Identification of Lipid Deposits and Quantification of Carotid Endarterectomy Plaque Components Using High Resolution MRI and Image-guided Proton MRS at 11.7T

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Introduction Patients with carotid plaque undergo endarterectomy based on empirical guidelines, primarily the magnitude of stenosis. There is significant peri-operative morbidity and mortality associated with this procedure, and unfortunately many patients undergoing endarterectomy are found to have stable fibrotic plaque unlikely to cause thromboembolus. Patients who would derive benefit from carotid endarterectomy are those with lipid rich, vulnerable plaque at high risk of rupture [1]. We hypothesis that non-invasive magnetic resonance imaging (MRI) technique can provide distinguishable signal features of plaque components such as fibrous tissue, lipid rich necrotic core (LRNC), intra plaque hemorrhages, and calcifications, therefore can help identify at-risk patients preoperatively [2,3]. The purpose of this study is to demonstrate the capability of MRI and MR spectroscopy (MRS) methods for characterizing plaque composition and quantifying lipid deposition, and to explore potential imaging biomarkers that are associated with clinical phenotypes, thereby facilitating development of noninvasive, quantitative predictor of plaque stability.

Methods

Patient Characteristics Twenty carotid plaque specimens were obtained from patients (n=20, 10 symptomatic, 10 asymptomatic; male: n=13, female: n=7; mean age 68 years old, range from 50 to 91) undergoing endarterectomy. Among these patients, 19 are hypertension, 18 are hypercholesterolemia/hyperlipidemia, 2 have history of peripheral arterial disease, 4 have history of cerebrovascular stroke, and 5 have history of coronary artery disease.

Carotid Endarterectomy Specimens The endarterectomy specimens were preserved in RNAlater solution (SIGMA R 0901, St Louis, MO) at -20 degree. RNAlater is proposed for conservation of DNA and RNA in tissue specimens [4]. The specimens were 2-4 cm in length, and 1-1.5cm in diameter. They were immersed in RNAlater within plastic syringe (1.5cm ID) during experiments. There is no RNA degradation for specimens as a result of exposure to imaging processes at room temperature.

Standard Lipid Phantoms To demonstrate the influence of the lipid species found in plaque [5], specifically Cholesteryl Ester (CE), Free Cholesterol (FC), and trygliceride (TG), on the MR spectra intensity of two major fatty acyl chains (methyl and methylene), image-guided proton MRS was performed on standards of purified CE (cholesteryl linoleate; Sigma-Aldrich), FC (cholesterol), TG (glyceryl trioleat), and their mixtures. Particularly, the ratio of methyl and methylene spectra integrals (CH2:CH3) was studied for the evaluation of cholesterol content in various carotid plaque components (e.g., lipid rich, lipid poor). The line width of the lipid methyl spectra was broadened to about 100 Hz, simulating the line width of the methyl spectra of the specimens.

Ex Vivo MRI Protocol MRI was performed on a Bruker Biospin 500WB 11.7T spectrometer (Bruker NMR, Inc., Billerica, MA) with an 89 mm vertical bore. A 3-D, interleaved water-fat MRI protocol was implemented [6] to assess lipid deposition and plaque composition. High resolution water and fat images of the carotid plaque at the identical slice location were acquired, with an RF coil of 20 mm ID, TR = 2500ms, 2 echoes: TE = 5 and 10ms (to obtain R_2), FOV = 12.8 mm, acquisition matrix $= 256 \times 256 \times 16 \sim 32$, slice thickness = 1 mm, and number of averages = 4, to assure accuracy of estimation of lipid fractions. The acquisition time was about 8 hours.

MR spectroscopy experiments Image-guided localized proton MRS were performed using PRESS (Voxel size = $2 \times 4 \times 2mm3$, TR~5s, TE=10ms, and NEX=256) with selective water suppression. Voxels were selected at plaque locations with varying lipid levels based on the lipid images (lipid poor vs. lipid rich). Automatic and manual shimming was performed resulting in line-widths of 100-200 Hz for specimens preserved in RNAlater solution. The ratio of CH2:CH3 was calculated and compared to that from the standard lipid phantoms, and the content of lipid species including CE and TG at selected locations were estimated.

Photographic Microscopic Imaging and Immunohistology After MRI/MRS, the carotid plaque was sectioned along its length into 1 mm slices for high resolution microscopic imaging, which can be used to verify the plaque composition especially lipid rich component and intra-plaque hemorrhage in MR images. Some slices were sectioned and subjected to histological analyses, and immunohistological stating including CD68, CD3, SMC and HLA-DR were used for identification of macrophages, lipid rich necrotic core, smooth muscle cells, and fibrous tissues in plaque, to validate the plaque composition by MRI/MRS assessment.

Results MRI microscopy was able to provide plaque morphology, especially the definition of lipid-rich/necrotic-core by direct water-fat imaging. The lipid fraction can be assessed pixel by pixel based on the intensity of the water and lipid images. Figure 1 demonstrates the definition and characterization of plaque components such as fibrous tissue, calcification, intra-plaque hemorrhage, and lipid rich components by high resolution MRI, MRS, and lipid fraction histograms. The size of non-lipid, lipid poor, lipid rich, and calcification components can be estimated based on the histogram distribution of the lipid fraction within the plaque. The plaque components such as lipid rich, hemorrhage, and calcification (dark area) were confirmed by matched slice microscopy and histology (CD 68). R_2 relaxation rate was found higher in region containing intra-plaque hemorrhage (Figure 1b). With MRS of standard CE, FC, TG lipids and their mixtures, the effect of TG fraction on the ratio of CH2:CH3 is demonstrated in Figure 1c. As expected, the increase in the cholesterol fraction resulted in an increase in CH3, leading to a decrease in CH2:CH3. For carotid plaque, CH2:CH3 ratios are ranged from 1.2~2, indicating a high fraction of cholesterol (liquid phase) in selected regions. Lipid rich plaques are associated with inflammation and vulnerability. By MRI we were able to correlate the size and composition of a plaque with the clinical status of the patients. Overall, plaques from symptomatic patients were either large (>320 mm^3) or >45% lipid rich and <10% calcified. Plaques from asymptomatic patients were either small (<320 mm^3) or >10% calcified. The fraction of a plaque that was lipid rich inversely correlated with patient's HDL level (r=0.65). Plaque calcification may be associated with plaque stabilization.

Conclusions and discussions Plaques that contain larger lipid-rich components and advanced lesions may be more prone to being broken down. The present study suggested the feasibility of MRI/MRS in assessing the microstructure as well as lipid content in different components (lipid poor vs. lipid rich) of human carotid plaque. The lipid signal, specifically that from triglycerides, and cholesteryl ester in liquid phase found in human atherosclerotic plaques, can be assessed by MR chemical shift imaging technology and image guided MR spectroscopy methods. MRI/MRS assessment of plaque components is being validated by histology and biochemical analyses. Longer term prospective noninvasive imaging studies could be warranted to investigate the relationship between events and lipid content in the plaque.

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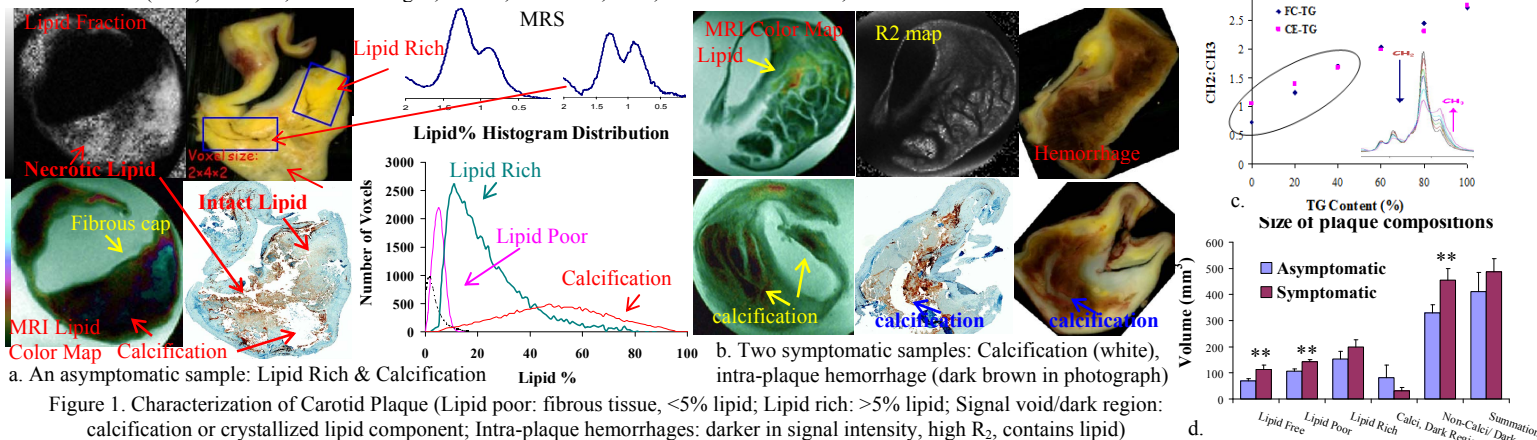
Figure 1. Characterization of Carotid Plaque (Lipid poor: fibrous tissue, <5% lipid; Lipid rich: >5% lipid; Signal void/dark region: calcification or crystallized lipid component; Intra-plaque hemorrhages: darker in signal intensity, high R$_2$, contains lipid)

a. An asymptomatic sample: Lipid Rich & Calcification

b. Two symptomatic samples: Calcification (white), intra-plaque hemorrhage (dark brown in photograph)