Fluorine-19 MR molecular imaging of angiogenesis on Vx-2 tumors in rabbits using $\alpha_v\beta_3$-targeted nanoparticles

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Introduction

Anti-angiogenic therapy in combination with established chemotherapy or radiation therapy has entered clinical practice for lung, colon and breast cancer [1]. However, effectiveness and optimal timing of anti-angiogenic pre-treatment is substantially varying among patients. Given the high cost and severe side effects, there is a strong clinical need for enhanced patient stratification, which could be based on MRI of angiogenesis using targeted imaging agents. $\alpha_v\beta_3$-integrin targeted nanoparticle (NP) emulsions [2], labeled with R1-enhancing Gd-chelates, were previously shown to allow three-dimensional MR mapping of tumor angiogenesis for a variety of tumor models [3, 4] in small animals. These studies were based on ΔR1 mapping from two image sets taken before and after NP injection. The present study shows, that the perfluorocarbon (PFC) core of the same targeted NP can be used as a ^{19}F MR label to map angiogenesis around Vx-2 tumors (adenocarcinoma) in rabbits. With simultaneous ^{19}F and ^{1}H MR [5], diagnostic imaging is only required at a single time point post-injection injection and may offer the ability of direct absolute quantification.

Methods

Male New Zealand White rabbits (~2 kg, N=3) were implanted in one hind leg with 2-3 mm Vx-2 carcinoma tumors (National Cancer Institute, MD), which grew to 15 mm within 2 weeks. Imaging was performed 3h post-injection of 1.0 ml/kg of $\alpha_v\beta_3$-targeted 15-5 perfluoro-crownether (20 vol%) NP, incorporating Gd-DTPA-bis-oleate in the outer surfactant (which also reduces fluorine T1 to about 400ms). The rabbits were initially anesthetized with xylazine/ketamine *i.m.* and maintained with a 20 ml/h ketamine infusion. All animal care and protocols were in accordance with institutional guidelines. The study was performed on a 3T clinical whole-body scanner (Achieva, Philips Healthcare, The Netherlands) using a dual-tuned transmit/receive surface coil (7×12 cm) and a dual ^{19}F/^{1}H spectrometer system [5]. 3D gradient-echo sequences with concurrent dual-frequency RF/acquisition for ^{19}F and ^{1}H were used [5] at two different resolutions: (i) 2.9×2.9×4.0 mm^3, matrix 48^2, 15 slices, TR/TE=13/6.0 ms, flip angles α_{19F}/α_{1H} = 40°/10°, pixel bandwidth=100 Hz, 128 averages, scanning time 24 minutes. (ii) 2.19×2.19×4.0 mm^3, matrix 64^2, 140 averages, TR/TE=12/6.0 ms, 35 min. High-resolution T1-weighted GRE images were recorded for anatomical co-registration (resolution 0.55×0.55×4.0 mm^3, TR/TE=24/6.5 ms, α=35°). For sensitivity calibration of the surface coil, actual flip-angle imaging sequences (AFI) were used, and the signal decay with distance from the surface could be corrected using a GRE signal model. For signal comparison, a calibration sample with PFC NP (0.3 mol$_{19F}$/l) in agar was added for all experiments at a fixed position on the surface coil (not visible in the selected image planes).

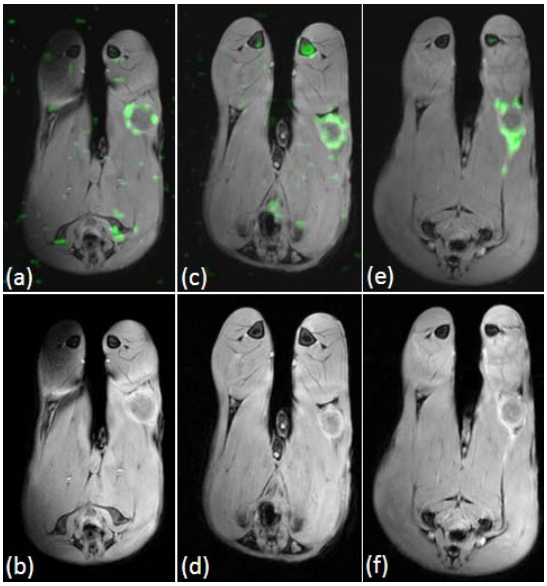

Figure 1: Selected slices from image sets of 3 different rabbits with implanted Vx-2 tumors taken on a clinical 3.0 T scanner (Achieva, Philips Healthcare) using a dual-tuned surface coil: (a,c,e) overlay ^{1}H and ^{19}F (green), (b,d,f) T1-weighted ^{1}H images. ^{19}F images were taken at a resolution of 2.9 mm within 24 minutes.

Results and Discussion

Figure 1 displays 3 selected image planes from different rabbits, with T1-weighted ^{1}H images in the lower row and overlays with the ^{19}F images (green) using parameter set (i) in the upper row. All tumors developed to a diameter of 15±1 mm, and the contrast-enhanced images clearly revealed the angiogenesis, which can be identified by the bright rim in the T1-weighted images as well as by annular hot-spots of fluorine signal intensity. Angiogenesis in this tumor model is a heterogeneous process [3], which is clearly appreciated in the non-uniform ^{19}F signal distribution at the periphery of the tumor. Other ^{19}F enhancing regions comprise tissue structures adjacent to the tumor, where angiogenesis can be expected as well (e.g. in supplying vessels). Some fluorine signal is also observed in the bone marrow and epiphyseal heads. In order to demonstrate 3D mapping capability of the applied simultaneous GRE sequences, Figure 2 shows a series of 5 adjacent slices around the tumor, which represent an overlay of ^{19}F images from the simultaneous sequence with parameter set (ii) and T1-weighted ^{1}H images. ^{19}F signal is visible all around the tumor, with varying intensity relating to regional angiogenesis. There is no ^{19}F signal from the tumor center, which is not involved in the neovascular growth front. The calibrated signal intensity on the tumor rim shows a maximum local concentration of NP corresponding to 70 mmol$_{19F}$/l, or 2.4 µmol$_{19F}$/voxel, which is well above the detection limit of about 300 nmol$_{19F}$/voxel (SNR=5;10 minutes) [6].

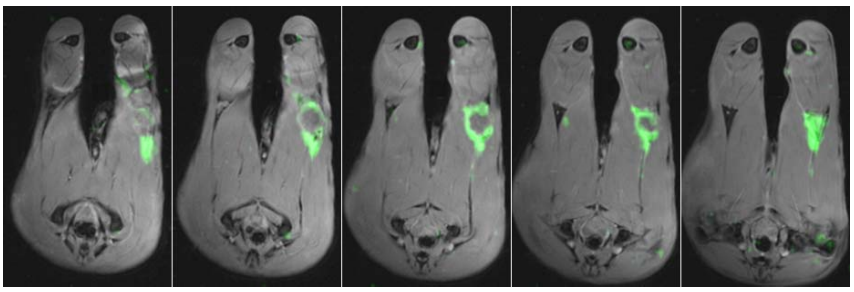

Figure 2: 3D imaging of a Vx-2 tumor at an in-plane resolution of 2.19 mm (slice thickness 4 mm): 5 adjacent image planes out of 15 are shown. Simultaneous ^{19}F and ^{1}H acquisition was completed within 35 minutes. Anatomical co-registration is demonstrated by overlay of ^{19}F (green) and high-resolution T1-weighted ^{1}H GRE images.

Conclusion

The present study demonstrates pre-clinical feasibility of ^{19}F/^{1}H MR using integrin targeted NP for non-invasive, 3D quantitative assessment of tumor angiogenesis, which may be relevant for patient segmentation and management. The ^{19}F signal offers a high specificity and the workflow and precision is enhanced, because only post-injection imaging is required. Moreover, this diagnostic application can be combined with anti-angiogenic therapy for a new "theranostic" approach [4]: Anti-cancer drugs linked to the targeted NP, could be strongly effective locally even in minute dosage and thus largely avoid side effects.

References

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