Dynamic Contrast-Enhanced MRI and Vessel Size Imaging Sensitively Indicate Antiangiogenic Therapy Effects on Tumor Xenografts in Mice

S. Zwick[1,2], E. C. Woenne[2,3], R. Strecker[1], M. Bock[3], A. Hengerer[1], M. Taupitz[4], J. Schnorr[4,5], B. Misselwitz[6], W. Semmler[3], and F. Kiessling[2,3]

[1]MR Molecular Imaging, Siemens Medical Solutions, Erlangen, Germany, [2]Junior Group Molecular Imaging, German Cancer Research Center, Heidelberg, Germany, [3]Medical Physics in Radiology, German Cancer Research Center, Heidelberg, Germany, [4]Institute for Radiology, Charité, Berlin, Germany, [5]Ferropharm, Teltow, Germany, [6]Schering, Berlin, Germany

Introduction:

New antiangiogenic tumor therapy regimens demand for stable and early indicators of therapy response. In this context, dynamic contrast enhanced (DCE) MRI in combination with blood pool contrast agents has proven promising [1]. Vessel size imaging (VSI) describes a novel method to determine the mean vessel size for a tissue of interest [2,3]. However, to our best knowledge the use of VSI to assess antiangiogenic therapy effects on tumors has not been reported. Thus, nude mice bearing squamous cell carcinoma xenografts were treated with an antiangiogenic therapy and investigated by DCE MRI and VSI.

Methods:

In total 7 nude mice bearing subcutaneous squamous cell carcinoma xenografts (HaCaT-ras-A5RT3) were investigated with T1w DCE MRI and 24 h later with VSI. Four of these mice were treated for 1 week with the VEGFR2-blocking antibody DC101 (800µg/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 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 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 a two compartment model [4] 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 bases on the susceptibility differences between vessels and the surrounding tissue, which was assessed by measuring the relaxation rate changes ΔR2 and ΔR2* induced by injection of an intravascular superparamagnetic contrast agent. ΔR2 was estimated from the signal ratio of T2w images before and after contrast agent administration. The vessel size index R was then calculated according to the formula proposed by Troprès and coworkers [2]:

$$R = 0.425 \cdot \left(\frac{D}{\gamma \Delta \chi B_0} \right)^{1/2} \left(\frac{\Delta R_2^*}{\Delta R_2} \right)^{3/2}$$

assuming a diffusion coefficient *D* of 10^{-3} mm²/s [5] and a susceptibility difference Δχ of 0.571 ppm [2]. T2w images (TSE, TR 3930ms, TE 85ms, averages 5, FOV 62x31, res. 0.5x0.5x1.5mm³) were acquired and T2* was measured (FLASH 2D, TR 200, TE 6-24ms (7 Echos), α = 45°, 5 averages, FOV 62x50, res. 0.5x0.5x1.5mm³) before and 3 min after contrast agent administration (Very Small Superparamagnetic Iron Oxide Nanoparticles, VSOP, 9µmol/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. Differences in vessel density between treated and untreated tumors were also confirmed by immunofluorescence microscopy measuring area fractions of CD 31 positive vessels.

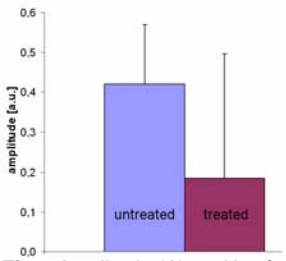

Fig.1 *Amplitude (A) and k_{ep} for untreated and treated tumors.*

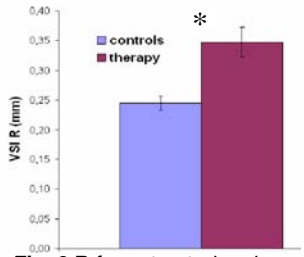

Fig. 2 *R for untreated and treated tumors.*

Results:

Amplitudes (A) of treated tumors (0.19 ±0.06) were lower than those of untreated ones (0.42±0.16), whereas k_{ep} was elevated in treated (0.70±0.31 min^{-1}) over untreated (0.36±0.15 min^{-1}) tumors (Fig. 1). Also VSI was capable to mirror antiangiogenic therapy response showing significantly ($p<0.002$) higher vessel size indices (R) in treated than in untreated tumors. In detail R of untreated and treated tumors was 0.25±0.01mm and 0.35±0.03mm, respectively (Fig. 2). Histological analysis proved the success of the antiangiogenic therapy and showed lower mean vessel area fractions in treated tumors, which was particularly true for small vessels in the tumor centers (Fig. 3).

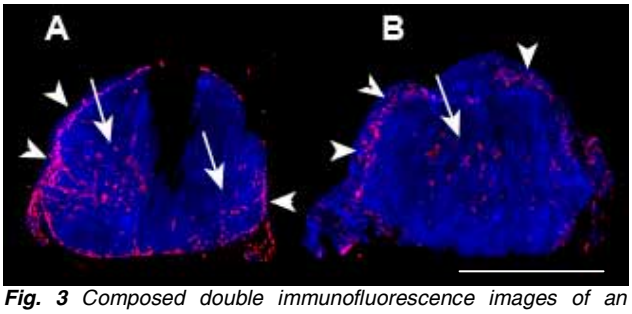

Fig. 3 *Composed double immunofluorescence images of an untreated (A) and 6-day treated tumor. Vessels were stained with an anti CD31 primary and a Cy2-labelled secondary antibody (red). Counterstaining was performed by Hoechst DNA stain (blue). Arrows indicate small vessels inside the tumors, which are clearly reduced in number after therapy. Arrowheads indicate large mature vessels at the tumor periphery. Bar: 5 mm.*

Discussion:

These preliminary data suggest that VSI provides a sensitive indicator of early antiangiogenic therapy effects on tumors that valuably supplements DCE MRI data. However, relatively high R may point to a systematical overestimation of vessel diameters, which may rely on the chosen *D*. *D* is known to vary between different tumors and at different stages of tumor development. Thus the additional determination of D prior to the vessel size measurement may be suited to overcome this problem. The decrease of A in treated tumors may rely on a shrinkage of the interstitial space and on a decrease of vessel density. The increase of k_{ep} is most probably a consequence of vessel normalization and thus of the improved blood flow conditions. The increase of R after therapy can be explained by the destruction of small immature tumor vessels and the persistence of large mature vessels. Thus, it is plausible that the mean tumor vessel diameter increases, although there is a decrease in the mean tumor blood volume.

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