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Potential of Magnetic Resonance Plaque Imaging Using Superparamagnetic Particles of Iron Oxide for the Detection of Carotid Plaque

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Abstract

Macrophages and by-products are important in plaque destabilization in atherosclerosis. Ultra-small superparamagnetic particles of iron oxide (SPIO)-enhanced magnetic resonance (MR) imaging may be suitable for the detection of macrophages in atherosclerotic plaques. The present study investigated the potential of MR plaque imaging using SPIO in 10 patients scheduled for carotid endarterectomy before and 24–43 hours after administration of SPIO (fercarbotran, 0.016 ml/kg). Three-dimensional gradient recalled acquisition in the steady state (3D-GRASS) was used for detecting macrophages within plaques. Signal loss on the post-contrast 3D-GRASS images was found in 5 of 10 cases, and accumulation of SPIO particles in the vessel wall was confirmed in 4 of these 5 cases. Intracytoplasmic localization of SPIO particles within recruited macrophages was verified by double staining analysis. A correlation between MR plaque imaging using SPIO and localization of macrophages was demonstrated in 6 of 10 patients. This study indicates that MR plaque imaging using SPIO is a potential functional imaging tool to detect infiltration of macrophages in human atherosclerotic carotid plaque.

Key words: carotid plaque, functional imaging, high-resolution magnetic resonance imaging, macrophage, superparamagnetic particles of iron oxide

Introduction

Atherosclerosis is currently considered to be a chronic inflammatory disease.[12] The risk of an acute event mediated by plaque rupture depends on the plaque components rather than luminal narrowing.[3] Increased infiltration of inflammatory cells is one of the characteristics of vulnerable plaque, as macrophages are key in atherosclerosis and are involved in plaque destabilization.[7,8,12,16] Therefore, macrophages provide a sensitive and specific marker for atherosclerosis and inflammation within carotid plaques, and are an attractive target for plaque stabilization therapies.

High-resolution magnetic resonance (MR) imaging is a promising noninvasive tool for characterizing atherosclerotic plaque composition and provides excellent contrast images of the arterial walls using cardiac-gated, fat suppression, and black-blood techniques.[1,2,5,19] Atherosclerotic plaque has recently been imaged in hyperlipidemic rabbits using ultra-small superparamagnetic particles of iron oxide (SPIO) for functional MR imaging.[4,9,13,14] More recently, the ultra-small SPIO agent Sinerem® (Guerbet, Paris, France) has been used for the evaluation of human carotid plaque.[6,15,17,18] Ultra-small SPIO consists of iron oxide nanoparticles stabilized with low molecular weight dextran with a diameter of 30 nm and has a long plasma half-life. Ultra-small SPIO particles can migrate through inter-endothelial junctions and capillary pores with diameters ranging between 5 and 100 nm.[10] Ultra-small SPIO particles are taken up by inflamed plaques rich in macrophages as intracellular deposits that induce areas of signal loss on T_2^*-weighted imaging, which suggests the potential for visualizing macrophages within

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carotid plaques. Unfortunately, the use of ultra-small SPIO in humans is not yet permitted in Japan. SPIO consist of larger particles (mean particle diameter, 57 nm) than ultra-small SPIO particles and are usually used for detecting hepatic tumors. SPIO particles are taken up in the mononuclear phagocytic system (Kupffer cells, macrophages, etc.) and are a strong positive enhancer in blood.[6,13,14,18]

The present study investigated the potential for plaque imaging with SPIO.

Materials and Methods

Eleven consecutive patients underwent carotid endarterectomy (CEA) at our institution between May 2005 and November 2006. Ten of these patients, all males aged 66.6 ± 8.1 years, underwent high-resolution MR imaging using SPIO contrast agent. Written informed consent could be obtained from 9 of the 10 patients. The institutional review board of the hospital approved the study. Seven patients were symptomatic and three were asymptomatic (Table 1).

The carotid atherosclerotic plaques of the patients were imaged with a 1.5-Tesla MR imaging scanner (Signa; GE Medical System, Cleveland, Ohio, U.S.A.) using a phased array coil with a diameter of 3 inches. After a survey to determine the position of the carotid plaque, the following MR imaging sequence was used. Three-dimensional gradient recalled acquisition in the steady state (3D-GRASS): repetition time (TR)/echo time (TE) 33.3/9.6 msec, flip angle 20°, field-of-view 13 cm, matrix size 256 × 128 with zero-fill interpolation technique, slice thickness 2.5 mm, and number of excitations 1. 3D-GRASS imaging was performed before and 24–43 hours after the administration of SPIO contrast agent (fercarbotran, Risovist® [0.016 ml/kg, Fe; 0.45 mg/kg = 8 μmol/kg]; Bayer Schering Pharma, Berlin, Germany). In addition, the following sequences were used to investigate general carotid plaque components. T_1-weighted imaging: two-dimensional fast-spin echo (FSE), TR/TE 800/11 msec, echo train length (ETL) 4. Proton density-weighted/T_2-weighted imaging: FSE, black-blood (double-inversion recovery) technique, TR 2 heart beats (1400 to 2000 msec, depending on heart rate), TE 20 msec for proton density-weighted and 80 msec for T_2-weighted, ETL 16. Two-dimensional time-of-flight imaging: TR/TE 50/4.2 msec, flip angle 45°, field-of-view 13 cm, matrix size 256 × 128, slice thickness 2.5–3.0 mm. Fat suppression was used for T_1-, proton density-, and T_2-weighted imaging.

Pre- and post-contrast MR images were matched, and analyzed retrospectively by two experienced neurosurgeon and radiologist unaware of the histological findings and clinical status of the patients. The 3D-GRASS images were analyzed to detect signal loss changes in the vessel wall in the post-contrast image compared with the corresponding pre-contrast image, and whether these changes were positive or negative.

Carotid plaque specimens obtained at CEA were formalin-fixed, cut into 5 mm transverse slices,

Table 1 Cases of magnetic resonance (MR) plaque imaging using superparamagnetic particles of iron oxide (SPIO) contrast agent

Case No.	Sex/ Age (yrs)	Presentation	Signal on GRASS	Period between pre- and postcontrast imaging (hrs)	Period between MR imaging and CEA (days)	SPIO uptake	Presence of macrophages	Histology (AHA type)
1	M/69	TIA	−	42	13	+	+	VI
2	M/75	CI	−	40	6	−	−	VI
3	M/79	CI	+	42	13	+	+	VI
4	M/75	−	+	42	6	−	+	VI
5	M/66	CI	+	24	27	+	+	VI
6	M/71	−	−	40	4	−	−	Vb
7	M/54	CI	+	40	19	+	+	Va
8	M/68	TIA	−	43	19	−	+	VI
9	M/53	−	−	42	5	−	+	Va
10	M/59	TIA	+	30	8	+	+	VI

Presentation: CI, cerebral infarction; TIA, transient ischemic attack; −, asymptomatic. Signal on gradient recalled acquisition in the steady state (GRASS): +, positive; −, negative. CEA: carotid endarterectomy. SPIO uptake: +, positive; −, negative. Presence of macrophages: +, rich; −, poor or none. Histology (American Heart Association [AHA] type: I, initial lesions; II, fatty streak; III, intermediate lesions; IV, atheroma; Va, fibroatheroma; Vb, calcified lesions; Vc, fibrous lesions without lipid core; and VI, complicated lesions characterized by rupture, thrombus, or intraplaque hemorrhage.

briefly decalcified, and embedded in paraffin. Subsequently, 4-μm sections were subjected to histological analysis of plaque phenotype after hematoxylin and eosin staining according to the American Heart Association (AHA) classification. The AHA histological score for atherosclerotic lesions distinguishes 6 major plaque types: I, initial lesions; II, fatty streak; III, intermediate lesions; IV, atheroma; Va, fibroatheroma; Vb, calcified lesions; Vc, fibrous lesions without lipid core; and VI, complicated lesions characterized by rupture, thrombus, or intra-plaque hemorrhage. Immunohistochemically, macrophages were identified with CD 68 (1:100; Dako, Glostrup, Denmark). Berlin blue stain was used to confirm the accumulation of SPIO within atherosclerotic carotid plaques. Double staining with CD 68 and Berlin blue was used to verify the uptake of SPIO particles by macrophages. The analysis was independently performed by two experienced pathologists, who were unaware of the imaging results and the clinical status of the patients.

Results

The period between pre- and postcontrast GRASS imaging was 24 to 43 (mean ± standard deviation [SD] 38.5 ± 5.9) hours. The period between SPIO administration and CEA was 4 to 27 (mean ± SD 12 ± 7.2) days, and no patient suffered an ischemic event. Signal loss changes on postcontrast GRASS images were found in 5 of 10 cases (Figs. 1A, B and 2A, B). All specimens were demonstrated to be advanced carotid plaques (AHA classification: type Va, Vb, or VI) (Figs. 1C and 2C). Accumulation of SPIO particles in the vessel wall by Berlin blue stain was confirmed in the corresponding tissue slices in 4 of 5 cases (Fig. 1D). Moreover, numerous macrophages were found in these slices (Figs. 1E and 2D, Table 1), and intracytoplasmic localization of SPIO particles within these recruited macrophages was verified by double immunohistochemistry and iron stain analysis (Fig. 1F). Uptake of SPIO particles by some smooth muscle cells and endothelial cells was also observed (Fig. 2E).

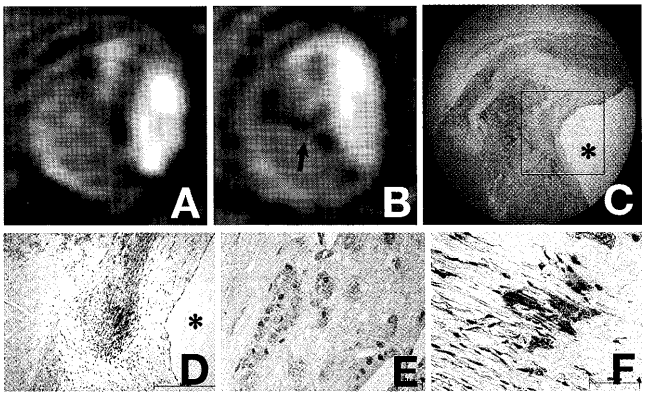

Fig. 1 **A 79-year-old male with symptomatic right carotid artery stenosis. A, B: Pre- (A) and post-contrast (B) three-dimensional gradient recalled acquisition in the steady state images showing signal loss around the intra-plaque hemorrhage (*arrow*). C–F: Photomicrographs showing inflammatory cells around the intra-plaque hemorrhage in the corresponding slice (C: hematoxylin and eosin stain, ×25), accumulation of superparamagnetic particles of iron oxide (SPIO) within the plaque (*square* in C), corresponding to the plaque shoulder (D: Berlin blue stain, ×100), numerous macrophages in the area of SPIO accumulation (E: CD68 stain, ×200), and intracytoplasmic localization of SPIO particles within recruited macrophages (F: double CD68 and iron stain, ×200). *Asterisks* indicate lumen.**

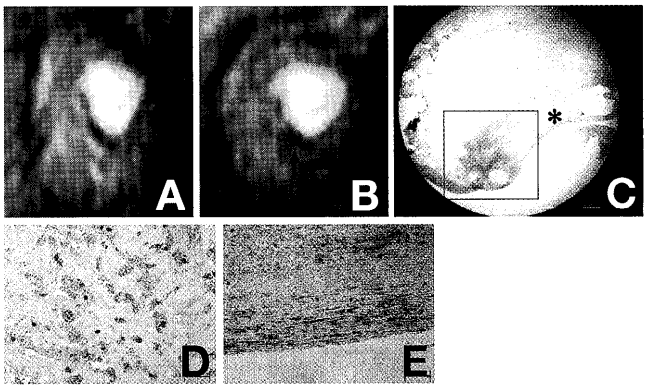

Fig. 2 **A 75-year-old male with asymptomatic right carotid artery stenosis. A, B: Pre- (A) and post-contrast (B) three-dimensional gradient recalled acquisition in the steady state images showing signal loss in the plaque shoulder (*arrow*). C–E: Photomicrographs showing inflammatory cells in the corresponding slice, but no accumulation of superparamagnetic particles of iron oxide (SPIO) (C: hematoxylin and eosin stain, ×25), numerous macrophages (*square* in C) (D: CD68 stain, ×200), and uptake of SPIO particles by some smooth muscle cells and endothelial cells (E: Berlin blue stain, ×100). *Asterisk* indicates lumen.**

Discussion

In the present study, signal loss changes on postcontrast GRASS images were found in 5 of 10 cases, and uptake of SPIO particles by macrophages was confirmed in the corresponding tissue slices in 4 of these 5 cases. SPIO uptake by macrophages may be regulated by various factors. First, the rate of internalization by macrophages may be determined by particle size, so is likely to be higher for ultra-small SPIO than for SPIO. However, a broad range of individual particle cluster diameters can be expected because individual particles tend to aggregate. Second, the effect of SPIO concentration on macrophage uptake may be important, as macrophages internalize SPIO in a dose-related manner.[11] In the present study, SPIO was administered according to the prescribed dose (0.016 ml/kg, Fe; 0.45 mg/kg = 8 μmol/kg). Furthermore, although the way in which ultra-small SPIO particles enter the atherosclerotic plaques remains unknown, the degree of permeability of the endothelium or the presence of neovascularization within plaques may be related to the rate of internalization.

The present study showed that macrophages were present within plaques, yet accumulation of SPIO particles in the vessel wall by iron staining was not confirmed in 3 cases. There are several possible explanations. A heterogeneous population of macrophages may exist with differing phagocytic capacities for SPIO or the labeling of macrophages by SPIO is a dynamic process, dependent on individual cell kinetics. Two of our cases showed a discrepancy between the signal loss changes on postcontrast GRASS imaging and uptake of SPIO particles by macrophages in the corresponding tissue slices. We suggest that the detection of signal loss may also be influenced by factors including the period between pre- and postcontrast imaging, SPIO concentration, duration between MR imaging examination and CEA, and the effect of cells with phagocytic capacity other than macrophages (smooth muscle cells or endothelial cells). The present study found a correlation between MR plaque imaging using SPIO and localization of macrophages in 6 of 10 cases.

The present preliminary study has other limitations. Seven patients had plaques complicated with intra-plaque hemorrhage (AHA classification type VI). Intra-plaque hemorrhage may also have an effect on the visualization of macrophages by MR plaque imaging using SPIO because iron from hemoglobin may also appear positive by Berlin blue staining. Berlin blue staining is not a very sensitive marker for SPIO and other iron staining methods may be needed to resolve this problem. We found that MR plaque imaging using SPIO also has the potential to discriminate macrophage-rich from macrophage-poor lesions, and may be useful for detecting vulnerable plaques with increased infiltration of macrophages prior to surgery or during follow up. However, further work is required to overcome problems caused by motion artifacts, lack of resolution, and interpretation of signal heterogeneity before this technique can realistically be used to assess carotid plaque composition.

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References

1) Cai JM, Hatsukami TS, Ferguson MS, Small R, Polissar NL, Yuan C: Classification of human carotid atherosclerotic lesions with in vivo multicontrast magnetic resonance imaging. *Circulation* 106: 1368–1373, 2002
2) Fayad ZA, Fuster V: Clinical imaging of the high-risk or vulnerable atherosclerotic plaque. *Circ Res* 89: 305–316, 2001
3) Fuster V, Badimon L, Badimon JJ, Chesebro JH: The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med* 326: 242–250, 1992
4) Herborn CU, Vogt FM, Lauenstein TC, Dirsch O, Corot C, Robert P, Ruehm SG: Magnetic resonance imaging of experimental atherosclerotic plaque: comparison of two ultrasmall superparamagnetic particles of iron oxide. *J Magn Reson Imaging* 24: 388–393, 2006
5) Honda M, Kitagawa N, Tsutsumi K, Nagata I, Morikawa M, Hayashi T: High-resolution magnetic resonance imaging for detection of carotid plaques. *Neurosurgery* 58: 338–346, 2006
6) Kooi ME, Cappendijk VC, Cleutjens KB, Kessels AG, Kitslaar PJ, Borgers M, Frederik PM, Daemen MJ, van Engelshoven JM: Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. *Circulation* 107: 2453–2458, 2003
7) Krams R, Segers D, Mousavi Gourabi B, Maat W, Cheng C, van Pelt C, van Damme LC, de Feyter P, van der Steen T, de Korte CL, Serruys PW: Inflammation and atherosclerosis: mechanisms underlying vulner-

able plaque. *J Interv Cardiol* 16: 107–113, 2003
8) Peiser L, Gordon S: The function of scavenger receptors expressed by macrophages and their role in the regulation of inflammation. *Microbes Infect* 3: 149–159, 2001
9) Priest AN, Ittrich H, Jahntz CL, Kooijman H, Weber C, Adam G: Investigation of atherosclerotic plaques with MRI at 3 T using ultrasmall superparamagnetic particles of iron oxide. *Magn Reson Imaging* 24: 1287–1293, 2006
10) Renkin EM: Multiple pathways of capillary permeability. *Circ Res* 41: 735–743, 1977
11) Rogers WJ, Basu P: Factors regulating macrophage endocytosis of nanoparticles: implications for targeted magnetic resonance plaque imaging. *Atherosclerosis* 178: 67–73, 2005
12) Ross R: Atherosclerosis is an inflammatory disease. *Am Heart J* 138: S419–420, 1999
13) Ruehm SG, Corot C, Vogt P, Kolb S, Debatin JF: Magnetic resonance imaging of atherosclerotic plaque with ultrasmall superparamagnetic particles of iron oxide in hyperlipidemic rabbits. *Circulation* 103: 415–422, 2001
14) Schmitz SA, Coupland SE, Gust R, Winterhalter S, Wagner S, Kresse M, Semmler W, Wolf KJ: Superparamagnetic iron oxide-enhanced MRI of atherosclerotic plaques in Watanabe hereditable hyperlipidemic rabbits. *Invest Radiol* 35: 460–471, 2000
15) Schmitz SA, Taupitz M, Wagner S, Wolf KJ, Beyersdorff D, Hamm B: Magnetic resonance imaging of atherosclerotic plaques using superparamagnetic iron oxide particles. *J Magn Reson Imaging* 14: 355–361, 2001
16) Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, Mailhac A, Villareal-Levy G, Fallon JT, Regnstrom J, Fuster V: Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation* 92: 1565–1569, 1995
17) Trivedi RA, Mallawarachi C, U-King-Im JM, Graves MJ, Horsley J, Goddard MJ, Brown A, Wang L, Kirkpatrick PJ, Brown J, Gillard JH: Identifying inflamed carotid plaques using in vivo USPIO-enhanced MR imaging to label plaque macrophages. *Arterioscler Thromb Vasc Biol* 26: 1601–1606, 2006
18) Trivedi RA, U-King-Im JM, Graves MJ, Cross JJ, Horsley J, Goddard MJ, Skepper JN, Quartey G, Warburton E, Joubert I, Wang L, Kirkpatrick PJ, Brown J, Gillard JH: In vivo detection of macrophages in human carotid atheroma: temporal dependence of ultrasmall superparamagnetic particles of iron oxide-enhanced MRI. *Stroke* 35: 1631–1635, 2004
19) Yuan C, Mitsumori LM, Beach KW, Maravilla KR: Carotid atherosclerotic plaque: noninvasive MR characterization and identification of vulnerable lesions. *Radiology* 221: 285–299, 2001

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Commentary

The authors critically discuss the use of a new functional method to identify "in vivo" the presence of instable, possibly embolizing, plaque, in patients with atherosclerotic carotid artery disease. As suggested recently by other papers, they employed carotid MRI before and after administration of ultra small supermagnetic particles of iron oxide, that are picked up by macrophages when present in the plaque itself. Macrophages are known to cause, or to be a sign of, plaque destabilization. The present paper is interesting and the results are promising: a good correlation between MRI images and post-operative histological findings has been demonstrated. However, as the authors point out, there are still significant limitations in this method, and much has to be clarified before using routinely this procedure to study patients in clinical setting.

In my opinion, the main contribution of this work is to bring the attention to the fact that intraluminal narrowing is not the most important element of risk for patients with atheromatous carotid disease; therefore, ultrasonography, that is the most widely diffused, sometimes unique, technique to assess surgical indication, should be integrated with more sensitive "functional" investigation. In particular, this special carotid MRI may provide information not just on plaque morphology, but also about composition and stability, thus allowing a more sound indication for surgery.

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The present study explores the potential for a non-invasive evaluation of human carotid plaques utilizing magnetic resonance (MR) imaging with superparamagnetic particles of iron oxide (SPIO) instead of ultra-small SPIO (SPIO consists of larger particles than ultra-small SPIO). For the evaluation, T2-weighted MR imaging (T2*WI) with administration of the ultra-small SPIO agent has been used, detecting the iron oxide particles as hypointensity deposits in macrophages within the inflamed plaques. However, ultra-small SPIO is not available for clinical use in Japan, which might have encouraged the authors to*

*conduct the present study. The authors examined ten patients scheduled for carotid endarterectomy using T2*WI before and 24–43 hours after administration of SPIO to detect macrophages in atherosclerotic carotid plaques. The plaque specimens obtained at surgery were also assessed histopathologically using a double staining technique. In 5 of the 10 cases, hypointensity deposits were detected on T2*WI with SPIO. In 4 of these 5 cases, SPIO particles were confirmed in macrophages within the plaques. These results seem to contain several pieces of valuable information. From a practical perspective, SPIO-enhanced T2*WI is potentially capable of distinguishing macrophage-rich from macrophage-poor lesions, and may consequently be useful for evaluating the vulnerability of plaques and the risk of plaque rupture. From a scientific viewpoint, differences in the internalization of iron particles within the plaques between SPIO and ultra-small SPIO may promote a further understanding of the mechanism underlying carotid plaque formation as well as the possible factors regulating the internalization, such as the degree of permeability, the heterogeneity of phagocytic capability, and the difference in neovascularization within the plaques. In conclusion, the present study seems to contribute to both the establishment of SPIO-enhanced MR imaging to evaluate the unstableness of carotid plaques and the elucidation of plaque formation to develop various plaque stabilization therapies.*

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