Sustained Acceleration in Carotid Atherosclerotic Plaque Progression With Intraplaque Hemorrhage

A Long-Term Time Course Study

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OBJECTIVES This study sought to determine the immediate and long-term effects of intraplaque hemorrhage (IPH) on plaque progression in the carotid artery.

BACKGROUND Previous studies have associated IPH in the carotid artery with more rapid plaque progression. However, the time course and long-term effect remain unknown. Carotid magnetic resonance imaging is a noninvasive imaging technique that has been validated with histology for the accurate in vivo detection of IPH and measurement of plaque burden.

METHODS Asymptomatic subjects with 50% to 79% carotid stenosis underwent carotid magnetic resonance imaging at baseline and then serially every 18 months for a total of 54 months. Subjects with IPH present in at least 1 carotid artery at 54 months were selected. Subsequently, presence/absence of IPH and wall volume were determined independently in all time points for both sides. A piece-wise progression curve was fit by using a linear mixed model to compare progression rates described as annualized changes in wall volume between periods defined by their relationship to IPH development.

RESULTS From 14 subjects who exhibited IPH at 54 months, 12 arteries were found to have developed IPH during the study period. The progression rates were $-20.5 \pm 13.1$, $20.5 \pm 13.6$, and $16.5 \pm 10.8$ mm$^3$/year before, during, and after IPH development, respectively. The progression rate during IPH development tended to be higher than the period before ($p = 0.080$) but comparable to the period after ($p = 0.845$). The progression rate in the combined period during/after IPH development was $18.3 \pm 6.5$ mm$^3$/year, which indicated significant progression ($p = 0.008$ compared with a slope of 0) and was higher than the period before IPH development ($p = 0.018$). No coincident ischemic events were noted for new IPH.

CONCLUSIONS The development of IPH posed an immediate and long-term promoting effect on plaque progression. IPH seems to alter the biology and natural history of carotid atherosclerosis. Early identification of patients with IPH may prove invaluable in optimizing management to minimize future sequelae. (J Am Coll Cardiol Img 2012;5:798–804) © 2012 by the American College of Cardiology Foundation

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There is increasing evidence that intraplaque hemorrhage (IPH) is associated with high-risk atherosclerotic carotid plaque. Prospective studies have identified a compelling link between the presence of IPH at baseline and the development of future ischemic cerebrovascular events in both previously asymptomatic and symptomatic individuals (1–3). IPH has also been associated with a more rapid growth of the lipid-rich necrotic core and accelerated progression in plaque burden that seem to induce luminal narrowing regardless of initial stenotic severity (4,5). However, the time course of these deleterious effects remains undefined.

Understanding the immediate and long-term implications of IPH is particularly compelling. IPH has been identified across a wide spectrum of stenosis and/or plaque burden (6–8). The acceleration of plaque growth on IPH development, if visualized in the same artery, will support IPH as a direct promoter for plaque progression. The duration of its effects, however, may primarily determine the clinical significance of IPH, especially in those lesions without luminal stenosis and with minimal plaque burden (6,7).

In this study, our goal was to determine the time course of effects of IPH in the carotid artery. We designed a retrospective study consisting of participants enrolled in an ongoing natural history study of carotid atherosclerotic disease imaged every 18 months for 54 months with carotid magnetic resonance imaging (MRI). Carotid MRI is a noninvasive imaging technique validated with histology for the accurate in vivo detection of IPH and has become an established approach for identifying IPH during cross-sectional and longitudinal studies (9–11).

**METHODS**

**Study sample.** As part of a natural history study of carotid atherosclerotic disease, participants were recruited from the diagnostic vascular ultrasound laboratories at the University of Washington Medical Center, Veterans Affairs Puget Sound, and the Virginia Mason Medical Center with the following inclusion criteria: 1) 50% to 79% stenosis in at least 1 carotid artery; and 2) no cerebrovascular symptoms in the 6 months before enrollment. Study procedures and consent forms were reviewed and approved by the institutional review board at each site. Written informed consent was obtained before enrollment.

Subjects underwent carotid MRI at baseline and then serially every 18 months for a total of 54 months. Routine medical care was directed by the subjects’ primary care physicians during the entire study duration. At the baseline MRI, a standardized health questionnaire was completed and physical examination was performed. Regular follow-ups were conducted every 3 months by telephone interview to record recent cerebrovascular events, occurrence of carotid endarterectomy (CEA), and current medications. Subjects who provided a history suggestive of an ischemic event during the telephone interviews were evaluated and confirmed by the study neurologist.

Imaging criteria for study inclusion were: 1) 54 months of imaging follow-up; and 2) presence of IPH (see the Image Review section for criteria to determine presence/absence of IPH) in at least 1 carotid artery at the most recent scan. Exclusion criteria were: 1) history of CEA; and 2) image quality <2 (see the Image Review section for description of image quality).

**MRI protocol.** All images were acquired on a 1.5-T scanner (Signa Horizon EchoSpeed, GE Medical Systems, Milwaukee, Wisconsin) by using phased-array surface coils (Pathway MRI, Seattle, Washington). A standardized carotid MRI protocol was used to acquire multicontrast, cross-sectional images (three-dimensional time-of-flight [TOF], T1-weighted [T1w], proton density–weighted [PDw], and T2-weighted [T2w]) centered at the bifurcation of the carotid artery with 50% to 79% stenosis, referred to as the index artery, as previously described (12,13). When both arteries demonstrated 50% to 79% stenosis at baseline, 1 artery was randomly chosen as the index artery for centering of coverage during image acquisition. During subsequent acquisitions, the index artery was always used for centering unless a CEA was performed on the index artery, in which case centering of coverage was switched to the contralateral side. Images were obtained with a field-of-view of 13 to 16 cm, matrix size of 256 × 256, and 2-mm slice thickness. There was 1 mm of overlap between adjacent slices for TOF, and no interslice spacing for other sequences. Scan coverage was 40 mm for TOF, 20 to 24 mm for T1w, and 30 mm for PDw and T2w. Total acquisition time was approximately 30 min.

**Image review.** All image interpretation was performed using a custom-designed image analysis software package (CASCADE, Seattle, Washington).
(14) by researchers trained in carotid MRI and blinded to clinical data. Image quality was rated per scan on a 4-point scale (1 = poor, 4 = excellent), consistent with previous studies (15). Scans with image quality <2 were excluded. Different weightings were registered during image review using the carotid bifurcation as a reference. Scans without a carotid bifurcation within the field-of-view were excluded.

The presence/absence of IPH in either carotid at the final (54 months) time point (TP) was determined by a hyperintense signal on T1w and TOF images consistent with the presence of IPH as previously validated with histology at 1.5-T (9). Participants with IPH at the 54-month TP in at least 1 carotid artery were selected for additional image review that included both carotid arteries (i.e., IPH arteries and their contralateral arteries) and also included all previous TPs (TP1 = baseline scan, TP2 = 18 months, TP3 = 36 months, TP4 = 54 months). Multiple scans were matched, blinded to time sequence, using the carotid bifurcation for each participant. Only slices covered at all TPs were used for analysis. Wall volume and presence of IPH were determined independently at all TPs. PDw/T2w images were used to measure wall volume blinded to presence/absence of IPH. TOF/T1w images were used to determine presence/absence of IPH blinded to the results from wall volume measurements.

**Statistical analysis.** Summary statistics of baseline demographics are presented as mean ± SDs for continuous variables and count with percentage for categorical variables. Common coverage across multiple serial scans is presented as range with median for both TOF/T1w and PDw/T2w series. Serial data on IPH status were used to identify arteries with new IPH. Wall volume was calculated by summing wall areas on matched slices multiplied by slice thickness, which was then normalized for varying numbers of slices to yield a volume for 10 slices (wall volume/number of slices × 10 slices) (4).

In arteries with new IPH during the study period, the interscan interval when new IPH occurred was defined as the IPH-new period. Periods before and after the IPH-new period were defined as the IPH-absent and IPH-present periods, respectively. To compare progression rates between the 3 periods, and between the IPH-absent and the combined IPH-new/IPH-present period, a linear mixed model approach was used (16). After centering the scan times around the occurrence of IPH (time = 0 when IPH was first observed), a piecewise linear curve was fit between wall volume and time, with knots set at boundaries between periods. A random intercept was included in the model to account for the dependence between multiple scans of the same artery. Slopes ± SEs of the piecewise linear curve correspond to progression rates in wall volume during different periods, which were compared with 0 and with each other by using Wald tests.

For the other arteries that did not show new IPH during the study period, the same linear mixed model approach was used to estimate their long-term average progression rates by fitting a single line over the entire study period (16).

All data analysis was performed by using R version 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as p < 0.05.

**RESULTS**

Fourteen subjects with 54-month follow-up had imaging evidence of IPH in at least 1 carotid artery at their most recent scan. Baseline demographic characteristics are shown in Table 1. In these 14 participants (n = 28 carotid arteries), there were 17 carotid arteries with IPH present on MRI, 10 carotid arteries without IPH on MRI, and 1 artery was excluded due to CEA during the 54-month study period. The arteries at 54 months with (n = 17) and without (n = 10) IPH were selected to have their previous time point scans analyzed. Accordingly, 108 scans (27 arteries × 4 time points) were available for review.

During the review of TOF/T1w series for IPH, 1 (3.7%) of the 27 arteries was excluded due to lack of coverage of the carotid bifurcation at TP1 and TP2. The remaining 26 arteries had serial data on

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<th>Table 1. Clinical Characteristics at Baseline (N = 14)</th>
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Values are mean ± SD or n (%). No subject was taking anticoagulant agents at enrollment. *All subjects with hypertension and diabetes were receiving corresponding medications at enrollment. CHD = coronary heart disease.
IPH status at all 4 TPs. The common coverage of TOF/T1w images across 4 scans ranged from 10 to 20 mm (median 16 mm). During the review of PDw/T2w series for wall volume measurements, 16 (14.8%) scans were excluded due to poor image quality. This left 26 arteries with wall volume measurements at 2 to 4 TPs. The common coverage of PDw/T2w images ranged from 10 to 28 mm (median 24 mm).

Occurrence of IPH. Of the 26 arteries with serial data on IPH, 4 (15.4%) had IPH present at all TPs (IPH-present group; Fig. 1); 12 arteries (46.2%) did not have IPH at the baseline scan and had IPH on all subsequent scans after the occurrence of IPH (IPH-new group; Fig. 2); 10 arteries (38.5%) did not have IPH at any TPs (IPH-absent group).

Resolution of IPH was not observed. Of the 12 arteries with new IPH, 10 (83.3%) were being treated with statins when IPH occurred (Fig. 3).

Long-term effects of IPH. The annualized change in wall volume during the IPH-absent period was $-20.5 \pm 13.1 \text{ mm}^3/\text{year}$ ($p = 0.129$ compared with a slope of 0 [Fig. 4]). Of the 7 arteries with an IPH-absent period, 6 (85.7%) were being treated with statins, and in 1 case, statin therapy was initiated during this period (Fig. 3). During the 18-month IPH-new period, the annualized change in wall volume was $20.5 \pm 13.6 \text{ mm}^3/\text{year}$ ($p = 0.141$ compared with a slope of 0 [Fig. 4]). Nine (81.8%) of the 11 arteries with an IPH-new period were on statin therapy during this period. The annualized change in wall volume in the IPH-present period was $16.5 \pm 10.8 \text{ mm}^3/\text{year}$ ($p = 0.138$ compared with a slope of 0 [Fig. 4]).

Figure 1. A Representative Case of Arteries With IPH at All Timepoints
White arrows indicate the presence of intraplaque hemorrhage (IPH) at all time points (TPs) shown as bright areas on time-of-flight (TOF) images. L = lumen of internal carotid artery; T1w = T1-weighted.

Figure 2. A Representative Case of Arteries With Incident IPH
White arrows indicate the presence of IPH at TP3/TP4, shown as bright areas on TOF images. The IPH signal was absent at TP1/TP2. Abbreviations as in Figure 1.
(75.0%) of the 8 arteries with an IPH-present period were being treated with statins, and in 1 case, statin therapy was initiated during this period (Fig. 3). The annualized change in wall volume during the IPH-new period tended to be greater than that during the IPH-absent period ($p = 0.080$ for difference of slope) but comparable to that during the IPH-present period ($p = 0.845$ for difference of slope). The annualized change in wall volume during the IPH-new/IPH-present period was $18.3 \pm 6.5$ mm$^3$/year ($p = 0.008$ compared with a slope of 0), which is significantly higher than that during the IPH-absent period ($p = 0.018$ for difference of slope).

The average progression rates over the entire study period of IPH-present and IPH-absent arteries were $34.2 \pm 9.0$ mm$^3$/year ($p = 0.004$ compared with a slope of 0) and $5.3 \pm 7.2$ mm$^3$/year ($p = 0.466$ compared with a slope of 0), respectively. All IPH-present arteries were being treated with statins during the study period of IPH-present and in 1 case, statin therapy was initiated before its TP2 scan.

All 14 subjects with IPH at TP4 remained clinically asymptomatic during the entire study period.

**DISCUSSION**

In this study using serial images of the in vivo carotid artery over a 54-month period, the development of IPH was found to be associated with an immediate long-term acceleration of plaque progression compared with the period before. These observations expand our understanding of the potentially central role that IPH contributes to carotid atherosclerotic disease in 2 important ways. First, acceleration of plaque growth was seen coincidently with new IPH, suggesting IPH is a direct promoter rather than a bystander in plaque progression. Second, the accelerating effects of IPH did not resolve after an extended period of observation. These findings substantiate previous animal studies (17) and human studies of a shorter duration (4,5), which conjectured that the presence of IPH may fundamentally alter the biology of atherosclerotic disease. Therefore, the early identification of patients with IPH regardless of stenotic severity or plaque burden may prove invaluable in optimizing management to minimize future sequelae.

Although previous studies have identified an association between IPH and stroke (1–3,18), the results have largely been presented in the form of...
increased event risk. In the current study, no coincident clinical symptoms were noted for new IPH. The observation is supported by histopathologic studies that have identified neovasculature originating from the adventitia as a potential source of IPH (19,20), which enables the development of IPH without disruption of the plaque surface. Collectively, it is implied that the identification of IPH may stratify patients with an advanced rate of plaque growth, but additional features such as surface disruption may need to be identified to better delineate patients at greatest risk for stroke (21,22). Nevertheless, due to the small sample size in this study, whether the occurrence of new IPH is directly linked with cerebrovascular events should be investigated further.

Statin therapy improves prognosis of cardiovascular disease and has been shown to decrease plaque burden in multiple imaging studies (23–25). We found that 12 arteries developed new IPH during the study period despite the fact that 10 (83.3%) of them were being treated with statin therapy when IPH occurred. The 4 arteries with IPH present throughout the 54-month study period were progressing despite statin therapy, as did the 12 arteries with new IPH after IPH developed. These findings suggest that statin therapy alone may be insufficient to prevent arteries from developing IPH and that the effects of IPH may outweigh statin therapy in determining plaque progression. Further studies are needed to verify these observations in clinical trials.

Methemoglobin from hemoglobin breakdown after erythrocyte extravasation has been proposed to account for the high signal intensity of IPH. However, the magnetic resonance signal of IPH is distinctively durable compared with hemorrhage in other pathological settings (26,27). Using a T1w sequence for IPH detection, Yamada et al. (18) reported that only 1 of 30 high signal intensity carotid arteries changed to low signal intensity during a median interval of 279 days (range 10 to 1,037 days). Two other studies using combined TOF and T1w sequences for IPH detection did not report any disappearance of IPH over an 18-month period (4,5). Although the current study was not designed to study the resolution of IPH, we found that the hyperintense signal of IPH could last for as long as 54 months. One possible explanation for this phenomenon is that erythrocytes may continue to extravasate from leaky vessels once IPH occurs (4,20). Alternatively, the intraplaque environment may be unique and yield an atypical degradation of hemoglobin. Regardless, hemorrhage as detected by MRI clearly persists in carotid atherosclerotic disease, and further studies targeted at understanding this unique occurrence may benefit the understanding of the atherosclerotic disease process.

Study limitations. This study was limited by its small sample size, which was primarily because the design required that study participants not only have 4 serial scans using the same carotid imaging protocol but also exhibit IPH on the last scan. However, this is the first study, to the best of our knowledge, to serially monitor at regular intervals the natural history of atherosclerosis over an extended period. In so doing, we found that the common coverage of 4 serial scans was generally smaller compared with each individual scan alone but was comparable to previous longitudinal studies that used only 2 TP scans (5,28). In addition, the available coverage was sufficient to cover lesions at the carotid bifurcation, as is consistent with previous reports (8). This study thus provides compelling evidence that monitoring plaque progression by using serial imaging with a validated MRI protocol is a promising approach for long-term, large-scale studies of in vivo human atherosclerosis.
CONCLUSIONS

MRI monitoring of carotid arteries over a 54-month period demonstrated the development of IPH and the concurrent acceleration of plaque growth despite a high prevalence of statin therapy in affected arteries. IPH seems to fundamentally alter the biology and natural history of carotid atherosclerosis.

REFERENCES


Key Words: atherosclerosis ■ carotid arteries ■ intraplaque hemorrhage ■ magnetic resonance imaging.