Combined use of DCE-MRI and VSI to monitor an antiangiogenic therapy against VEGF in tumor xenografts

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Introduction:

Vessel Size Imaging (VSI) is a non invasive method to analyze the mean vessel size (vessel size index R) in a tissue of interest and might provide an interesting surrogate to characterize pathologies and to monitor antiangiogenic tumor therapy effects. Thus, nude mice bearing squamous cell carcinoma xenografts were treated with an antiangiogenic therapy and investigated by DCE MRI and VSI.

Methods:

In total 6 treated and 8 untreated nude mice bearing subcutaneous squamous cell carcinoma xenografts (HaCaT-ras-A5RT3) were investigated twice with T1w DCE MRI and 24 h later with VSI. Six of these mice were treated for 1 week with the VEGF-blocking antibody Avastin (1mg every third day). MRI measurements were performed on a clinical 1.5T whole-body MRI system (Siemens Magnetom Vision, Erlangen, Germany) using a custom-made small animal Tx/Rx radiofrequency coil. Animal and tumor morphology were assessed using T1w gradient echo sequences (FLASH) and T2w turbo spinecho sequences. DCE MRI was performed using a T1w Saturation Recovery Turbo FLASH sequence (TR 13ms, TE 5.3ms, TI 300ms, Flip 12, averages 4, FOV: 60x22.5 mm², voxel size: 0.5x0.5x2mm³). The contrast agent (CA) Gadomer (Bayer-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 a two compartment model [1] using the software package DynaLab (Mevis, Bremen, Germany). Amplitude (A), which is highly influenced by the tumor blood volume and the size of the interstitial space and the exchange rate constant k_{ep} (predominantly influenced by vessel permeability and perfusion) were determined. VSI was performed in a static way as described by Irène Troprès [2]. ΔR2 was estimated from the signal ratio of T2w SE images before and after contrast agent administration and ΔR2* by quantifying T2*. The vessel size index can then be calculated out of the ratio of ΔR2* and ΔR2. T2w images (SE, TR 6000ms, TE 100ms, averages 1, FOV 62x50mm², res. 0.5x0.5x1.5mm³) were acquired and T2* was measured (FLASH 2D, TR 320, TE 4,76-47,6ms (10 in phase echos before and 8 after CA administration), flip angle = 45°, averages 3, FOV 62x50mm², res. 0.5x0.5x1.5mm³) before and 3 min after CA administration (Very Small Superparamagnetic Iron Oxide Nanoparticles, VSOP, 12µl/mouse, 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 VSI scans. The mean vessels size index was calculated using a self implemented VSI Taskcard using RadBuilder. Differences in mean vessel size and vessel density in treated and untreated tumors were also confirmed by immunofluorescence (IF) microscopy measuring area fractions of vessels (CD31) and mean vessel diameter.

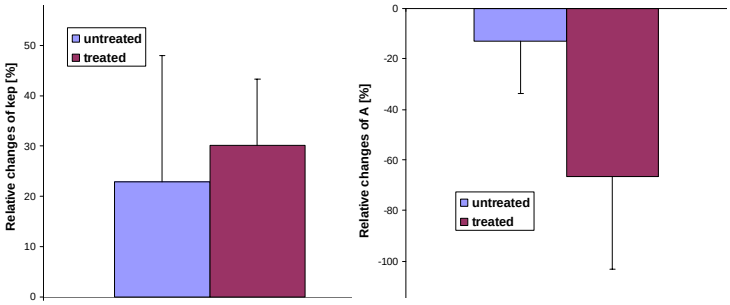

Fig.1 *Relative changes of Amplitude (A) and k_{ep} for untreated and treated tumors.*

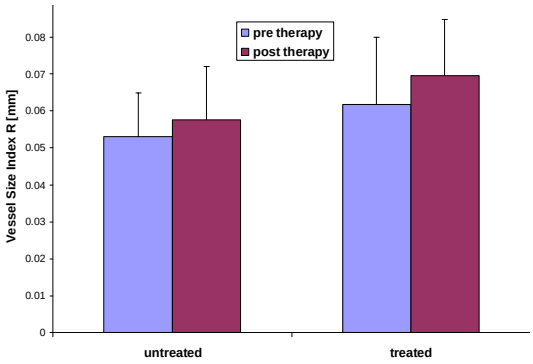

Fig.2 *Vessel size index R in treated and untreated tumors before and after therapy.*

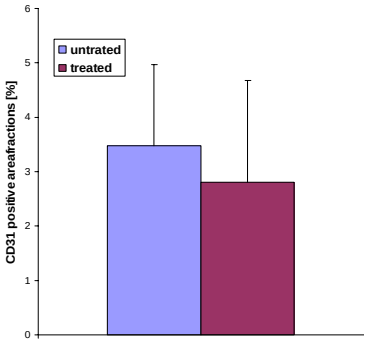

Fig.3 *CD31 positive area fractions for treated and untreated tumors.*

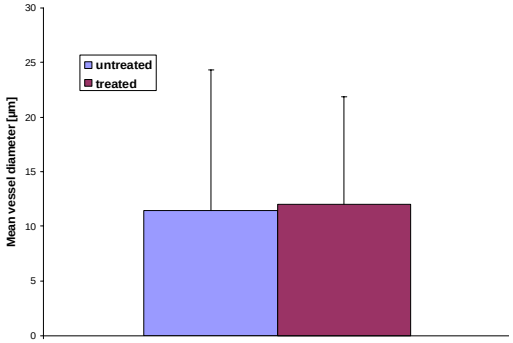

Fig.4 *Mean vessel diameter determined by immunofluorescence microscopy.*

Results:

Amplitudes (A) in treated tumors decreased under therapy (-66.6%) and a decent decrease was observed in untreated ones (-16.4%). Controversially, k_{ep} tended to increase more under therapy (+30%) as in untreated (+21.2%) tumors (Fig.1). Surprisingly, VSI did only tend to increase during treatment in this study and was not able to significantly indicate antiangiogenic therapy response. In detail, relative changes of R under therapy for untreated and treated tumors were +8% and +4.8%, respectively (Fig.2). Histological analysis confirmed the non invasive results and showed lower mean vessel area fractions in treated than in untreated tumors (Fig.3) but no significant changes in the mean vessel size (Fig.4).

Discussion:

In our previous work we had shown that VSI provides a sensitive indicator of early effects of an antiangiogenic therapy against VEGFR2 [3]. However in this study VSI was not able to detect significant early effects of a therapy against VEGF, while DCE MRI again clearly indicated a decreased tumor vascularization. Histological analysis confirmed the results from non invasive imaging. As compared to our previous study, the absence of significant changes in mean vessel diameter under treatment might be explainable by the addressed therapeutic targets. Blocking VEGFR2 leads to vessel regression and maturation, while blocking VEGF indirectly also reduces signaling via VEGFR1. Additional inhibition of VEGFR1 signaling can have antagonistic effects on vessel maturation [4]. The decrease of A in treated tumors may rely on the size reduction of the interstitial space and the decrease in vessel density. The increase of k_{ep} is most probably the consequence of vessel normalization and thus higher perfusion. In summary, again we could demonstrate the potential and robustness of VSI. However, when using VSI and DCE MRI as indicators of therapy response one has to consider that different therapies lead to diverging early effects on neovasculature.

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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] Zwick S et al., Proccedings ISMRM 2007; 564; [4] Yancopoulos GD et al., Nature 2000; 407: 242-248