Adventitial Perfusion and Intraplaque Hemorrhage
A Dynamic Contrast-Enhanced MRI Study in the Carotid Artery

Jie Sun, MD*; Yan Song, MD, PhD*; Huijun Chen, PhD; William S. Kerwin, PhD; Daniel S. Hippe, MS; Li Dong, MD; Min Chen, MD, PhD; Cheng Zhou, MD; Thomas S. Hatsukami, MD; Chun Yuan, PhD

Background and Purpose—Autopsy studies have suggested a relationship between intraplaque hemorrhage (IPH) and vasa vasorum, which arise primarily from the adventitia. Adventitial vasa vasorum can be characterized in the carotid arteries by estimating perfusion parameters via dynamic contrast-enhanced MRI. The purpose of this investigation was to use dynamic contrast-enhanced MRI to test in vivo in a clinical population whether adventitial perfusion, indicative of vasa vasorum microstructure, is associated with IPH.

Methods—Symptomatic patients with carotid plaque ipsilateral to the ischemic event underwent bilateral carotid artery MRI examination, which included multicontrast sequences for detecting IPH and a dynamic contrast-enhanced MRI sequence for characterizing adventitial perfusion. Kinetic modeling of the dynamic contrast-enhanced MRI time series was performed to estimate adventitial $v_p$ (fractional plasma volume, reflecting local blood supply) and $K^{trans}$ (transfer constant, reflecting vessel surface area, and permeability).

Results—From the 27 patients (22 men; 69±10 years of age) recruited, adventitial perfusion parameters were obtained in 50 arteries. The presence of IPH was associated with a significantly higher value in adventitial $K^{trans}$ (0.142±0.042 vs 0.112±0.029 min$^{-1}$; $P<0.001$) but not in $v_p$ (0.163±0.064 vs 0.149±0.062; $P=0.338$). This relationship remained after adjusting for symptomatic status, degree of stenosis, and other confounding factors.

Conclusions—This study demonstrated an independent pathophysiological link between the adventitia and IPH and related it to the microstructure of adventitial vasa vasorum. Adventitial perfusion imaging may be useful in studying plaque pathogenesis, but further examination through prospective studies is needed.

Key Words: carotid artery ▪ hemorrhage ▪ MRI ▪ vasa vasorum

Intraplaque hemorrhage (IPH) has emerged as a key feature to further define the severity of carotid atherosclerosis beyond luminal stenosis. It contributes to atherosclerotic plaque progression and has been associated with cerebrovascular ischemic events in prospective cohort studies. Understanding the pathophysiology of IPH has important clinical implications given that the noninvasive detection of IPH can now be achieved using MRI.

In contrast to the accumulating evidence on its clinical correlates, the biology of IPH remains poorly understood. Autopsy studies examining IPH and angiogenesis have shown the colocalization of IPH with neovessels in both intima and adventitia, yet their exact relationship with IPH is unclear and has not progressed beyond hypothesis. The prevailing viewpoint considers these leaky vessels as the origin of IPH. Alternatively, the occurrence of IPH, by aggravating hypoxia in the intima, may promote angiogenesis in the adventitia. Highly clinically relevant is whether there is a resulting vicious cycle between IPH and adventitial angiogenesis, leading to poor outcome without intervention. As such, in vivo human studies via imaging are imperative to gain concrete evidence on disease mechanisms related to IPH, and to assess whether the adventitial vasa vasorum (VV) is a reasonable biological target to study.

Recent studies measuring adventitia thickness in human carotid arteries indicate that imaging modalities with submillimeter spatial resolution can reliably visualize the adventitia in extracranial carotid arteries. Indeed, previous studies using MRI have shown enhancement of the adventitia after contrast injection. Furthermore, the feasibility of applying dynamic contrast-enhanced MRI (DCE-MRI) with kinetic modeling to study adventitial perfusion has been shown, which generates physiological parameters of VV. In this study, we examined adventitial perfusion in a clinical population by using DCE-MRI with the objective to test whether perfusion parameters of adventitial VV, indicative of their microstructure, are associated with IPH.
Methods

Study Population
All study procedures were approved by the institutional review board. Patients with a history of transient ischemic attack or ischemic stroke in the distribution of carotid arteries within the past 6 months were recruited with informed consent from the Departments of Neurology and Neurosurgery at the Beijing Hospital if plaque was identified by ultrasound in the carotid artery ipsilateral to the ischemic event. Patients with atrial fibrillation or intracranial carotid stenosis as well as those with any contraindication for MRI or contrast injection (eg, pace-maker, claustrophobia, renal insufficiency) were not included.

Radiologists measured carotid stenosis from MR angiography according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria and categorized degree of stenosis as <16%, 16% to 49%, 50% to 69%, or ≥70%. Referring clinicians collected patient demographics and clinical risk factors. Lipid profiles were also recorded, if available, before MRI.

Carotid MRI Protocol
All patients were scanned using a 3T scanner (Achieva, Philips, the Netherlands) and phased-array carotid surface coils (Chenguang Inc, Shanghai, China). A previously published multicontrast protocol was used for IPIH detection (3-dimensional time-of-flight, MP-RAGE [magnetization-prepared rapid acquisition gradient-echo], and turbo spin-echo based T1-, proton-density- and T2-weighted sequences). All images shared the following parameters: field-of-view=14x14 cm, matrix=256x256, slice thickness=2 mm, and no interslice gap.

A 2-dimensional spoiled gradient recalled echo sequence was performed for DCE-MRI with the following parameters: repetition time/echo time=115/4.6 ms, flip=50°, time interval of 14 seconds. Coincident with the second acquisition, 0.1 mmol/kg of gadopentetate dimeglumine (Magnevist, Bayer, Germany) was injected at a rate of 0.7 mL/s by a power injector.

Image Analysis
Images were analyzed using a custom-designed image analysis software package (CASCADE, University of Washington, Seattle, WA). Bright-blood and black-blood images were used in combination to determine the lumen and outer wall boundaries. A hypertensive signal within the wall area on MP-RAGE was considered to be IPIH. DCE-MRI analysis was performed using a kinetic modeling approach. Briefly, a 2-compartment kinetic model focusing on the influx of contrast agent from plasma to tissue is used. Contrast concentration in the tissue will then depend on 2 physiological parameters: the fractional plasma volume ($v_p$) and the transfer constant ($K_{trans}$). $K_{trans}$ is the mathematical product of endothelial surface area and permeability, whereas $v_p$ reflects local blood supply.

$$C(t) = v_p C_p(t) + K_{trans} \int_0^t C_p(\tau) d\tau$$

where $C_p$ and $C_t$ are contrast concentrations in the tissue and plasma, respectively, $C_p$ and $C_t$ are estimated over time by changes in signal intensity during dynamic imaging in the tissue and plasma to solve for $K_{trans}$ and $v_p$. A linear relationship is assumed between contrast concentration and signal intensity.

DCE-MRI time series were processed to produce a color-coded parametric map (VV image) for each slice that shows $K_{trans}$ in green and $v_p$ in red. The lumen boundary was processed around the area of high $v_p$ defining the lumen. The outer wall boundary was placed to coincide with the rim of high $K_{trans}$ defining the adventitia (Figure I in the online-only Data Supplement). Adiventitial $K_{trans}$ and $v_p$ were computed per slice by averaging all pixels within 0.625 mm (1 pixel) of the outer wall boundary and over a region of the artery with plaques (defined as having a wall thickness in excess of 1.5 mm). The maximum value across all slices was then calculated for each artery.

Statistical Analysis
Data were presented as means±SD, median [interquartile range], or count (percentage) as appropriate. The prevalence of IPIH was compared between symptomatic and asymptomatic sides using the McNemar test. Associations between IPIH and clinical risk factors were assessed and tested using logistic regression with a random intercept (generalized linear mixed model) to account for the pairing of arteries. Similarly, linear mixed models were used to assess associations of the continuous perfusion parameters ($K_{trans}$ and $v_p$) with IPIH and other risk factors. To examine the independent association between adventitial perfusion and IPIH, a multivariate model was fitted, including risk factors that were associated with either adventitial perfusion or IPIH. Additionally, Pearson correlation coefficient ($r$) was used to summarize the correlation between adventitial $K_{trans}$ and $v_p$ of the same artery. All data analysis was performed using R 2.14.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as $P<0.05$.

Results

Patient Characteristics
A total of 27 patients were recruited, and their clinical characteristics are summarized in Table 1. All patients were affected unilaterally, with a contralateral asymptomatic artery. Eleven (40.7%) patients had a diagnosis of transient ischemic attack, and others had ischemic stroke. The time interval between carotid MRI and the most recent event was 16.3±18.7 days (range, 1–60 days).

IPH was detected in 12 (44.4%) arteries on the symptomatic side compared with 4 (14.8%) arteries on the asymptomatic side ($P=0.022$ by McNemar test). Additionally, the prevalence of IPIH increased with increasing degrees of stenosis ($P=0.005$; Table 1)

<table>
<thead>
<tr>
<th>Median (IQR) or n (%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>67 [60–77]</td>
</tr>
<tr>
<td>Male sex</td>
<td>22 (81.5)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.1 [23.3–27.0]</td>
</tr>
<tr>
<td>Current smoker</td>
<td>20 (74.1)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14 (51.9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>17 (63.0)</td>
</tr>
<tr>
<td>Lipid profile*</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.5 [3.7–4.9]</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.7 [2.2–3.3]</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.9 [0.8–1.1]</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.6 [1.3–2.2]</td>
</tr>
<tr>
<td>Degree of stenosis on symptomatic side</td>
<td></td>
</tr>
<tr>
<td>0% to 15%</td>
<td>5 (18.5)</td>
</tr>
<tr>
<td>16% to 49%</td>
<td>10 (37.0)</td>
</tr>
<tr>
<td>50% to 69%</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>70% to 99%</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>History of coronary artery disease</td>
<td>11 (40.7)</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>15 (55.6)</td>
</tr>
</tbody>
</table>

HDL-C indicates high-density lipoprotein cholesterol; IQR, interquartile range; and LDL-C, low-density lipoprotein cholesterol.

*Lipid profile was available in 20 (74.1%) patients.
Adventitial Perfusion and IPH

DCE-MRI was successfully performed in all patients. One subject with poor image quality attributable to gross movement was excluded from kinetic modeling. In addition, 2 arteries from separate individuals had no adventitial perfusion readouts because no plaque was detected (wall thickness <1.5 mm). The remaining 50 arteries were used in data analysis. Both adventitial $K^{\text{trans}}$ (0.121±0.036 min$^{-1}$; range, 0.044–0.231 min$^{-1}$) and $v_p$ (0.153±0.062; range, 0.002–0.282) were widely distributed, with a positive correlation to each other ($r=0.45; P=0.001$) in the whole study sample.

The presence of IPH was associated with significantly higher adventitial $K^{\text{trans}}$ (0.142±0.042 vs 0.112±0.029 min$^{-1}$, $P<0.001$; Figure 2A). Adventitial $v_p$ was higher in arteries with IPH compared with arteries without IPH, but this difference was not statistically significant (0.163±0.064 vs 0.149±0.062, $P=0.338$; Figure 2B). The enhancement process of adventitia in a representative case with IPH is shown in Figure 3.

Independent Pathophysiological Link Between Adventitial Perfusion and IPH

Among clinical risk factors, symptomatic status, degree of stenosis, and male sex were significantly associated with adventitial $K^{\text{trans}}$ (Table 2). In multivariate analysis, arteries with IPH still had significantly higher adventitial $K^{\text{trans}}$ after adjusting for these factors ($P=0.018$; Table 3). Although adventitial $v_p$ was not significantly associated with IPH, it was associated with symptomatic status, body mass index, and LDL-C (Table 2).

Discussion

To our knowledge, this is the first in vivo study to examine adventitial perfusion in relation to IPH in a clinical population. Of the 2 physiological parameters studied, $K^{\text{trans}}$ is the mathematical product of endothelial surface area and permeability, whereas $v_p$ reflects regional blood supply. Arteries with IPH showed increased $K^{\text{trans}}$, compared with those without IPH, indicative of greater vessel surface area, more leaky vessels, or both. This finding remained after considering confounding factors. Therefore, the present study supports a pathophysiological link between the adventitia and IPH, and relates it to the microstructure of adventitial VV.

There is continuing debate on the origin of IPH and its atherogenic mechanisms attributable to the lack of animal models of spontaneous IPH, and the inability to image relevant biological targets in humans. However, the latter may have changed given recent advances in contrast-enhanced ultrasound and MRI. Kinetic modeling differentiates physiological parameters that present unique information on adventitial VV by reflecting local vascular volume, vessel surface area, and permeability. Association between those parameters and IPH has not been established previously but may shed light on the pathophysiological role of VV and establish the ground for larger, prospective studies.

In this study, it is our hypothesis that adventitial perfusion in arteries with IPH differs from those without IPH as a result of changes in the microstructure of adventitial VV. Previous knowledge on the relationship between VV and IPH is mainly from autopsy studies and has been limited to observation of their colocalization. Our findings in adventitial perfusion not only demonstrate in vivo that arteries with and without IPH are different in terms of VV, but they also provide additional insights on the microstructure of adventitial VV. Specifically, the observed high value in adventitial $K^{\text{trans}}$ implies an increase in endothelial surface area, vessel permeability, or both, in arteries with IPH. In contrast, the increase in blood supply, as quantified by adventitial $v_p$, was slight and did not reach statistical significance. The increase in endothelial surface area without a parallel increase in blood supply may indicate the proliferation of capillaries/terminal arterioles during angiogenesis because terminal vessels tend to primarily expand surface exchange area without significant contribution to vascular volume. It is also possible that VV in arteries with IPH
are more leaky because of impaired vessel wall integrity. In an autopsy study, Sluimer et al found that adventitial microvessels were thin walled, with infrequent mural cells and compromised endothelial integrity. Although the study did not look at IPH specifically, it did provide histopathologic evidence of increased vessel permeability. Given that angiogenesis and increased vessel permeability are closely related biological processes, both mechanisms may be involved to account for the high adventitial $K_{trans}$.

Cardiovascular risk factors are known to be associated with the presence of IPH. In our study, IPH was more prevalent on the symptomatic side and associated with increasing carotid stenosis and age, consistent with previous studies using histology or MRI. On the other hand, associations of cardiovascular risk factors with VV are less studied. Because both IPH and VV are implicated in atherogenesis and clinical complications, it is critical to consider potentially confounding effects from commonly associated risk factors in the present study. Indeed, previous studies in which adventitial VV was qualitatively or semiquantitatively evaluated suggested the association between adventitial VV and clinical presentation. Our finding that adventitial $v_p$ was higher on the symptomatic side appears to agree with contrast-enhanced ultrasound. But direct comparison between the 2 approaches has yet to be performed. Nonetheless, adjusting for these confounding factors reinforces the independent link between adventitial perfusion and IPH.

### Study Limitations

Findings from this study provide proof-of-concept for the independent association between adventitial perfusion and IPH. However, further studies will be needed to elucidate the important time sequence between increase in adventitial perfusion, IPH occurrence, and plaque progression. Notably, it appears feasible to perform prospective longitudinal studies to address those questions given that both IPH and adventitial perfusion can be assessed in vivo. Although our study focused on the adventitia, it cannot exclude the possibility of IPH coming from the lumen (eg, microdissection). Histological or imaging studies that summarize the extent and relative location of hemorrhage in a large sample with IPH may provide insights in the future.

### Table 2. Univariate Analysis Between Clinical Variables and Adventitial Perfusion Parameters (n=50 Arteries)

<table>
<thead>
<tr>
<th>Variable</th>
<th>$K_{trans}$</th>
<th>$v_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic status</td>
<td>0.028</td>
<td>0.001</td>
</tr>
<tr>
<td>Degree of stenosis, per 1 grade higher</td>
<td>0.013</td>
<td>−0.007</td>
</tr>
<tr>
<td>Age, per 5 y</td>
<td>0.000</td>
<td>−0.006</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.028</td>
<td>0.033</td>
</tr>
<tr>
<td>Body mass index, per 25% increase</td>
<td>0.007</td>
<td>0.047</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0.014</td>
<td>0.033</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.002</td>
<td>0.010</td>
</tr>
<tr>
<td>Hypertension</td>
<td>−0.001</td>
<td>0.018</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0.008</td>
<td>0.022</td>
</tr>
<tr>
<td>Lipid profile*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, per 25% increase</td>
<td>0.003</td>
<td>0.011</td>
</tr>
<tr>
<td>LDL-C, per 25% increase</td>
<td>0.004</td>
<td>0.015</td>
</tr>
<tr>
<td>HDL-C, per 25% increase</td>
<td>−0.001</td>
<td>−0.011</td>
</tr>
<tr>
<td>Triglyceride, per 25% increase</td>
<td>−0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>History of coronary artery disease</td>
<td>−0.007</td>
<td>0.012</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>−0.001</td>
<td>0.023</td>
</tr>
</tbody>
</table>

* Lipid profile was available in 20 (74.1%) patients.

### Table 3. Association Between Adventitial $K_{trans}$ and IPH After Adjusting for Confounders

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Adjustment</th>
<th>After Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of stenosis, per 1 grade higher</td>
<td>0.013</td>
<td>0.008</td>
</tr>
<tr>
<td>Symptomatic status</td>
<td>0.028</td>
<td>0.017</td>
</tr>
<tr>
<td>Age, per 5 y</td>
<td>0.000</td>
<td>−0.002</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.028</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Variables were selected as associated with either adventitial $K_{trans}$ or IPH in univariate analysis. $\Delta$ indicates mean difference in $K_{trans}$ (min$^{-1}$) or $v_p$; and IPH, intraplaque hemorrhage.
Current parameters of the DCE-MRI protocol represent a tradeoff between signal-to-noise ratio, spatial resolution, and temporal resolution. Although previous studies have indicated that the spatial resolution used in the current protocol is sufficient to image the adventitia, especially in the diseased segment, improvements in temporal resolution will enable more accurate estimation of perfusion parameters by capturing contrast kinetics in more detail. Alternatively, contrast agents with larger molecular weight and, thus, slower kinetics can be helpful for adventitial perfusion imaging. Contrast efflux was not taken into account in our model, which precludes estimation of extravascular extracellular space. However, efflux should be minor compared with influx during the short DCE-MRI session, attributable to much increased extracellular space in atherosclerosis.8

Conclusions
The presence of IPH was associated with high adventitial perfusion, independent of symptomatic status, degree of stenosis, and traditional risk factors. DCE-MRI provides a noninvasive way to characterize adventitial VV in clinical studies, but further examination through prospective studies is needed.

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Disclosures
Dr Chen received research support from the American Heart Association. Dr Kerwin is a part-time employee of VP Diagnostics. Dr Hatsukami receives research support from the National Institutes of Health and Philips Healthcare. The other authors have no conflicts to report.

References


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Supplemental Figure S1: Measuring adventitial perfusion parameters on VV image.

(A) DCE time series shows the dynamic enhancement process of adventitia (arrows). (B) Measurements are computed by averaging all pixels within 0.625 mm (1 pixel) of the outer wall boundary and over a region of the artery with a wall thickness in excess of 1.5 mm.

VV = vasa vasorum.