Comparison of Gadofluorine M and Gd-DTPA relaxivities for Quantitation and Characterization of Atherosclerotic Plaque in Mouse at 11.7T

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Introduction

Recent advances in MRI technology and novel imaging contrast agents have made MRI an important imaging modality for detecting and characterizing atherosclerosis [1]. The contrast agent Gd-DTPA has been used clinically to help discriminate the fibrous cap from the necrotic core in advanced carotid atherosclerotic plaques [2]. We have established a preclinical *in vivo* high resolution MRI technique for the quantitation of the progression of atherosclerotic plaque in a mouse model using Gd-DTPA [3]. Signal enhancement by Gd-DTPA increased the sensitivity and efficiency of MRI. However as a non-specific contrast agent, the diffusion of Gd-DTPA into the surrounding tissue and its existence in circulation during plaque enhancement may result in estimation error in the quantitation of plaque size. This is especially true at the vessel wall and lumen interface where there is minimum blood flow causing incomplete suppression of blood signal. Molecular MRI technologies are emerging, which show promise in allowing evaluation of plaque composition at cellular and molecular level, thus improving the detection of vulnerable plaque and accuracy of plaque quantitation. These methods are very valuable for monitoring the efficacy of therapies where tracking changes in plaque load and characterization particularly in small arteries are desired. Gadofluorine M (Bayer Schering Pharma AG, Germany) has been reported to bind to plaque for prolonged time after its blood clearance and specifically target the extracellular matrix of plaque [4], thus making it an important marker of plaque staging [1]. The purpose of our study is to evaluate the ability of Gadofluorine M to quantify plaque burden through selective contrast enhancement in plaque in aortic and carotid arteries. Comparisons of relaxivities and dose response in plaque are made between Gd-DTPA and Gadofluorine M at 11.7 Tesla. The results demonstrate that Gadofluorine M improves the identification and quantitation of plaque burden in major arteries of mouse model of atherosclerosis, and may provide better characterization of plaque components at different stages.

Methods

Phantom experiment: MRI was performed on a Bruker Biospin 500WB spectrometer (Bruker NMR, Inc., Billerica, MA) with an 89 mm vertical bore magnet of 11.7 T. Diluted Gd-DTPA and Gadofluorine M phantoms were prepared with 6 Gd solutions in saline (0, 62.5, 135, 187.5, 250, and 312.5 umol Gd/L). A saturation recovery spin echo (SE) sequence with TE=10ms and 10 varied TR (50ms~2s) and mono-exponential fitting were used to estimate the R_1 relaxivites. Multi-echo SE sequence with inter echo spacing 10ms, Necho=8, and TR=3s was used to estimate the R_2 relaxivites.

In vivo experiment: All experiments were approved by the Institutional Animal Care and Use Committee. ApoE -/- mouse model was used in the study. Mice were anesthetized with 1.5% isoflurane in O_2 gas mixture during imaging within a birdcage coil of 25-mm ID. A cardiac triggered T1-weighted 3D-FSE sequence (rare factor 2) with fat saturation and blood flow suppression was implemented to allow detection of plaque in aortic root (AR), aorta arch (AA), innominate artery (IA), right carotid (RC), left carotid (LC), and left subclavian (LS) arteries. Images were acquired with an in-plane resolution 50×100μm^2 and through-plane resolution 300μm.

For *in vivo* comparison of the non-specific and specific contrast agents with regard to sensitivity of plaque detection and contrast efficiency, a mouse was injected each of the two doses of Gd-DTPA (100μmol/kg and 600μmol/kg), and a dose of Gadofluorine M (50μmol Gd/kg), through tail vein at three different days. Imaging acquisitions were taken 20 minutes after Gd-DTPA injections, and 24 hours after Gadofluorine M injection.

A progression study was conducted to evaluate the ability of Gadofluorine M to quantify plaque burden at different stages of atheroscerosis. Mice (n=7) were given Western diet beginning at 4 weeks of age. The baseline imaging was taken at 24 weeks of age. Serial imaging scans were taken following 5, 12, and 16 weeks of diet. After the final imaging session, the inominate arteries were sampled for histopathology validation.

Results

Figure 1 shows the measurement of R_1 relaxivities of Gd-DTPA and Gadofluorine M at 11.7T. Similar R1 relaxivities were found, which is 4.1±0.2 mmol^{-1} sec^{-1} for Gd-DTPA and 4.0±0.2 mmol^{-1} sec^{-1} for Gadofluorine M at room temperature. The relaxivities are consistent with the literature value for Gd-DTPA relaxivity at lower field strengths (1.5T) [5]. Previous studies have shown that at high field (8.5T) there were no significant differences in Gd-DTPA relaxivities in saline and various types of tissues [6]. This suggests that the *in-vitro* calibration of Gadofluorine M at 11.7T could be used to characterize the relaxivity of water protons in plaque extracellular compartment. Although the R_2 relaxivity of Gadofluorine M is found greater than that of Gd-DTPA (22.7 mmol^{-1} sec^{-1} vs. 5.6 mmol^{-1} sec^{-1}), no detectable signal changes due to T2 effects are observed for short TE (<20ms) sequences and the selected doses. Figure 2 shows the plaque enhancement in mouse carotid arteries using different doses of Gd-DTPA and Gadofluorine M. Images shown are at the peak contrast enhancement period. Gadofluorine M provided the best plaque enhancement and specificity, thus could be used as an effective contrast agent for monitoring plaque burden at different stages. Note that high dose of Gd-DTPA (12 times higher compared to Gadofluorine M) is required to achieve the same contrast enhancement in plaque due to its fast excretion kinetics. Moreover, the blood contamination of Gd-DTPA and its non-specificity of contrast enhancement may lead to inaccuracy in plaque quantitation. In the progression study, significant plaque size increases were detected in the selected arteries after 12 weeks of Western diet (see Figure 3). The histology is being conducted to validate the MRI plaque area measurements.

Conclusions and discussions

Gd-DTPA and Gadofluorine M both enhance the atherosclerotic plaque with increased extracellular volume [1]. This study shows that Gadofluorine M greatly helps in the identification and quantification of plaque with improved sensitivity and efficiency compared to Gd-DPTA. Further *in-vitro* studies will be conducted to investigate the relaxivities of Gadofluorine M in various types of tissue environments at 11.7T. The *in vivo* high resolution MRI protocol quantifying plaque burden in small animal models of atherosclerosis may help in the discovery of novel therapeutics that slow or reverse diseases, and could be directly applicable to the clinic.

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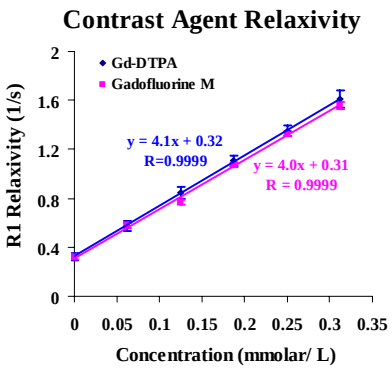

Figure 1. Measurent of R1 relaxivities of Gd-DTPA and Gadofluorine M at 11.7T

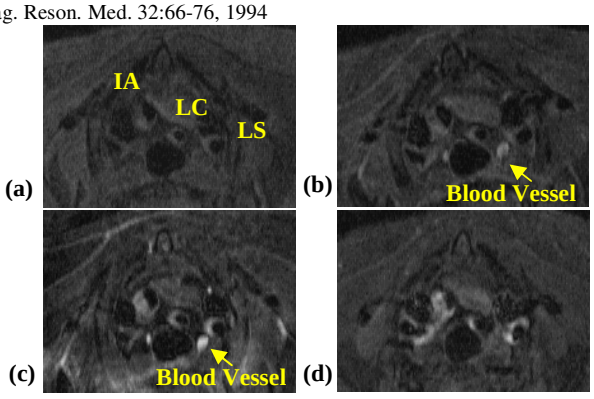

Figure 2. Plaque detection in mouse carotid arteries: (a) Pre contrast; (b) Gd-DTPA 100umol/kg; (c) Gd-DTPA 600umol/kg; (d) Gadofluorine M 50umol/kg.

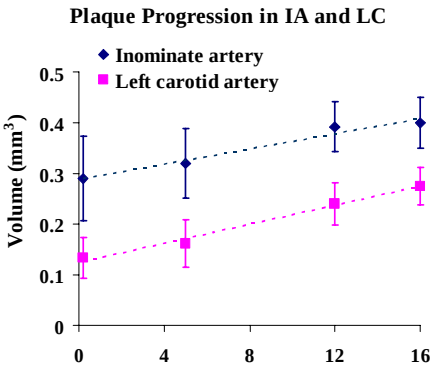

Figure 3. Plaque progression in mouse inominate and left carotid arteries