and a T2w turbo spin echo sequence. DCE-MRI was performed using a T1w saturation recovery turboFLASH sequenz (TR=13ms, TE=5.3ms, TI=300ms, α=12°, averages 4, FOV: 60x22.5mm², voxel size: 0.5x0.5x2mm³). The contrast agent Gadomer (Schering, Berlin; 0.05mmol/kg diluted in 0.9% NaCl to a total volume of 100 µl) was injected via the tail vein. Post-processing was done based on an open two compartment model [1] using the software package DynaLab (Mevis, Bremen, Germany), calculating the parameters *Amplitude* and k_{ep}. *VSI* was calculated by the method published by Troprès et al. [2] using a self implemented VSI Taskcard using RadBuilder. Therefore, T2w images (SE, TR=6000ms, TE=100ms, averages 1, FOV: 60x50.4mm², voxel size: 0.5x0.5x1.5mm³) and T2* quantification (TR=380ms, TE=4.76-47.6ms (10 inphase echoes), Flip 45°, averages 3, FOV 62x50.4mm², voxel size: 0,5x0,5x1,5mm³) was performed before and 3 min after contrast agent administration (Very Small Superparamagnetic Iron Oxide Nanoparticles, VSOP, 200µmol Fe/kg, Ferropharm, Teltow). Large liquid tumor areas, which could faithfully be identified on T2w and T2*w images, were excluded from analysis of DCE-MRI and vessel size imaging data. The changes of the parameters *Amplitude*, k_{ep} and *VSI* were analyzed over time. Differences in vessel density and mean vessel size between treated and untreated tumors were also measured by immunofluorescence.

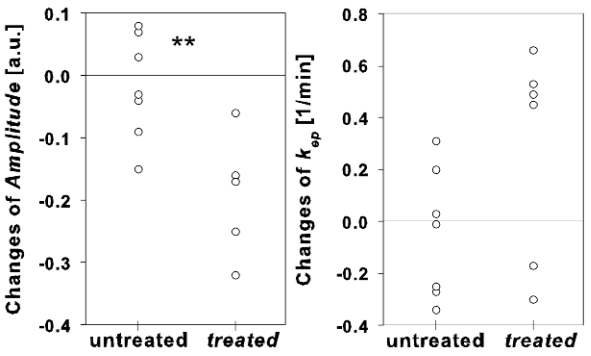

Fig.1 Results from DCE-MRI after post-processing using the two compartment model of Brix. *Amplitude* was significantly lower in treated than in untreated tumors (a). In contrast, no significant change were detected for k_{ep}, which tended to increase under therapy in A431 tumors (b) (** = $p < 0.01$).

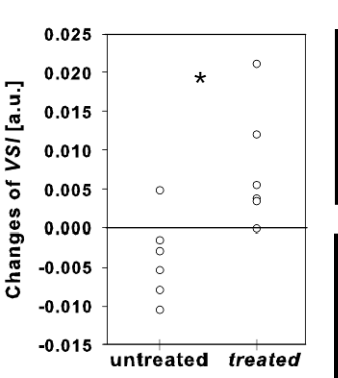

Fig.3 *VSI* values of untreated and treated A431 tumors. A significant ($p < 0.05$) increase of *VSI* was found in SU11248 treated tumors compared to untreated ones.

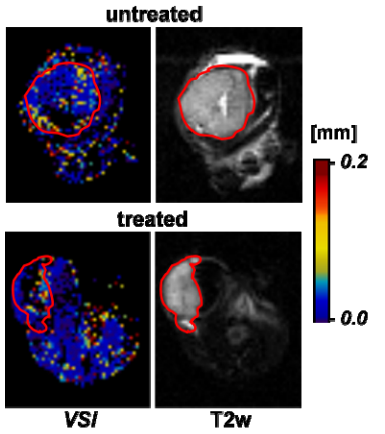

Fig.2 *VSI* and T2w images of untreated and treated tumors. Compared to control tumors a decrease in pixel density and a prevalence to higher *VSI* pixels is seen for the SU11248 treated A431 tumor in *VSI* images.

Results:

The parameter *Amplitude* decreased significantly ($p < 0.01$) over time in treated tumors (0.20 ± 0.09 a.u.) compared to untreated ones (0.02 ± 0.08 a.u.), whereas k_{ep} showed no significant change (treated: 0.28 ± 0.41 1/min; untreated: -0.05 ± 0.25 1/min) (Fig. 1). Also the change of the *VSI* was capable to mirror antiangiogenic therapy response showing significantly ($p < 0.05$) higher values in treated than in untreated tumors (Fig. 2). In detail the change of *VSI* over time of untreated and treated tumors was 7.6 ± 7.7 µm and -3.9 ± 5.4 µm, respectively (Fig. 3). Histological analysis proved the success of the antiangiogenic therapy indicating lower mean vessel area fractions and higher mean vessel size in treated compared to untreated A431 tumors (Fig. 4).

Discussion:

Histological analysis indicated a decrease of CD31 positive area fractions under treatment compared to the control group and thus a decrease in vessel density. Since it was shown previously in a comparative study between contrast-enhanced ultrasound and DCE-MRI that *Amplitude* highly correlates with the maximum accumulation of microbubbles (which remain strictly intravascular) [3], we postulate that the decrease in *Amplitude* is caused mainly by the reduction of the relative blood volume. The tendency of k_{ep} to increase is more difficult to explain and might reflect higher perfusion due to vessel normalisation and higher vessel permeability due to the destruction of immature vessels. The increase in *VSI* under treatment with the multitargeted tyrosine kinase inhibitor can be explained by a combination of vessel regression and vessel maturation. Histological evaluation indicated that vessel regression mainly occurred for small immature vessels, while larger mature vessels persisted, which leads to larger mean vessel diameters. In conclusion we can firmly state that DCE-MRI and vessel size imaging are in excellent agreement with histology and that the *VSI* might be a promising biomarker to assess early antiangiogenic therapy response.

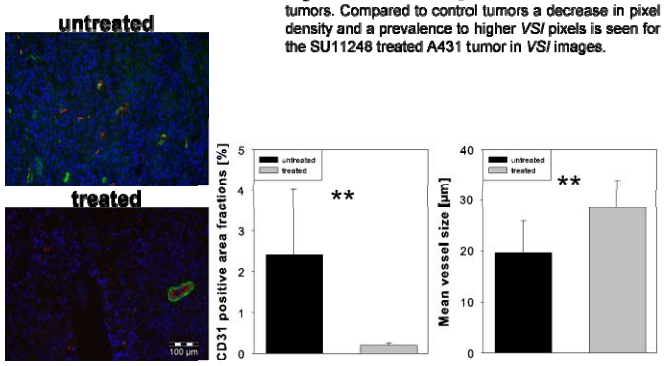

Fig.4 Immunofluorescence images of treated and untreated tumors. Endothelial cells were stained with a CD31 antibody (red). Smooth muscle cells, pericytes and myofibroblasts were labelled using a SMA antibody (green). Cell nuclei were counterstained with Hoechst DNA (blue). Quantitative analysis showed a highly significant reduction in CD31 positive area fractions and an highly significant increase of the mean vessel size (** = $p < 0.01$).

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References: [1] Brix G et al., J Comput Assist Tomogr 1991; 15: 621-628; [2] Troprès I et al., Magn Reson Med 2001; 45: 397-408; [3] Kiessling F et al. , Invest Radiol 2003:38:516-24.