Assessment of Inflammatory Burden Contralateral to the Symptomatic Carotid Stenosis Using High-Resolution Ultrasmall, Superparamagnetic Iron Oxide–Enhanced MRI

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Background and Purpose—It is well known that the vulnerable atheromatous plaque has a thin, fibrous cap and large lipid core with associated inflammation. This inflammation can be detected on MRI with use of a contrast medium, Sinerem, an ultrasmall superparamagnetic iron oxide (USPIO). Although the incidence of macrophage activity in asymptomatic disease appears low, we aimed to explore the incidence of MRI-defined inflammation in asymptomatic plaques in patients with known contralateral symptomatic disease.

Methods—Twenty symptomatic patients underwent multisequence MRI before and 36 hours after USPIO infusion. Images were manually segmented into quadrants, and the signal change in each quadrant was calculated after USPIO administration. A mixed mathematical model was developed to compare the mean signal change across all quadrants in the 2 groups. Patients had a mean symptomatic stenosis of 77% compared with 46% on their asymptomatic side, as measured by conventional angiography.

Results—There were 11 (55%) men, and the median age was 72 years (range, 53 to 84 years). All patients had risk factors consistent with severe atherosclerotic disease. All symptomatic carotid stenoses had inflammation, as evaluated by USPIO-enhanced imaging. On the contralateral sides, inflammatory activity was found in 19 (95%) patients. Contralaterally, there were 163 quadrants (57%) with a signal loss after USPIO when compared with 217 quadrants (71%) on the symptomatic side (P=0.007).

Conclusions—This study adds weight to the argument that atherosclerosis is a truly systemic disease. It suggests that investigation of the contralateral side in patients with symptomatic carotid stenosis can demonstrate inflammation in 95% of plaques, despite a mean stenosis of only 46%. Thus, inflammatory activity may be a significant risk factor in asymptomatic disease in patients who have known contralateral symptomatic disease. Patients with symptomatic carotid disease should have their contralateral carotid artery followed up. (Stroke. 2006;37:2266-2270.)

Key Words: atherosclerosis ■ carotid stenosis ■ contrast media ■ inflammation ■ magnetic resonance imaging ■ USPIO

Carotid endarterectomy (CE) has been shown to be beneficial for patients with severe (>70%) symptomatic stenosis. The benefit of surgery is attenuated in patients with moderate or asymptomatic stenosis. The Asymptomatic Carotid Artery Surgery (ACAS) trial showed a statistically significant benefit of surgery for patients with an asymptomatic stenosis >60%, but the number of patients needed to treat to prevent 1 event in 2 years was high, ≈67. Men seem to benefit from surgery only at 5 years (absolute risk reduction, 8%; relative risk reduction, 66%), with women deriving no advantage at all (absolute risk reduction of 1.4% at 5 years; relative risk reduction, 17%). The European Asymptomatic Carotid Surgery Trial (ACST) reported recently and has corroborated the findings of the ACAS trial. The ACAS trial revealed that there is almost certainly a subgroup of asymptomatic patients who harbor vulnerable plaque and may be at increased risk of stroke who would benefit from intervention. In both trials, however, there was very little reference regarding patients with contralateral disease and outcome, whether symptomatic or asymptomatic, although contralateral occlusion was associated with a poorer prognosis.

It has been shown that “vulnerable” atherosclerotic plaque is that which has a thin, fibrous cap, extensive lipid core, and an associated inflammatory infiltrate. In contrast, stable or
“safe” plaque is fibrous, with little lipid and minimal inflammation. These detailed aspects of plaque morphology and physiology have been impossible to detect in vivo until recently.

High-resolution MRI allows accurate quantification of plaque components, thereby improving risk stratification in these patients. The use of Sinerem (Guerbet, Roissy, France), an ultrasmall superparamagnetic iron oxide (USPIO), allows the direct visualization of macrophage infiltration of carotid atheroma in vivo. USPIO is taken up by activated macrophages and, when clumped in the phagolysosome, produces a strong susceptibility effect, visible at 1.5 T in T1-, T2-, and T2*-weighted sequences. In addition, we have observed a T2 shortening effect after USPIO infusion, allowing better visualization of the fibrous cap and suggesting that this contrast medium not only could be used to detect inflammation within vulnerable plaque but also aid in identification of “safer” plaque with a significant fibrous component.

Although the incidence of macrophage activity in asymptomatic disease appears low, we aimed to explore the incidence of MRI-defined inflammation in asymptomatic plaques in patients with known contralateral symptomatic disease.

Subjects and Methods

Twenty patients with symptomatic carotid stenosis, all scheduled for CE, were imaged before and 36 hours after USPIO infusion. Baseline characteristics were collected prospectively and documented in dedicated case report files. Details recorded included a history of cardiovascular and peripheral artery disease, risk factors, and family history of vascular disease. Preoperatively, a blood sample was taken to assess total cholesterol, triglycerides, and HDL.

The USPIO agent (Sinerem, Guerbet, Roissy, France) was supplied as a dry powder and initially made up to a volume of 10 mL with normal saline and given as a slow infusion through an indwelling cleidomastoid muscle in each quadrant was calculated after USPIO administration. Regions of interest showing focal signal drop after USPIO infusion were manually segmented into quadrants with signal enhancement after USPIO infusion than did the contralateral asymptomatic side (0.89 versus 0.61 regions per slice), although this was found not to be statistically significant (P = 0.05).

Pre- and post-USPIO MRI images were manually coregistered according to plaque morphology and distance from the carotid bifurcation. After this, images were manually segmented into quadrants with the use of objective “rules” and therefore, are less susceptible to interobserver error, excluding the luminal blood pool (CMR Tools, London, UK). The signal change normalized to the adjacent stenocleidomastoid muscle in each quadrant was calculated after USPIO infusion. Regions of interest showing focal signal drop after USPIO were also delineated. Conduction of the MRI studies did not cause a delay in surgical intervention in any of the study subjects. This study Committee. All patients gave informed, written consent.

Statistical Methods

As a preliminary analysis, the percentage of quadrants showing a signal decrease on T2*-weighted imaging was calculated for each side, and asymptomatic and symptomatic sides were compared with a paired Student’s t test. The mean number of regions of focal signal drop per slice was calculated for each side in each patient and also compared with a paired t test.

It should be noted, however, that these analyses do not account for any dependence or correlation between multiple measurements made on a single patient. A statistical model was therefore developed that explicitly modeled the correlation between signal loss in different quadrants within a slice and different slices within a plaque. Furthermore, this model also allowed quantification of the mean signal drop with the correct degree of precision and provided a method for testing for differences between groups and controlling the type I error rate.

The actual model used was a repeated-measures mixed model with USPIO-enhanced MRI signal drop as the outcome variable. Side (symptomatic/asymptomatic contralateral) was fitted as a fixed effect and patient as a random effect. Slice and quadrant were used to define the within-patient correlation within each side. The best fitting correlation structure was selected according to the Akaike information criterion. Estimates of signal change within each side were calculated with appropriate 95% CIs. An estimate of the difference between sides with appropriate 95% CI was also calculated, together with a probability value testing the hypothesis of no difference between sides. Model assumptions regarding homogeneity of variance were verified by inspection of residual plots. Distributional assumptions regarding normality were verified by assessment of normal probability plots. The analysis was carried out with PROC MIXED in SAS for Windows, version 8.2 (SAS Institute, Cary, NC).

Results

There were 11 (55%) men, and the median age was 72 years (range, 53 to 84 years). All patients had risk factors consistent with severe atherosclerotic disease. Baseline characteristics are presented in supplemental Table I, available online at http://stroke.ahajournals.org. Patients had a mean asymptomatic stenosis of 77% compared with 46% on their asymptomatic side, as measured by conventional angiography, according to NASCET criteria. The mean time (±SD) from USPIO infusion to CE was 6.9 days (±4.8 days; range, 40 hours to 18 days). Nineteen patients (95%) showed a USPIO signal loss on both the symptomatic and asymptomatic sides (Figure 1).

Symptomatic plaque demonstrated normalized signal loss in significantly more quadrants after USPIO infusion than on the contralateral side (72% of quadrants versus 51%, P = 0.007; supplemental Figure I, available online at http://stroke.ahajournals.org). The contralateral side had more quadrants with signal enhancement after USPIO infusion than did symptomatic plaques (43% versus 29%, P = 0.009; Figure 2). The symptomatic side also showed more signal loss in focal regions of interest than did the contralateral asymptomatic side (0.89 versus 0.61 regions per slice), although this was found not to be statistically significant (P = 0.05).

According to the mixed mathematical model described earlier, the symptomatic plaques showed a significant mean signal intensity decrease of 10.9% ±4.3% after USPIO infusion (P = 0.02). In quadrants that showed signal loss, the mean signal change over all quadrants was significantly greater in the symptomatic group than on the asymptomatic contralateral side (P = 0.0069), with a mean signal difference between the 2 groups of 13.6 ±4.5% (supplemental Figure II, avail-
able online at http://stroke.ahajournals.org). The contralateral plaques showed a mean signal intensity increase (ie, enhancement) after USPIO infusion of 2.7%±4.4% (P=0.55).

**Discussion**

If one is serious about developing optimal management strategies for reducing stroke in the long term (eg, 10 years), simple discrimination between “asymptomatic” and “symptomatic” patients may be inappropriate. There is a need for new adjuncts to risk-stratify patients: patient symptomatology and the degree of luminal narrowing, which have been previously used as clinical grounds for surgical intervention, are no longer appropriate to be considered solely for decision making.

The primary findings of our study are as follows. First, despite a mean contralateral carotid stenosis of 46%, 95% of asymptomatic plaques demonstrated USPIO uptake, suggesting an inflammatory burden within their carotid atheromas bilaterally. This finding highlights the truly systemic nature of vulnerable atheroma. This raises the possibility of whether patients showing inflammatory activity on 1 side may be more likely to have it contralaterally. Truly asymptomatic patients would certainly be an interesting cohort for a future USPIO study. Second, symptomatic carotid plaques showed significantly more inflammatory activity than did the contralateral side, as demonstrated by normalized signal loss in significantly more quadrants and a significant mean signal intensity decrease after USPIO infusion. Finally, the contralateral side had significantly more quadrants with signal enhancement (implying plaque stability) after USPIO infusion than did symptomatic plaques, but there was also a subset of quadrants showing signal loss.

Routine clinical measures of atherosclerotic severity are derived from simple angiographic determinations of lumen narrowing and pay no attention to the morphology, inflammatory infiltrate, and biomechanical stress of the plaque. An additional limitation of angiographic studies is frequent underestimation of the degree of atherosclerotic burden owing to the phenomenon of expansive vascular remodeling. There has been recent interest in the detection of inflammatory activity in vivo in atheroma, denoting plaque vulnerability, by high-resolution carotid MRI. Such imaging allows accurate quantification of carotid plaque components and the experience in patients with matched MR carotid imaging and histology shows a strong correlation between imaging and histopathological assessments of carotid lesions. The use of the USPIO contrast medium, which is taken up by the macrophage population in vivo, has allowed the direct imaging of carotid plaque inflammation at 1.5 T and visible in T₁- and T₂-weighted sequences and on T₂* gradient echo imaging. This has now been validated in high-grade carotid lesions as a means of detecting inflammation with >90% sensitivity.

**Figure 1.** T₁-weighted imaging of the common carotid artery of a patient before and after USPIO infusion. A, Symptomatic side. B, Asymptomatic contralateral side. Focal signal drop is clearly shown bilaterally (white arrows).

**Figure 2.** Pre- (A) and post- (B) USPIO imaging, showing significant fibrous cap enhancement in the postinfusion image in a contralateral asymptomatic carotid artery stenosis (white arrow).
The idea that USPIO may be useful in the assessment of inflammatory activity in atherosclerotic plaques is strongly supported by the endothelial dysfunction theory. This hypothesis assumes that the dysfunctional endothelium represents a key factor contributing to atherosclerosis. Dysfunctional endothelium initiates and sustains an inflammatory reaction within the arterial wall and allows for the accumulation of plasma components, such as LDLs, in the subendothelial intima. Oxidized LDL and its products are then phagocytosed by macrophages via the scavenger receptor pathway, ultimately forming foam cells. USPIO particles of a diameter similar to that of LDL (15 to 25 nm) enter atherosclerotic plaques with increased endothelial permeability and accumulate in plaques with high macrophage content. This hypothesis is supported by experimental investigations in hyperlipidemic rabbits, an atherosclerotic animal model. Double-staining histology and immunohistochemistry have localized USPIO to activated macrophages in the plaque. Histopathology data have shown marked iron uptake in macrophages embedded in atherosclerotic plaques, and electron microscopy has shown multiple (within the phagolysosomes or late endosomes of the macrophage) cytoplasmic iron particles in macrophages. These changes were not observed in animals that did not receive USPIO. It was concluded herein that USPIO is phagocytosed by macrophages in atherosclerotic plaques of the aortic wall of hyperlipidemic rabbits in a quantity sufficient to cause susceptibility artifact detectable by MRI. These data suggested an uptake of intravenously administered USPIO particles by macrophages of atherosclerotic lesions that were identified on MRI images by a pronounced signal loss of the arterial wall.

The role of surveillance of the contralateral carotid artery remains unclear. It was previously argued that the onset of symptoms preceded recognition of disease progression on each occasion and that none of the observed strokes could have been prevented by postoperative surveillance. However, contralateral surveillance in those studies was performed with either carotid angiography or duplex ultrasonography, which are unable to visualize plaque morphology or fully identify the vulnerable plaque. Natural history suggests that contralateral plaques have a 1% risk per year of ischemic sequelae. Although the long-term risk of stroke in the nonoperated internal carotid artery territory appears small, carotid artery disease progression was common in 7% to 26% of patients. Today, with improving imaging and a better understanding of the atherosclerotic process, disease progression could be better monitored and the risk of stroke predicted. It is worth noting that in these previous studies, there were a substantial number of patients who were lost to follow-up because they were either too frail, had too far to travel, or died of 1 of their other comorbidities, most often as a result of myocardial infarction. USPIO-enhanced MRI may prove to be a useful method of risk-stratifying patients with carotid atheroma in the future and may provide a means of disease surveillance for the contralateral carotid artery.

Although the absolute normalized signal showed mean enhancement in the contralateral group, there was a subset of quadrants showing signal drop. Although it is likely that this was due to macrophage accumulation within the plaque, a dysfunctional and leaky endothelium may also play a role, along with increased plaque perfusion via the vasa vasorum. The reality may well be that all of these factors play some role.

Quantification of macrophage burden by USPIO signal change is difficult. There is likely to be a nonlinear relation between signal loss on $T_2^*$-weighted imaging and overall macrophage burden. One reason for this is that clustering of iron oxide particles in tissue leads to additional $T_2^*$ shortening and the “blooming effect,” where protons some distance away from the iron oxide particle are dephased. There is, however, a suggestion that the inflammatory burden of the atheroma of the symptomatic side is greater than that of the contralateral side, which would agree with the relative risk of an event from the 2 sides as described in the literature. Despite an otherwise “stable” morphology on MRI (ie, thick fibrous cap, small lipid pool, absence of plaque ulceration and hemorrhage), a number of plaques demonstrated significant USPIO uptake, suggesting that it is this subset of patients that may need to be followed up more closely and may eventually require intervention.

Enhancement (Figure 2) may be related to low concentrations of USPIO within the plaque (as $T_1$ increases at low concentrations before a $T_2^*$ effect produces an exponential drop in signal at higher concentrations). Because there is a lack of phagocytic activity in these plaques, it is likely that USPIO concentrations locally will be reduced and little clumping of particles will occur in phagolysosomes. Although there are areas of signal loss, contralateral asymptomatic enhancement may be related to plaque stability and/or fibrous cap thickness. Thus, despite a possibly intense inflammatory milieu within the plaque, a thick fibrous cap reduces its chances of rupture. It is possible that this may go some way to explaining the relatively low event rate from the contralateral side.

One of the limitations of the study is that there was, by design, no corroborative evidence with histology from the contralateral side. Trivedi et al. have demonstrated that there is a good correlation between USPIO uptake on MRI and USPIO staining (Perls staining) of activated macrophages within the plaque. The relation between signal drop and macrophage number still remains unclear. Furthermore, we do not know whether the vasa vasorum play a role in USPIO uptake and macrophage infiltration. Although we have found the technique to be highly reproducible, numbers are still small who have had multiple USPIO infusions.

For the time being, however, we would suggest that patients who have a symptomatic carotid stenosis and who are found to have contralateral disease should be considered for close follow-up, possibly with a low threshold for intervention. If further validated by larger studies, USPIO-enhanced MRI may play a role in the future in the improved risk stratification of patients with asymptomatic carotid lesions.

Conclusions

USPIO-enhanced MRI is a promising noninvasive method to identify high-risk atheromatous plaques, of which inflammation plays a significant role. Contralateral asymptomatic plaques also seem to demonstrate inflammation but not to the
extent of the symptomatic side. Inflammatory activity may be a significant risk factor in asymptomatic disease. Neither the ACST nor ACAS trials were able to answer the question as to whether asymptomatic patients with 50% to 69% stenosis would benefit from surgical intervention. USPIO imaging may be a useful marker in the selection of patients eligible for C, if plaque inflammation can be correlated with the risk of developing clinical symptoms. A prospective study correlating future ischemic events with inflammatory plaque activity will be necessary to confirm this hypothesis.

Sources of Funding
This study was supported by GlaxoSmithKline and the Stroke Association.

Disclosures
S.R.M. and A.P.B. are employees of GlaxoSmithKline (GSK). J.H.G. is a GSK paid consultant. The remaining authors have no competing interests to disclose.

References
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Stroke. 2006;37:2266-2270; originally published online August 17, 2006;
doi: 10.1161/01.STR.0000236063.47539.99

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/37/9/2266