A novel approach to tracer-kinetic modeling of (macromolecular) multi-bolus DCE-MRI data, applied in a murine tumor model

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Purpose Dynamic contrast-enhanced MRI (DCE-MRI) is a widely applied tool for characterization of the tumor vasculature and treatment evaluation[1]. Using conventional tracer-kinetic models, it has been shown that macromolecular contrast agents may be preferred to assess changes in microvascular permeability and blood volume, while low-molecular-weight agents are more useful to assess changes in blood flow[2]. More advanced models, such as the two-compartment exchange (2CX) model, enable a separate estimation of blood flow and vascular permeability[3]. In this research a multi-bolus DCE-MRI protocol was developed, in which three contrast agents of different size but similar composition were injected sequentially under identical circumstances. A novel tracer-kinetic modeling strategy for the combined multi-bolus data was applied, based on the 2CX model, where blood flow and mean capillary transit time were constrained to be identical between the different boluses based on physiological grounds, while the extraction fraction and washout rate constant were allowed to differ between contrast agents. The feasibility of this approach was demonstrated in a murine tumor model. It is hypothesized that this approach is suitable to accurately assess tumor vascular status and treatment effects on the different vascular parameters.

Methods Contrast agents: modified poly(propylene imine) (PPI) dendrimers of generation 2 (G2-PPI-(dPEG$_6$-GdDOTA)$_8$) and 5 (G5-PPI-(dPEG$_6$-GdDOTA)$_{64}$) were synthesized by SyMO-Chem BV. Gd-DOTA (Dotarem) was purchased from Guerbet. The molecular weights of the G2 and G5 dendrimer and Dotarem are 7317, 59517 and 559 g/mol respectively. For the *in vivo* multi-bolus experiments an infusion line was filled with equal volumes of the G5 and G2 dendrimer and Dotarem (125 mM Gd^{3+}, ~ 0.1 mmol Gd^{3+}/kg), separated by equal volumes of saline. A small air bubble between all volumes prevented mixing of the agents.
In vivo multi-bolus experiments: Multi-bolus DCE-MRI experiments were performed on five CT26 colon carcinoma bearing Balb/c mice, on a 7 T Bruker BioSpec 70/30 USR equipped with a 1H 59/35 (outer/inner diameter) circular polarized MRI transceiver volume coil. All mice underwent MRI 3 times with 2 days of recovery in between. B1-mapping was performed based on the 180° signal-null method[4]. Pre-contrast T_1 mapping was performed using a variable flip angle approach. For the multi-bolus DCE-MRI measurements an RF-spoiled 3D FLASH was used with the following sequence parameters: TR = 2.5ms, TE=0.84ms, FA = 6°, acquisition matrix = 52x72x14 (reconstructed to 96x96x16), FOV = 30x30x24 mm^3, temporal resolution 1.890 sec, total scan time 47min. Contrast injections were performed at 2 (G5 dendrimer), 17 (G2 dendrimer) and 32 (Dotarem) minutes after start of the acquisition, using an infusion pump at a rate of 2 mL/min. ΔR_1 values were calculated on a pixel-pixel basis based on the standard signal-equation for a spoiled gradient-echo sequence, using the pre-contrast T_1 values and the post-contrast dynamic signal intensities. Regions of interest delineating the tumor tissue were hand-drawn using anatomic images.
Multi-bolus tracer-kinetic modeling: ΔR_1 curves were scaled by the contrast agent relaxivity, measured in mouse plasma at 7T at 37 °C. Arterial input functions were determined for each agent separately using the Monte Carlo Blind Estimation (MCBE)[5] algorithm. Multi-bolus data were simultaneously fit with a modified 2CX model; blood flow (F), mean capillary transit time (t$_c$), and bolus arrival time (t$_d$) were constrained to be identical between the different boluses, while extraction fractions (E) and washout rate constants (k$_{ep}$) were separately determined for each contrast agent. Blood volume fraction (v$_b$) was calculated by F*t$_c$. No scaling factor for the AIF was obtained yet, so AIF scaling was performed using a mouse-specific population AIF. This could affect the absolute values of F, t$_c$ and v$_b$. Median pharmacokinetic parameters were calculated in regions of the tumor where model estimates of blood flow were statistically significant. Median tumor parameter values for each measurement day were determined by taking the median of the median parameter values of all mice.

Results single-pixel ΔR_1 curves in the tumor tissue were of good quality and clear differences in enhancement pattern were observed for the different contrast agents (fig 1A). Areas of low and high contrast enhancement corresponded well with the measured blood volume fractions in these areas (fig 1B). Blood flow and capillary transit time were essentially identical for the different measurement days (fig 2A,B). The perfused tumor fraction was significantly lower at day 3 compared to day 1, presumably due to interval tumor growth (fig 2C). Extraction fractions of Dotarem were significantly higher than those of the G2 and G5 dendrimers (fig 2D). Washout rate constants of the G2 dendrimer and Dotarem were significantly higher than those of the G5 dendrimer (fig 2E).
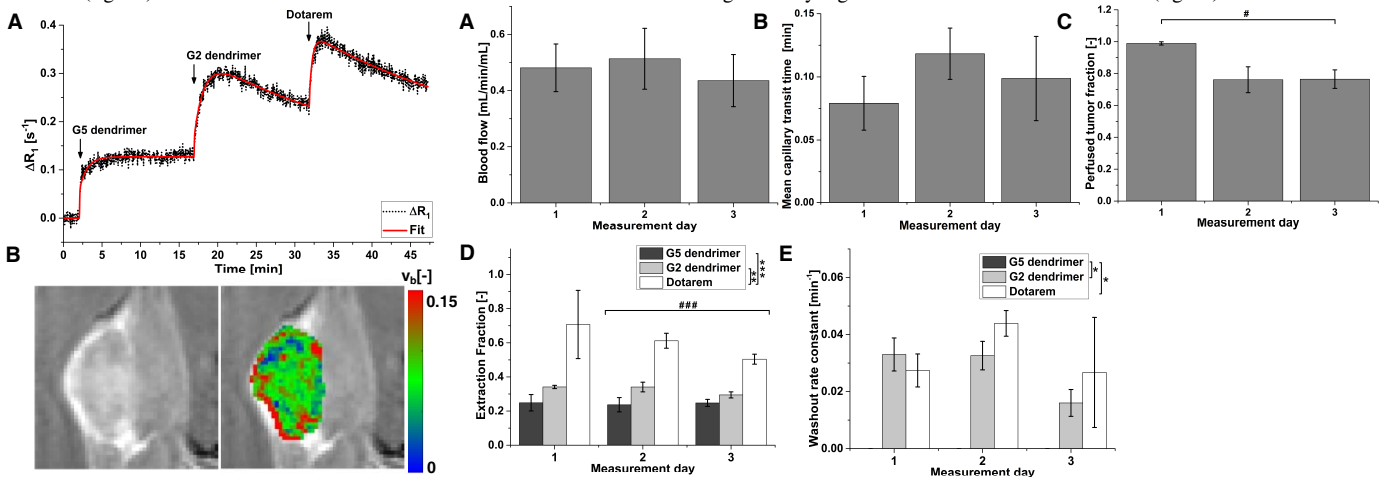

Figure 1: A) An exemplary ΔR_1 curve measured in a single tumor-pixel and the multi-bolus fit through the data. Pharmacokinetic parameters for this pixel were: F=0.71 mL/min/mL, E(G5)=0.29, k$_{ep}$ (G5)=0.0 min^{-1}, t$_c$=0.13 min, t$_d$=0.15 min, E(G2)=0.46, k$_{ep}$(G2)=0.05 min^{-1}, E(Dotarem)=1.0, k$_{ep}$(Dotarem)=0.03 min^{-1}. G5, G2 and Dotarem indicate to which contrast agent the parameter value applies. B) An exemplary slice of the tumor bearing hind limb at 45 min after the first injection, with on the right the v$_b$ values superimposed on the tumor tissue.

Figure 2: A-E) Median blood flow, capillary transit time, perfused tumor fraction, extraction fraction and washout rate constant respectively. Differences in pharmacokinetic parameters between measurement days and between the different contrast agents were analyzed using a mixed-model repeated-measures ANOVA with post-hoc Games-Howell test, with the time point as the within-subjects factor and the contrast agent as the between subject-factor. Data are reported as median±mad (median absolute deviation) of the five mice for each measurement day. The level of statistical significance was set at α=0.05. *,**,*** indicates a significant difference with p< 0.05,0.01,0.001 respectively between the different contrast agents. #,### indicates a significant difference with p<0.05, 0.001 respectively between the different measurement days.

Discussion and conclusion The feasibility of a novel tracer-kinetic modeling approach for fitting multi-bolus DCE-MRI data, acquired with contrast agents of various molecular weights, was shown in a murine tumor model. Blood flow was separately assessed from contrast agent specific vascular parameters associated with vascular permeability. Significant differences in extraction fractions and washout rate constants were observed between the different contrast agents. Future multi-bolus measurements could benefit from an increased temporal resolution and higher flip angle to be more sensitive to the first bolus passage. In addition, bolus specific scaling of the AIF could improve the modeling outcome. The approach can be used to obtain separate estimates of blood flow and permeability associated vascular parameters and as a next step the multi-bolus approach will be applied to assess treatment effects on the different vascular parameters.

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