Design and setup of dynamic contrast enhanced experiments for longitudinal preclinical studies of tumor response to anti-angiogenic therapy

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Purpose: Dynamic contrast enhanced (DCE)-MRI is seen as a promising biomarker for assessing tumor angiogenesis and the effects of antiangiogenetic therapy because of its sensitivity to properties of the microvasculature [1]. DCE provides measures of tumor vascular function, i.e. tumor vessel permeability during the course of the treatment. Particularly DCE experiments in preclinical models in small animals can help to better verify effects of anti-angiogenic drugs on certain tumors, by permitting repeated measurements with a given tumor during the course of the treatment [2]. Although, DCE-MRI is common in clinical routine it is not yet standardized in small animals and the experimental DCE designs and setups are only barely documented in literature. An accurate and reproducible setup is, however, crucial to conduct reliable DCE experiments. Thus, the purpose of this work was to report in detail our DCE design and setup in order to monitor the treatment effects of anti-angiogenic drugs in tumor bearing mice.

Materials and Methods: Non-selective saturation recovery (SR) and in slice direction flow compensated FLASH images were acquired on a 7T animal MRI (ClinScan, Bruker) to measure the magnetization recovery in a single shot (snapshot) with 16 time points for TI = 82-3344.5 ms with ΔTI = 217.5 ms, TE = 1.53 ms, FA = 5°, echo spacing = 2.7 ms, TR = 3562 ms, acquisition matrix: 128×80, FoV: 32×25 mm^2, 6/8 Partial Fourier, BW = 550 Hz/pixel. The SR-snapshot method was calibrated using a phantom made of four Eppendorf tubes filled with 2 ml saline and aqueous solutions of Gd-DTPA (0.1, 0.2, 0.3, 0.4 mM) at about 30°C. Healthy wild type mice were used to verify the DCE setup. The anesthesia was introduced with a gas mixture of by 2.5-3.5% Isoflurane in a 2.5 L/min O_2 flow. The respiration rate and body temperature were monitored by a small animal monitoring system (SA Instruments Inc.). The temperature was maintained by a water circulation system integrated into the probe holder. During the DCE-experiment the mice body temperatures are kept between 30 and 35 °C and the respiration rates between 20 and 30 min^{-1}. The contrast injection system consisted of two MR-compatible syringe pumps (Harvard Apparatus) placed 50 cm away from the bore and loaded with 1 ml plastic syringes containing saline and 10-fold diluted Magnevist (Bayer), respectively. The syringes were connected with 130 cm long Tygon Microbore tubing (Cole Parmer) (ID/OD = 0.76/2.29 mm) to a non-magnetic stainless steel T-junction (Cole Parmer) (ID/OD = 0.58/0.90 mm) which was joined to a 15 cm long small Tygon tubing (ID/OD = 0.25/0.76 mm) by using 1 cm of the thick tubing as a coupling sleeve. The blunt end of a 27G butterfly needle was connected to the end of the thin tubing. The dead volume from the T-junction to the tip of the needle was about 10 µL. The whole injection line was gas sterilized and filled first with the contrast agent solution up to the T-junction and then to the very end with saline through the other line. Special attention was paid to keep the syringes and injection line free of air bubbles. The contrast line was clamped shortly before the T-junction to prevent leaking of the contrast agent while the needle is placed into the tail vein. To prevent motion, a 1 cm wide coiled cradle was wrapped around the section of the mouse body to be scanned. A single dose of contrast agent (0.1 mmol/kg), which corresponds to 50 µL of diluted contrast agent for a 25g mouse, was injected at the 20th scan and immediately flushed with 25 µL saline. The injection speed of both pumps was 5 µL/sec. The SR-snapshot sequence was repeated 300 times for about 18 min. The dynamically measured R1 values were converted to the concentration time curve of the contrast agent by using its relaxivity and the mean R1 of the first 20 scans which served as baseline. The two compartment kinetic model was fitted to the DCE-curves with $Ct(t) = Ktrans \cdot Cp(t) \otimes \exp(kep \cdot t) + vp \cdot Cp(t)$ [3]. Where the tissue and plasma concentration of the agent is denoted by Ct and Cp, respectively, vp denotes the fractional plasma volume, Ktrans the transfer constant and kep the rate constant with kep = Ktrans / ve. Here, ve denotes the fractional interstitial space. Cp(t) was estimated for each mouse individually by a tri-exponential fit from the vascular input function of the aorta (Ca) corrected by the hematocrit (*hct*) with: Cp(t) = Ca(t) / (1-*hct*), where the hematocrit was assumed to be 0.45 [4]. Matlab (MathWorks Inc.) was used for data processing.

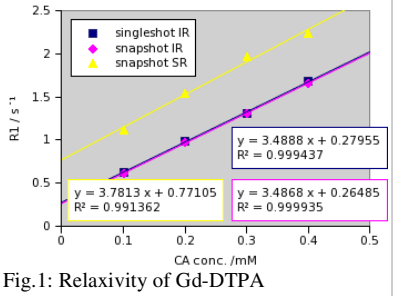

Fig.1: Relaxivity of Gd-DTPA

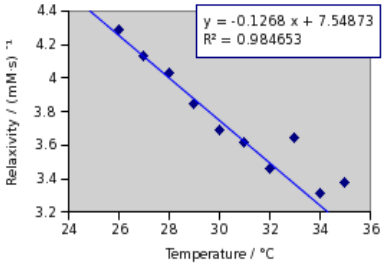

Fig.2: Temperature curve of relaxivity.

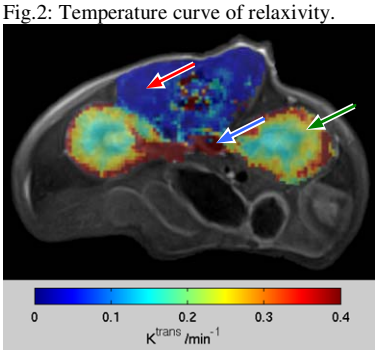

Fig.3: Abdominal slice of the wild type mouse with overlay of Ktrans. Arrows mark pixels for which the DCE curves were plotted in Fig.4.

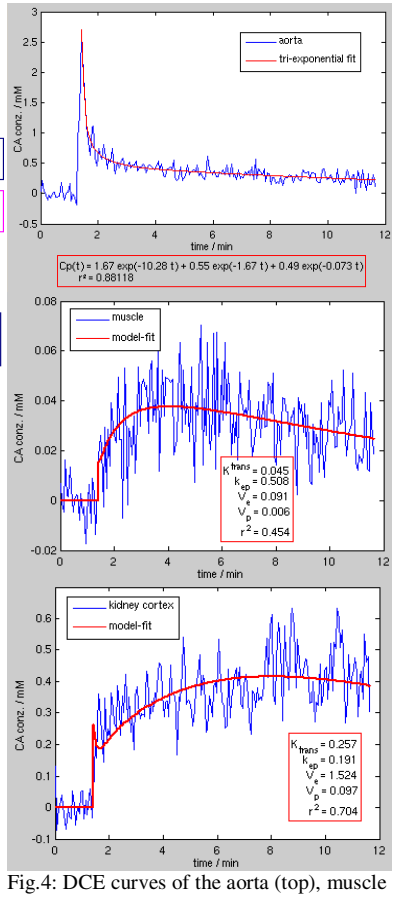

Fig.4: DCE curves of the aorta (top), muscle (middle) and the kidney cortex (bottom). The corresponding regions are marked by colored arrows in Fig.3. (blue arrow: aorta, red arrow: muscle, green arrow: kidney cortex)

Results: The relaxation calibration curve of the SR-snapshot FLASH sequence shows higher relaxation rates, but similar relaxivity, i.e. linear slope, compared to the IR-single shot or IR-snapshot method [5] (Fig.1). The temperature calibration curve permits adjustment of the relaxivity to the actual mouse body temperature (Fig.2). The transfer constant (Ktrans) is shown as an overlay to an anatomical image in Fig.3 and DCE curves with the fitted model parameters are shown for the aorta, muscle and kidney in Fig.4.

Discussion/Conclusion: We were able to design an experimental setup for reliable DCE measurements. Due to the small dead volume of our injection line we can keep the total injection volume low which is of importance in mice whose physiology is most likely affected by larger injection volumes. Another improvement of our design is the calibrated SR-snapshot sequence which dynamically measures the relaxation rate and makes the use of another method for acquisition of reference relaxation rates obsolete. This setup is used in our institution to consecutively measure the tumor response to anti-angiogenic drugs reliably and reproducibly. This is essential for the successful completion of longitudinal preclinical studies.

References:
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