Assessment of inflammation in a rabbit model of early atherosclerosis: reproducibility and accuracy of kinetic analysis approaches with black-blood DCE-MRI

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Introduction

Inflammation plays an important role in both atherosclerotic plaque progression and rupture [1]. Recently, dynamic contrast-enhanced (DCE) MRI has been shown to be sensitive to inflammatory content within plaque [2]. Unfortunately, this bright-blood technique is not compatible with early lesions or the evaluation of inflammatory conditions in the surface regions of the plaque. In either case, partial volumes, blurring, and motion of the bright vessel lumen can eradicate the enhancement signal in the adjacent wall. Calcagno et al. [3] addressed this issue with a black-blood spin echo DCE-MRI sequence in which the lumen signal is suppressed to avoid contamination of the wall enhancement signal. In an animal model of atherosclerosis, they showed that plaque neovessels were associated with higher area under the enhancement versus time curve (AUC) measured with this sequence. The AUC was used because suppression of the lumen precluded direct measurement of the arterial input function (AIF) for use in kinetic modeling; however, the AUC may not be as informative as actual estimates of the physiological kinetic parameters for partial plasma volume (v_p) and transfer constant (K^{trans}). In this investigation, we sought to develop and validate a reference region approach for determining K^{trans} and v_p in atherosclerotic plaque, using black-blood DCE-MRI without a measured AIF.

Methods and Materials

Population Our study utilized 7 male New Zealand White rabbits, with approval of the protocol from the Institutional Animal Care and Use Committee. Rabbits were fed a 0.2% cholesterol-enriched diet beginning one week prior to balloon injury of the descending aorta. Subsequently, all animals remained on the cholesterol-enriched diet to develop model atherosclerotic lesions. Four animals were kept on the diet for 3 months and 3 more on the diet for 6 months. At that point, all animals were scanned with a DCE-MRI protocol twice within 5 days, euthanized, and histologically evaluated.

MR Imaging All animals were imaged under anesthesia on a clinical 3T MRI scanner (Philips Achieva, Netherlands) using a human knee coil. Four dynamic image slices were prescribed at 8mm intervals along the abdominal aorta using a small FOV quadruple inversion recovery (sfQIR) [4] black-blood TSE protocol. Scan parameters were TR=750ms, TE=12ms, FOV=12cm x 4cm, matrix=256x48 and slice thickness=3mm. Imaging time was 4.5 seconds per slice. A total of 15 time points were obtained, wherein a bolus of gadobenate dimeglumine (Bracco, Milan, Italy) at 0.05 mmol/kg was injected into the marginal ear vein coincident with the third time point. In addition, 4 animals were also imaged using a standard bright-blood DCE-MRI sequence with the same injection protocol to measure kinetic parameters in a reference muscle region with a measured AIF.

Kinetic analysis Inner and outer vessel wall contours were drawn to identify the region of interest (ROI) for each DCE-MRI slice. Then, a reference-region approach was used to estimate the transfer constant (K^{trans}) and fractional plasma volume (v_p) within the vessel ROI. These estimates were based on the Patlak model used in [2]:

$$C_t(t) = v_p C_p(t) + K^{trans} \int_0^t C_p(\tau) d\tau \ ,$$

where $C_p(t)$ is the AIF and $C_t(t)$ is the tissue concentration. To address the lack of a measured AIF, we derived an equation to calculate the parameters in the ROI based on the kinetics of a reference region (RR) with known kinetic parameters v_p^{RR} and K^{trans}_{RR}:

$$v_p C_{RR}(t) + K^{trans} \int_0^t C_{RR}(\tau) d\tau = v_p^{RR} C_{ROI}(t) + K_{RR}^{trans} \int_0^t C_{ROI}(\tau) d\tau \ ,$$

where $C_{ROI}(t)$ and $C_{RR}(t)$ are the concentration functions of contrast agent in ROI and RR, respectively. Given known perfusion parameters within RR, the perfusion parameters within ROI can be calculated by a linear least square error fitting algorithm without a directly measured AIF. In this study, $C_{RR}(t)$ was extracted by averaging the time-dependent intensity curve in a user-identified muscle region for each animal. The values of v_p^{RR} and K^{trans}_{RR} were calculated from the bright blood DCE-MRI results in 4 rabbits. In addition, the area under the time-intensity curve (AUC) was also computed for each ROI to account for possible differences in coil positioning and contrast bolus characteristics that can affect absolute signal levels, the average muscle AUC for each animal was used to normalize the AUC of the vessel wall producing an "AUC ratio" between vessel and muscle.

Histological analysis For validation, the aortas of each animal were dissected after euthanization and multiple 5 micrometer sections were obtained from each of the 4 locations corresponding to the DCE-MRI slices. Sections from each location were stained using a modified Movat Pentachrome procedure and immunohistochemistry with the macrophage marker, RAM-11. To quantitatively assess macrophage content, the total area staining positive for RAM-11 was measured by color thresholding and divided by the total cross-sectional lesion area to obtain "percent macrophage staining." Neovasculature was quantified by qualitatively assessing the intima, media and adventitial regions in each of four quadrants per cross-section and assigning a score of 0 (no neovessels), 1 (few neovessels) or 2 (many neovessels). The sum of all quadrants and regions was used as a "neovessel score" (range = 0 to 24).

Data analysis Reproducibility of the K^{trans}, v_p and AUC ratio were computed using the intra-class correlation coefficient (ICC). After the repeated measurements were averaged, the association of the K^{trans}, v_p and AUC ratio with each histological parameter was evaluated at the slice level using Pearson correlation coefficients.

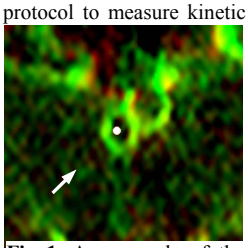

Fig 1. An example of the produced V-V image with K^{trans} in green channel and v_p in red channel. The point label the lumen of aorta. The arrow points the adjacent muscle.

Table 1. ICCs of the three parameters evaluated from black blood DCE-MRI.

	ICC
K^{trans}	0.614
v_p	0.721
AUC Ratio	0.566

Results

The reference region parameters were found to be $v_p^{RR} = 0.0265 \pm 0.0102$ and $K^{trans}_{RR} = 0.0281 \pm 0.0032$ min^{-1}. Fig 1. shows an example of the resulting parameter estimates with K^{trans} in the green channel and v_p in the red channel. To assess reproducibility, repeated measurements were obtained from all 28 locations and ICCs are summarized in Table 1. The parameters K^{trans} and v_p exhibit somewhat better reproducibility than the AUC ratio. For 27 locations histological results were also obtained (Table 2). Correlations with histological variables were stronger for K^{trans} and v_p than for the AUC ratio.

Table 2. Correlations between perfusion parameters evaluated from black blood DCE-MRI and histological bio-markers.

Pearson correlation	Macrophage Area (%)	Neovessel Score
K^{trans}	**0.667*** (**p<0.001**)	0.060 (p=0.766)
v_p	0.248 (p=0.212)	**0.648*** (**p<0.001**)
AUC Ratio	**0.421*** (**p=0.029**)	**0.568*** (**p=0.002**)

Conclusion

In this study, we demonstrated the potential for kinetic modeling of black blood DCE-MRI of atherosclerotic plaque using a reference region approach to the Patlak model. This approach appeared to be superior to the AUC analysis previously applied to the problem [3]. Although we found a similar link between AUC and neovessels as reported previously, our estimate of v_p was more strongly correlated with neovessel score. Furthermore, our estimate of K^{trans} exhibited very good correlation with macrophage content. Finally, both kinetic parameters measured with the reference region model exhibited better reproducibility than the AUC ratio. This result therefore suggests a further improvement in the ability to assess plaque inflammatory characteristics with black-blood DCE-MRI. In addition to applications to small lesions, as in this study, this technology may be useful for studies of localized plaque kinetics [5] adjacent to the lumen.

References:

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