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In Vivo Atherosclerotic Plaque Characterization Using Magnetic Susceptibility Distinguishes Symptom-Producing Plaques

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OBJECTIVES We investigated the role of iron deposition in atherosclerotic plaque instability using a novel approach of in vivo plaque characterization by a noninvasive, noncontrast magnetic resonance-based T2* measurement. This approach was validated using ex vivo plaque analyses to establish that T2* accurately reflects intraplaque iron composition.

BACKGROUND Iron catalyzes free radical production, a key step for lipid peroxidation and atherosclerosis development. The parameter T2* measures tissue magnetic susceptibility, which historically has been used to quantify hepatic and myocardial iron. The T2* measurement has not been used for in vivo plaque characterization in patients with atherosclerosis.

METHODS Thirty-nine patients referred for carotid endarterectomy were prospectively enrolled to undergo preoperative carotid magnetic resonance imaging (MRI) and postoperative analysis of the explanted plaque. Clinical history of any symptoms attributable to each carotid lesion was recorded. We could not complete MRI in 4 subjects because of their claustrophobia, and 3 patients scanned before the institution of a neck stabilizer had motion artifact, precluding quantification.

RESULTS Symptomatic patients had significantly lower plaque T2* values (20.0 $\pm$ 1.8 ms) compared with asymptomatic patients (34.4 $\pm$ 2.7 ms, $p < 0.001$). Analytical methods demonstrated similar total iron (138.6 $\pm$ 36.5 μg/g vs. 165.8 $\pm$ 48.3 μg/g, p = NS) but less low molecular weight Fe(III) (7.3 $\pm$ 3.8 μg/g vs. 17.7 $\pm$ 4.0 μg/g, $p < 0.05$) in the explanted plaques of symptomatic versus asymptomatic patients, respectively, which is consistent with a shift in iron from Fe(III) to greater amounts of T2*-shortening forms of iron. Mass spectroscopy also showed significantly lower calcium (37.5 $\pm$ 10.8 mg/g vs. 123.6 $\pm$ 19.3 mg/g, $p < 0.01$) and greater copper (3.2 $\pm$ 0.5 μg/g vs. 1.7 $\pm$ 0.1 μg/g, $p < 0.01$) in plaques from symptomatic patients.

CONCLUSIONS In vivo measurement of intraplaque T2* using MRI is feasible and distinguishes symptom-producing from non–symptom-producing plaques in patients with carotid artery atherosclerosis. Symptom-producing plaques demonstrated characteristic changes in iron forms by ex vivo analysis, supporting the dynamic presence of iron in the microenvironment of atherosclerotic plaque. (J Am Coll Cardiol Img 2008;1:49–57) © 2008 by the American College of Cardiology Foundation

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Atherosclerosis is a major cause of cardiovascular disease, including acute coronary syndromes and ischemic strokes. With increasing recognition that the plaque microenvironment determines clinical sequelae rather than degree of vessel stenosis alone, better strategies to characterize plaque are needed to improve prevention and treatment (1,2). Since Sullivan (3) first proposed that relative iron depletion was protective against cardiovascular disease, the quest to demonstrate iron's role in atherosclerosis has focused on its ability to catalyze the peroxidation of low-density lipoprotein (LDL). Microhemorrhage into atherosclerotic plaque with macrophage-mediated phagocytosis and degradation of aged red blood cells leads to accumulation of redox-active iron (4,5). Via Fenton chemistry, iron catalyzes the generation of oxidized LDL (Fig. 1) (6,7). Oxidized LDL, but not native LDL, binds the macrophage scavenger-receptor, leading to unregulated uptake, foam cell formation, and accelerated atherogenesis (8–10).

ABBREVIATIONS AND ACRONYMS

EPR = electron paramagnetic resonance

ICP-MS = inductively coupled plasma mass spectroscopy

LDL = low-density lipoprotein

MRI = magnetic resonance imaging

PDW = proton density-weighted

TE = echo time

T1W = T1-weighted

T2W = T2-weighted

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Despite these established pathophysiologic mechanisms, studies relating iron and atherosclerosis have provided conflicting results. Iron has consistently been found in greater concentrations in atherosclerotic plaque compared with normal arterial tissue (11,12).

Fenton chemistry

$$Fe^{3+} + O_2^{\cdot -} \longrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot} + OH^-$$

Haber-Weiss reaction

$$O_2^{\cdot -} + H_2O_2 \longrightarrow O_2 + OH^{\cdot} + OH^-$$

LDL peroxidation

$$OH^{\cdot} + LDL \longrightarrow H_2O + LDL\text{-}ox$$

Figure 1. LDL Peroxidation Catalyzed by Iron

The Haber-Weiss reaction and Fenton chemistry use iron in generating free radicals that oxidize low-density lipoprotein (LDL). Microhemorrhage into atherosclerotic plaque with macrophage-mediated phagocytosis and degradation of aged red blood cells leads to accumulation of redox-active iron. Oxidized LDL binds the macrophage scavenger-receptor, leading to unregulated uptake, foam cell formation, and accelerated atherogenesis.

Table 1. Iron Quantification Techniques

Method	Type of Iron Detected
Inductively-coupled plasma mass spectroscopy	Total iron
Electron paramagnetic resonance	Low molecular weight Fe(III)
T2* magnetic resonance imaging	Iron aggregates

In animal models, iron overload accelerates atherogenesis (13). Epidemiologic studies, however, have yielded equivocal results when comparing serologic markers of total body iron stores with the incidence of atherosclerotic disease (14–17).

Notably, little work has involved direct in vivo examination of plaque iron, particularly with an appreciation of the different species of iron in biologic tissues. Free or low molecular weight iron exists as Fe(II) and Fe(III) cations. Iron may be incorporated into hemoglobin or bound to the storage proteins ferritin and hemosiderin, both of which cause measurable changes in local magnetic field homogeneity. This change can be appreciated qualitatively using magnetic resonance T2*-weighted imaging (18) or quantified using the relaxation parameter T2*. T2* quantification allows for the accurate estimation of tissue iron content (19). Multiple in vivo and ex vivo techniques exist to measure these various forms of iron (Table 1). Inductively coupled plasma mass spectroscopy (ICP-MS) is used to measure total iron content. Electron paramagnetic resonance (EPR) is sensitive to several forms of iron, iron storage, and iron transport proteins; the $g \approx 4$ peak is specific for Fe(III) with rhombic coordination symmetry. Electron paramagnetic resonance does not detect the reduced state of iron, Fe(II). T2*-weighted magnetic resonance imaging (MRI) has been proven to be particularly sensitive to iron clusters as occurs in ferritin- or hemosiderin-bound iron (20) but, to date, a quantitative estimation of T2* has not been used to understand iron's role in the microenvironment of human atherosclerotic plaque (19,21).

Recognizing that MRI is already established as a means for high-resolution in vivo imaging of carotid artery atherosclerotic plaque (22), we hypothesized that T2*-weighted MRI could uniquely evaluate the relationship between atherosclerosis and iron. Specifically, we sought in this work to: 1) validate the feasibility of in vivo T2*-based MRI carotid plaque characterization; and 2) to use both in vivo and ex vivo analyses to identify changes in iron content that distinguish symptomatic from asymptomatic patients with carotid atherosclerosis.