Reproducibility of MR perfusion imaging

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Abstract
Dynamic MR biomarkers (T2*-weighted or susceptibility-based and T1-weighted or relaxivity-enhanced) have been applied to assess tumor perfusion and its response to therapies. A significant challenge in the development of reliable biomarkers is a rigorous assessment and optimization of reproducibility. The purpose of this study was to determine the measurement reproducibility of T1-weighted dynamic contrast-enhanced (DCE)-MRI and T2*-weighted dynamic susceptibility contrast (DSC)-MRI with two contrast agents (CA) of different molecular weight (MW): Gd-DTPA (0.5 kDa) and P792 (6.5 kDa). Each contrast agent was tested with eight mice that had subcutaneous MDA-MB-231 breast tumors. Each mouse was imaged three times within one week to achieve measures of reproducibility. DSC-MRI results were evaluated with a contrast to noise ratio (CNR) efficiency threshold. In all animals with both agents, there was a clear signal drop (> 95% probability threshold) in the DSC of normal tissue, while signal changes were minimal or non-existent (< 95% probability threshold) in tumors. Mean Within-subject coefficient variation (wCV) of CBV in normal tissue was 3.43% for Gd-DTPA group and 2.58% for P792 group. The intraclass correlation coefficient (ICC) of BV in normal tissue was 0.940 for Gd-DTPA group and 0.978 for P792 group. Inter-subject correlation coefficient was 0.092. DCE-MRI results were also obtained for both contrast agents in all animals. Calculated Ktrans showed comparable reproducibility (mean wCV, 2.26% for Gd-DTPA group; 2.87% for P792 group). High inter-subject reproducibility (Rho; 0.9990 ± 0.0055) and inter-subject heterogeneity (ICC = 0.774), for both CA measured on three different days. Histograms and cumulative histograms of Ktrans distributions for three measurements of each individual had high degrees of overlap. These results represent intra-subject measurement and heterogeneous inter-subject character of biological population, suggesting that perfusion MRI could be imaging biomarkers to monitor or predict response of disease.

Materials and Methods

Cell lines and animals model
MDA-MB-231 breast cancer cells were obtained from the American Type Culture Collection (ATCC) and grown in DMEM supplemented with 10% FCS and antibiotics in a 5% CO2 humidified incubator at 37 °C. Cells were routinely monitored for mycoplasma contamination and cell line authenticity. Orthotopic tumors were obtained by injecting female SCID mice with 10 7 cells into the mammary fat pad (MFP). Tumors were measured using electronic calipers and tumor volumes calculated as 0.5 × (long axis in mm) × (short axis in mm) 2.

Data acquisition

MRI experiments were performed with a Bruker Biospec 7 T MRI scanner equipped with a maximum gradient amplitude of 600 mT/m. All animals were anesthetized by inhaled isoflurane (1.5% in O2) at 1.0 LPM and camouflaged at the tail vein. A pressure-transducer balloon was inflated to the animal’s chest to allow for continuous monitoring of the respiration rate. The body temperature was continuously monitored by a rectal fluoroptic thermometer (SAII®, Stony Brook, NY). An external heater was used to maintain body temperature at 37.0 ± 0.2 °C during the course of the imaging experiments. The animal was gently secured in a plastic holder and loaded into a 34-mm-ID small-animal imaging Litz coil (Doxy Scientific, Columbia, SC, USA). T2-weighted images were acquired using a rapid acquisition with relaxation enhancement (RARE) MRI pulse sequence with a RARE factor of 8, giving an effective TE of 72 ms. A series of spin echo (SE) images at six different recovery time (TR) values were acquired prior to injection of the contrast agent (TR = 200, 400, 800, 1500, 3000, 5000 ms, TE = 8.5 ms, FOV = 35 x 35 mm, matrix = 128 x 128, spatial resolution = 273 x 273 μm). A 1.5-mm-thick axial slice was oriented through the center of the tumor and three additional slices were orientated through the thigh and tail to monitor the contrast agent in the femoral artery to obtain an Arterial Input Function (AIF). Both thigh and tail were well imaged to avoid motion artifacts. During bolus administration of a single dose of CA (0.1 mmol/kg for P792 or 0.25 mmol/kg for Gd-DTPA injected within 5s), a gradient-echo (GRE) MRI FLASH pulse sequence was applied for 60 seconds (TR = 10 ms, TE = 5 ms, flip angle = 5°, Slice = 1.5 mm, FOV = 35 x 35 mm, matrix = 64 x 64, spatial resolution = 547 x 547 μm, temporal resolution = 0.6 s) a followed by a dynamic series of spin echo T1-weighted images (TR = 150 ms, TE = 7.2 ms, Slice = 1.5 mm, FOV = 35 x 35 mm, matrix = 128 x 128, spatial resolution = 273 x 273 μm, dummy scan = 2, temporal resolution = 19 s) for 30 minutes. The reduced spatial resolution of DSC images is compromised to maximum the temporal resolution for making perfusion measurements. A 5° flip angle was used in FLASH sequence to minimize the T1 effect of DSC-MRI.

Data and statistical analysis

MRI data were analyzed using self-developed programs written for MATLAB (Mathworks, Natick, MA). A region of interest (ROI) encompassing the tumor was drawn manually on the T2-W anatomical image. The ROIs for the AIF were determined by a self-constructed automatic selection algorithm. This algorithm first segments all possible artery/vein ROIs by comparing the signal amplitude differences of pre- and post-CA, then calculates the slopes of time-course signal curve for these ROIs and sorts slopes within a reasonable range into a candidate pool, finally averages the most similar slopes into an AIF. A 3x3 matrix with center to edge ratio of 9 used for Gaussian spatial smoothing and a spline interpolation with number of degree of 6 was used for pixel-sized temporal smoothing. The values of Ktrans maps within the ROI were used to generate normalized distribution histograms and cumulative histograms. Cumulative histograms were constructed by counting the cumulative number of observed values at each bin. Area under curve (AUC) was calculated from each histogram. The differences of AUC for three repeated measurement of a single animal were used to quantify the reproducibility. The sum of these AUC curves stay at a range of 0 to 1. The more this number approaches to 0 the higher reproducibility. Statistics of reproducibility between three scans per mouse were determined using tCV and ICC.

Conclusion

Intra-subject reproducibility tests for perfusion MRI are fundamental basis of many biomarker studies. Inter-subject variability presents a blueprint of biological heterogeneity and complexity. We conclude that tumor vasculature characteristics can be measured reproducibly using DCE-MRI in the same mouse when imaging analysis was carefully performed. The reproducibility of DCE-MRI results gives confidence in measuring or predicting the effect of anti-angiogenic therapies. DSC-MRI consistently detected the first pass of each contrast agent in normal tissue, but did not detect the first passes in tumor tissues with immature blood vessels, suggesting that this technique has potential to assess normalized tumor vessels.

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