Complex Hemodynamics at the Apex of an Arterial Bifurcation Induces Vascular Remodeling Resembling Cerebral Aneurysm Initiation

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Background and Purpose—Arterial bifurcation apices are common sites for cerebral aneurysms, raising the possibility that the unique hemodynamic conditions associated with flow dividers predispose the apical vessel wall to aneurysm formation. This study sought to identify the specific hemodynamic insults that lead to maladaptive vascular remodeling associated with aneurysm development and to identify early remodeling events at the tissue and cellular levels.

Methods—We surgically created new branch points in the carotid vasculature of 6 female adult dogs. In vivo angiographic imaging and computational fluid dynamics simulations revealed the detailed hemodynamic microenvironment for each bifurcation, which were then spatially correlated with histologic features showing specific tissue responses.

Results—We observed 2 distinct patterns of vessel wall remodeling: (1) hyperplasia that formed an intimal pad at the bifurcation apex and (2) destructive remodeling in the adjacent region of flow acceleration that resembled the initiation of an intracranial aneurysm, characterized by disruption of the internal elastic lamina, loss of medial smooth muscle cells, reduced proliferation of smooth muscle cells, and loss of fibronectin.

Conclusions—Strong localization of aneurysm-type remodeling to the region of accelerating flow suggests that a combination of high wall shear stress and a high gradient in wall shear stress represents a “dangerous” hemodynamic condition that predisposes the apical vessel wall to aneurysm formation. (Stroke. 2007;38:1924-1931.)

Key Words: wall shear stress ■ gradient ■ intimal hyperplasia ■ intracranial aneurysm

Arterial bifurcations—or, more specifically, arterial expansions or sinuses opposite the apices—are known to be preferred sites for atherosclerosis. Pathologic remodeling of the vessel wall at these sites has been attributed to the low and oscillating hemodynamic stresses in such locations. Interestingly, bifurcations on or near the circle of Willis are common sites for a different type of pathologic remodeling: the formation of saccular aneurysms. However, in this case, remodeling occurs at or immediately adjacent to the apex of the bifurcation with high wall shear stress (WSS). Unlike the well-studied localization of atherosclerotic lesions, little is known about the factors that predispose apices of cerebral arterial bifurcations to aneurysm formation, although it is speculated that the high WSS near the flow divider is involved. Risk factors for cerebral aneurysm development, such as hypertension, smoking, and family history, are well recognized, but the consistent localization of aneurysms at arterial bifurcations suggests that the unique hemodynamics at bifurcation apices play a key role in aneurysm formation. Autopsies of human aneurysms and animal models reveal that the walls of cerebral aneurysms, in contrast to healthy cerebral arteries, are characterized by a disrupted internal elastic lamina (IEL), a thinned media, reduced smooth muscle cells (SMCs), and in some cases, disrupted endothelium and the presence of inflammatory cells. We believe these characteristics reflect maladaptive remodeling of the vessel wall in response to unique hemodynamics.

Our objectives were to identify the specific hemodynamic insults that lead to maladaptive vascular remodeling associated with aneurysm development and to identify early remodeling events at the tissue and cellular levels. For this purpose, we needed an in vivo model system in which (1) both upstream conditions (hemodynamics) and downstream events (pathologic remodeling) could be examined and correlated with each other and (2) there existed a “time-zero” point, such that vascular changes thereafter would be uniquely attributable to the hemodynamic conditions. Unfortunately, most existing animal models for cerebral aneurysms are inappropriate for this purpose because they are not “grown” but rather surgically created. An exception is the induction of...
cerebral aneurysms in the circle of Willis in rodents by increased flow and hypertension. These aneurysms are morphologically similar to human saccular cerebral aneurysms. However, the cerebral arteries in these animals are too small to image the flow in sufficient detail to permit mapping of hemodynamics with histology. Thus, it is difficult to correlate specific hemodynamic stresses with local tissue responses.

We recently developed a method for creating a new branch point in the carotid vasculature of adult dogs by exposing previously naïve vessel walls to impinging flow, elevated WSS, and localized wall shear stress gradients (WSSGs). In this model system, measured hemodynamics can be correlated spatially with tissue responses in specific microenvironments of the bifurcation. Furthermore, morphological changes that develop after establishing the bifurcation (time zero) can be attributed to the hemodynamic insults resulting from the increased and redirected flow. Thus, causal relations between altered hemodynamics and remodeling of the vascular wall at the cellular level can be studied.

In the present study, we further examined, at the cellular level, how arterial tissue adapts to the complex hemodynamic environment arising at created bifurcations. Our observations provide insight into how specific hemodynamic factors near the apex of an arterial bifurcation, including flow impinge-

Figure 1. A, Schematic of bifurcation creation. L (R)-CCA indicates left (right) common carotid artery. B, A created bifurcation. C, Overlay of CFD-calculated velocity vectors (color indicates magnitude) on a histologic section of a bifurcation (from dog No. 4) 2 months after creation (van Gieson’s staining). D, WSS and WSSG along the median wall of the bifurcation apex (left branch) reveal 3 regions with distinct flow characteristics: I, the impingement region; II, acceleration region; and III, recovery region. The range of normal physiologic WSS values for a straight vessel segment is indicated by the gray band.

Materials and Methods

We surgically created arterial bifurcations with a new branch point in common carotid arteries in 6 female dogs (Figure 1A) as previously described. In an attempt to induce hypertension, dogs were fed a high-salt diet starting 4 to 14 weeks before surgery until the end of the experiment, and in 3 of 6 dogs, the right renal artery was ligated before the bifurcation was created (the Table). However, we could not demonstrate induced hypertension: baseline blood pressure before surgery or the special diet was 122/81 mm Hg, and at the end of the experiment, blood pressure was 106/47 mm Hg with high-salt treatment alone and 130/48 mm Hg for renal ligation plus a high-salt diet. Failure to detect hypertension may have been due to excitability of the animals, making it difficult to obtain reliable blood pressure values. Alternatively, the effects of renal ligation may have been negated by the development of collateral renal arteries.

Two weeks or 2 months after bifurcation creation, bifurcation geometry and flow rates were obtained as described previously: the dogs were euthanized, and the bifurcations were examined histologically. For comparison, a segment of the unmanipulated left common carotid artery was excised from dog No. 4 during surgery before bifurcation creation, and the natural bifurcation between the right internal and external carotid arteries was harvested from a similar dog.
Histology and Immunohistochemistry

Vessels were fixed and embedded as described previously. Adjacent sections were stained with hematoxylin and eosin, van Gieson’s, and trichrome stains. For immunohistochemical analysis, sections were deparaffinized, rehydrated, and incubated in 3% H2O2 for 10 minutes to inactivate endogenous peroxidase. For proliferating cell nuclear antigen (PCNA) staining, the antigen was unmasked by boiling the sections in 10 mmol/L citrate buffer, pH 6.0, for 15 minutes, and then they were stained with biotinylated antibody against PCNA, followed by streptavidin-peroxidase visualized with 3,3’-diaminobenzidine with use of a Zymed PCNA staining kit (Zymed Laboratories Inc). For fibronectin staining, the antigen was unmasked by treating the sections with 20 μg/mL proteinase K for 10 minutes at 37°C, and the sections were stained with biotinylated antibody followed by streptavidin-peroxidase and diaminobenzidine chromogen with biotinylated anti-mouse immunoglobulins followed by monoclonal fibronectin antibody (BD Biosciences) and visualized with 3,3’-diaminobenzidine with use of a Zymed PCNA staining kit (Zymed Laboratories Inc). Specificity of all staining was confirmed by the absence of specific staining when nonimmune mouse immunoglobulin G was substituted for the primary antibody.

CFD Analysis and Flow-Histology Mapping

From in vivo 3-dimensional rotational angiography, we reconstructed the 3-dimensional lumen of the bifurcation, which, together with the measured flow rates in the vessel branches, served as boundary conditions for computational fluid dynamics (CFD) simulations. The 3-dimensional CFD solutions under both steady-state (average peak Re=390±252) and pulsatile-flow conditions were obtained under assumptions of a newtonian fluid (μ=0.0035 Pa·s) and incompressible flow in a rigid-wall model. Details of the CFD methodology have been previously published. To compare hemodynamics and histology, the 3-dimensional flow field of the bifurcation was sliced, morphed, and superimposed onto the photomicrograph of the corresponding histologic section. Mapping the CFD solutions onto the photomicrographs allowed the biologic properties of the cells to be related to the hemodynamic environments that the cells experienced in situ.

Proliferation Measurements

Proliferation of SMCs was indicated by staining the sections for PCNA, a material that is elevated in all phases of the cell cycle except G0. A hematoxylin/eosin-stained section at the same level was used to count the total number of SMC nuclei. Images were acquired consecutively from the apex of the impingement region into the left branch. Positively stained cells in each image were counted, and a proliferation index was expressed as PCNA-positive nuclei per 100 medial SMC nuclei.

### Results

#### Hemodynamic Microenvironment at the Created Arterial Bifurcations

The local flow fields in created bifurcations from CFD were aligned with histologic images to reveal the specific hemodynamic microenvironments along the wall of the bifurcation apex (Figure 1C). Based on the calculated flow field, the hemodynamic microenvironment along the wall of the bifurcation apex can be divided into 3 regions (I, II, and III) with different flow characteristics. These hemodynamic regions are exemplified in Figure 1D, which shows WSS and WSSGs along the median plane of a 2-month-old bifurcation (dog No. 4, left branch).

#### Impingement Region (I): WSS \( \leq 2 \) Pa and High WSSG

Blood from the feeding vessel impinged on the apex, creating a central stagnation point and raising the local pressure by \( \approx 1 \) mm Hg, and accelerated away from the apex into the branches. WSS peaked at 2 Pa (where 1 Pa=10 dynes/cm²) at the boundary of this region. The rapid increase of WSS over a short distance produced a large spatial gradient. In Figure 1D, the WSSG ranged from 11 to 10 Pa/mm in region I.

#### Acceleration Region (II): High WSS and High, Positive WSSG

Flow continued to accelerate until reaching the maximum WSS, which defines the distal edge of region II. In Figure 1D, WSS peaked at \( \approx 13 \) Pa, whereas WSSG persisted at elevated levels.

#### Recovery Region (III): Decelerating Flow With WSS Gradually Declining to Baseline

The WSSG was negative, and its magnitude overall was less than in the acceleration region. Eventually, the WSSG...
reached zero when the WSS returned to baseline levels seen in straight vessel segments.

Local Vascular Remodeling

The Table summarizes the vascular remodeling events observed by histologic analysis for all 6 dogs in this study. The response of the vessel wall was distinctly different between regions I and II. Three of 6 dogs showed hyperplasia of the vessel wall in the impingement region (region I). Five of 6 dogs showed moderate to extensive tissue loss in the acceleration region (region II). Remodeling in these 2 regions was examined in detail.

Hyperplastic Remodeling in the Impingement Region

In the 3 dogs showing hyperplastic remodeling in the impingement region, we observed 2 morphologies: early intimal hyperplasia (dog No. 1) and a more mature “intimal pad” (dogs No. 2 and 4). In the first type, intimal hyperplasia (Figures 2A3 and 2A4 for dog No. 1) was manifested by an increased number of cells on the luminal side of the IEL (Figure 2A3), beneath which was deposited an 8- to 16-μm-thick layer of largely acellular collagen (Figure 2A3). The additional cells on the luminal side of the IEL were demonstrated to be mainly SMCs by immunostaining for smooth muscle α-actin (data not shown).

The second type of hyperplastic remodeling is exemplified in Figures 2B3 and 2B4 (dog No. 4). There were fewer intimal cells in the hyperplastic region, but the subendothelial layer of collagen was much thicker (16 to 40 μm; Figure 2B3). Additional layers of elastin were present within this collagen matrix (Figure 2B4). This morphology resembled the “intimal pad” at the apex of a natural canine carotid bifurcation (Figures 2C3 and 2C4). A similar structure is found at many human and rat cerebral bifurcations.2,7 This suggests that intimal hyperplasia at the very apex is a normal, healthy, developmental response of a naïve vessel subjected to flow impingement at a new branch point.

Destructive Remodeling in the Acceleration Region

In 5 of 6 dogs, the acceleration region showed destructive remodeling. The specific events that constituted “destructive remodeling” are illustrated in the left branch of dog No. 4 (Figures 2B, 2B1 and 2B2). The acceleration regions contained a shallow “groove” in the vessel wall, ie, a decrease in the overall thickness of the media and intima. Closer exam-
ination of the groove revealed loss of the IEL (Figure 2B2), loss of overlying endothelium, and a prominent collagen matrix possessing fewer medial SMCs (Figure 2B1); this morphology was seen in 5 of 6 dogs. In 5 dogs, there was also loss of endothelium overlying the groove (see Figure 2B2). Such remodeling (ie, thinned vessel wall with severely disrupted IEL, reduced SMCs, and loss of endothelium) resembles early-stage intracranial aneurysm.7 Note that the lone bifurcation where destructive events were not observed (dog No. 1) was 1 of 3 shorter-term (2-week) experiments, and the bifurcations with the most destructive remodeling were 2-month experiments. So destructive remodeling is likely a gradual response to prolonged exposure to the new flow.

To determine the nature of the SMC loss in the groove, we examined cell proliferation by PCNA staining. Figure 3 shows medial SMC proliferation in the left bifurcation branches of dog No. 1 (which did not display medial thinning), dog No. 4 (which had substantial thinning), and a natural carotid bifurcation. The proliferation index stayed relatively constant along the walls of the natural bifurcation and the unthinned bifurcation of dog No. 1, whereas proliferation changed dramatically in dog No. 4; proliferation was markedly diminished in the acceleration region (Figures 3A and 3C, region II) of dog No. 4 while remaining similar to the natural bifurcation in the impingement (Figures 3A and 3B, region I) and recovery (Figures 3A and 3D, region III) regions.

As a potential molecular marker of preaneurysm remodeling,14,15 fibronectin was also examined. Immunostaining of tissue from dog No. 4 showed that fibronectin expression in the impingement region (Figure 4A) was generally similar to that of an unperturbed carotid artery (Figure 4D) but was locally elevated inside the hyperplastic intima. In the acceleration region, fibronectin was dramatically reduced (Figure 4B). This variation paralleled the trends in cell loss, elastin disruption, and reduction of SMC proliferation along the vessel wall. In the recovery region, fibronectin gradually increased to normal levels (Figure 4C).

### Localization of Remodeling Events

Figure 5 summarizes the spatial distributions of the remodeling events described earlier relative to the hemodynamic microenvironments at the created bifurcations (dogs No. 2, 4, and 6). Constructive, hyperplastic remodeling events, whenever present, were localized to the flow-impingement region (region I), whereas destructive remodeling events were localized to the acceleration region (region II), despite variations in bifurcation geometry and associated variations in lengths of the regions. Hyperplastic and destructive modes of remodeling were induced by the altered hemodynamics and not by the surgical procedure, because these remodeling processes were both behaviorally and spatially distinct from remodeling around the surgical wounds: remodeling around the sutures of the surgical anastomoses (Figure 2D1) consisted predominantly of collagen deposits that were attenuated within a short distance (Figures 2D and 2D2), and there was neither disruption nor hyperplasia of the endothelium evident by the time of euthanasia. Vessel wall morphology was normal ≈1 mm from the R2–L2 anastomosis, and neither hyperplasia nor destructive remodeling was observed in the intervening region between the anastomosis and regions I and II, which were a further 8.5 to 6 mm away from the suture site. At the R1–L2 junction, which was closer to the bifurcation apex, the suture sites were still separated from the flow-induced remodeling by 6 to 7 mm (half the circumference of the vessel), and serial sectioning through the bifurcations consistently showed that remodeling events in regions I and II were confined to the side of the wall, where flow impingement and acceleration occurred, ie, the opposite wall from the sutures.
Discussion

In our new branch-point model, 2 distinct patterns of arterial remodeling were observed: (1) hyperplasia that forms an intimal pad at the bifurcation apex and (2) destructive remodeling in the adjacent acceleration zone that resembles initiation of an aneurysm. The hyperplastic and destructive regions begin as biologically indistinguishable segments and differ only in the hemodynamic microenvironment that they experience after creation of the bifurcation. Therefore, the different remodeling behaviors represent distinct responses to specific hemodynamic microenvironments.

Constructive Remodeling in the Impingement Region

Conditions in the impingement region elicit healthy remodeling of the vessel wall. At the impingement point, where the

Figure 4. Fibronectin staining in regions I, II, and III (A–C) of the left branch of dog No. 4 and an unmanipulated left common carotid artery (D). Arrow indicates the apex. Note diminished staining of the media in region II (B).

Figure 5. Hemodynamic environments (as defined in Figure 1D) and localization of remodeling events in (A) dog No. 2 (right branch), (B) No. 4 (left branch), and (C) No. 6 (left branch). Hyperplastic responses were observed in region I, whereas all destructive events were in region II. Bifurcation lumen (reconstructed from in vivo imaging) with WSS distribution.
Aneurysm-Type Remodeling in the Acceleration Region

In contrast to the constructive response in the impingement zone, remodeling in the acceleration region consisted of destructive events (Figure 5). A shallow groove developed, with disruption of the IEL and loss of SMCs and endothelium. These events are characteristic of aneurysm development in hypertensive rats and humans. IEL disruption is necessary in aneurysm initiation to allow the vessel wall to expand outward. Whereas IEL disruption in abdominal aortic aneurysms is due to matrix metalloproteinases, mostly secreted by infiltrating macrophages that precede aneurysm formation, in cerebral aneurysms there is no evidence that inflammatory cells are present during initiation, and they were not found in our created bifurcations. We speculate that in the acceleration region, the combination of a high WSS and a high WSSG induced matrix metalloproteinase production by ECs and SMCs. Matrix metalloproteinases are elevated in carotid arteriovenous fistulas. However, in the elevated WSS of our acceleration region, SMCs were instead lost. We attribute this difference to the high WSSG in the acceleration region. The combination of high WSS and high WSSG may cause EC dysfunction, eventually leading to denudation of the vessel wall. High WSSG is associated with other vascular pathologies, particularly atherosclerosis, that involve dysfunction of ECs, leading to increased endothelial permeability and local inflammation. However, a high WSSG has previously been studied only in low-WSS environments, and little is known about the effects of WSSG under high WSS conditions. We do not think that atherosclerosis is involved in the early changes at our bifurcations, as we saw no evidence of inflammation, deposition of lipid molecules, or a continuous, localized increase of intimal layers.

In addition to IEL and EC disruption, SMC loss was another prominent feature of destructive remodeling in the acceleration region. SMC loss contributed to medial thinning and creation of a groove that we believe to be a nascent aneurysm. Decreased PCNA in the media of the acceleration region indicated that SMCs became more quiescent under high WSS and WSSG conditions. This may have resulted indirectly from endothelial dysfunction and the consequent loss of endothelium-secreted trophic factors for SMCs. Inhibition of SMC proliferation, which was apparent in the groove of dog No. 4, would locally thin the media, as SMCs failed to reproduce sufficient numbers to maintain wall thickness. In addition, matrix degradation may reduce cell-matrix contact and cause SMC apoptosis.

Further dysfunction of SMCs in the acceleration region is suggested by reduced fibronectin expression in the groove. Diminished or disorganized fibronectin is observed in both rat and human aneurysms. Fibronectin is an important extracellular matrix component for tissue remodeling and wound healing. Loss of fibronectin could impair SMC adhesion and proliferation, as fibronectin stimulates cell growth and survival. Thus, fibronectin loss may lead to further SMC reduction and a thinning, weakening vessel wall, eventually producing an aneurysm.

Limited by the small number of dogs in this study, we cannot clearly define the time course of hemodynamically induced remodeling. However, dog No. 1 (2 weeks) exhibited strong early-stage hyperplastic remodeling at the impingement region and no destructive remodeling at all, whereas dog No. 4 (2 months) had more mature hyperplastic remodeling at the apex and a distinct groove with all of the destructive remodeling events, indicative of early aneurysm, in its acceleration region in the left branch. Other dogs displayed these remodeling events to various degrees with consistent localization in the hemodynamic regions.

In summary, the complex flow at the apices of arterial bifurcations produces correspondingly complex tissue responses that are determined by the local hemodynamic microenvironments. It has long been suggested that a high WSS contributes to aneurysm initiation and development. The present observation that aneurysm-type remodeling is localized in the acceleration zone, where both WSS and WSSG are elevated, suggests that WSSG is an important hemodynamic factor in aneurysm initiation. Future investigation into the effects of high WSSG and the interaction between WSSG and WSS may provide important insights into the mechanism of intracranial aneurysm formation.
The new branch-point model captures both healthy adaptation of a naïve vessel to impinging flow and maladaptation in a region of flow acceleration. The destructive, aneuryism-like remodeling events observed in the latter implicate the combination of high WSS and high WSSG as a potentially "dangerous" hemodynamic insult. We conjecture that this hemodynamic microenvironment predisposes a vessel wall to aneurysm formation, thus explaining why cerebral aneurysms often form at or near bifurcation apices. In cerebral bifurcations, destructive, aneurysm-like remodeling may be initiated either by an increase in the "dangerous" hemodynamic insult through chronic increase of flow (caused by cardiovascular changes) or by deficiencies in the vessel response stemming from known risk factors, such as hypertension, smoking, and connective tissue disease (eg, Ehlers-Danlos syndrome). The specific, proaneurysm hemodynamics and early remodeling events identified herein may help identify targets for hemodynamic and pharmacological approaches to aneurysm treatment.

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Disclosures

None.

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