Cerebral blood flow patterns at major vessel bifurcations and aneurysms in rats

HIDEYUKI NAKATANI, M.D., NOBUO HASHIMOTO, M.D., YOO KANG, M.D., NAOHIRO YAMAZOE, M.D., HARUHIKO KIKUCHI, M.D., SABURO YAMAGUCHI, PH.D., AND HIDEYUKI NIIMI, PH.D.

Department of Neurosurgery, Kyoto University Medical School and Hospital, Kyoto, and National Cardiovascular Center Research Institute, Osaka, Japan

Cerebral arterial bifurcations in rats were treated to induce cerebral aneurysms experimentally, and flow patterns of latex particles introduced under a constant flow rate were analyzed with a 16-mm cine-camera and videocassette recorder. Cerebral aneurysms were produced by ligating one common carotid artery, inducing experimental hypertension, and feeding the animals β-aminopropionitrile. After perfusion and fixation, samples of cerebral arterial bifurcations with shallow invaginations and with small aneurysms were obtained and used for analysis. Bifurcations in rats without experimental treatment were used as control specimens. Flow studies in the control bifurcations showed that the apical intimal pad, not the apex itself, acted as the flow divider. Small particles tended to accumulate at the region just distal to the apical intimal pad, where the initial aneurysmal changes are known to occur. This indicates stagnation of flow at that site. In the bifurcations with shallow invaginations and small aneurysms, a marked pressure gradient was present at the proximal end of the aneurysm orifice. A tendency for stagnation of small particles near the aneurysm wall was also observed. The wall shear stress was highest at the distal end of the aneurysmal orifice, which may be responsible for the development of these lesions.

KEY WORDS • aneurysm • cerebral blood flow • rheology • pathogenesis

Materials and Methods

Preparation of the Bifurcation

Male rats of the Sprague-Dawley strain, aged 6 or 7 weeks, were used for this study. In each rat, the left common carotid artery and the posterior branches of both renal arteries were ligated under sodium pentobarbital anesthesia (40 mg/kg intraperitoneally). One week after ligation, 1% of NaCl solution was given with the animals' drinking water. Two weeks later, β-aminopropionitrile fumarate was added to a standard laboratory diet in a 0.12% concentration. About 3 months after ligation, the rats were cannulated through the descending aorta under general anesthesia, and perfused with heparinized phosphate-buffered saline (PBS, 0.15 M, pH 7.4), followed by 2% buffered formaldehyde. After perfusion and fixation, the Major arteries at the base of the brain were carefully removed under a dissecting microscope. The junctions of the anterior cerebral artery (ACA) and the olfactory artery (OA) on the
Cerebral blood flow patterns in aneurysms in rats

**Flow System and Perfusion Fluid**

The experimental system for flow analysis is illustrated in Fig. 2. The vascular preparation was cannulated using a tapered polyethylene tube with a tip diameter of about 200 μm. It was connected to a head cylinder with a No. 22 needle and a polyethylene tube (internal diameter approximately 1 mm). The perfusion agent was a diluted suspension mixture of polyethylene latex particles* in various sizes (diameter 5, 8, 15, 25, and 47 μm, density 1.06 g/cm³) in 0.15 M of the PBS (viscosity 1.0 × 10⁻² Pa/sec at a temperature of 21°C). The specimens were mounted on a small glass plate under a microscope and always kept moist with PBS to minimize optical distortion. Care was taken to maintain the original angle of the bifurcation.

* Polyethylene particles manufactured by Polysciences, Inc., Warrington, Pennsylvania.

---

**Results**

**Flow in Control Bifurcations**

If particles in the lumen were clearly and individually observed on the 16-mm cinefilm (Fig. 3 left). The paths of each particle could be traced by superimposition of several frames of 16-mm cinefilm showing the sequence of movements of the particles long and short arrows.

---

**Flow Visualization and Recording**

The perfusion fluid was controlled as a constant (nonpulsatile) flow with a constant head pressure system. The flow velocity of the parent ACA was limited to less than 20 mm/sec by changing the height of the head cylinder. For the illuminating system, a 300-W tungsten halogen lamp was used. Particle motions were observed through the microscope at magnifications ranging from ×12 to ×100. Recordings were made by a videocassette recorder, and a 16-mm cine-camera at a speed of 64 frames/sec.†

---

**Image Analysis**

The developed cinefilm was projected onto a screen frame-by-frame, and the motions and paths of the latex particles were plotted and analyzed. To improve the quality of the image, a film-to-video converter and a real-time image improvement system were used.‡ The flow behavior of the particles was monitored by repeated inspection of the video recordings.

---

* Videomicroscope, Model AG-7300, manufactured by Matsushita Electric, Inc., Kadoma, Japan; cine-camera manufactured by Bolex Co., Geneva, Switzerland.
† Film-to-video converter, Model TRV-16 manufactured by Elmo Co., Nagoya, Japan; Real-time image improvement system, Model DVS-3000, manufactured by Hamamatsu Photonics, Hamamatsu, Japan.
FIG. 4. Diagram showing the streams and velocity profiles in a control bifurcation. Arrows indicate the velocity vectors on each stream. ACA = anterior cerebral artery; PACA = parent ACA; DACA = daughter ACA; DOA = olfactory artery; a, b, and c indicate the points where the diameter of each vessel shown in Table 1 was measured.

Flow in Bifurcations With an Aneurysm

In bifurcations with a shallow invagination and in specimens with a small aneurysm, the diameters of the parent ACA’s were about twice as large as those in control bifurcations (Table 1). In bifurcations with a shallow invagination, some of the particles in the stream of the parent ACA on the OA side entered the dome from the proximal end of the aneurysmal orifice, with a sudden decrease of flow velocity. They then moved along the luminal surface of the aneurysms very slowly, with little change in their velocity. They emerged from the aneurysmal lumen at the distal end of the orifice to enter the stream along the medial wall of the daughter ACA (Fig. 6 left).

In bifurcations with a small aneurysm, the particles flowing in the stream of the parent ACA on the OA side reached the proximal end of the orifice and entered the cavity, changing their flow direction at the lateral angle toward the fundus. Then, they showed almost the same flow behavior as in the bifurcations with a shallow invagination (Fig. 6 right).

Streams in the aneurysm were not parallel to the main stream of the daughter ACA; they deviated toward the fundus and the deviation pattern depended on the size and shape of the aneurysm. The nearer the streams were to the fundus, the more they deviated toward the fundus. In bifurcations with a small aneurysm, some streams crossed the main stream of the daughter ACA (Fig. 6).

In the bifurcation with shallow invagination, the wall shear rate ($T$, which is the velocity gradient in the direction normal to the vessel wall in sec$^{-1}$) was roughly estimated using the data of flow velocity near the wall. Assuming a parabolic velocity distribution near the wall, the wall shear stress ($\tau$) was calculated in terms of the equation, $\tau = \eta \times \dot{T}$, at the following four points: 1) the proximal end of the aneurysmal wall; 2) near the fundus; 3) the distal end of the aneurysmal wall; and 4) just distal to the distal end of the aneurysmal wall (Fig. 6).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Diameter (µm)</th>
<th>$U_{\text{max}}$ (mm/sec)</th>
<th>Flow Ratio (Q_{DACA}/Q_{DOA})</th>
<th>Angle of Bifurcation</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>PACA</td>
<td>180</td>
<td>15.3</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>DACA</td>
<td>160</td>
<td>14.1</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>DOA</td>
<td>130</td>
<td>13.6</td>
<td>1.8</td>
</tr>
<tr>
<td>shallow invagination</td>
<td>PACA</td>
<td>360</td>
<td>13.9</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>DACA</td>
<td>280</td>
<td>11.2</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>DOA</td>
<td>130</td>
<td>10.4</td>
<td>1.4</td>
</tr>
<tr>
<td>small aneurysm</td>
<td>PACA</td>
<td>320</td>
<td>14.7</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>DACA</td>
<td>220</td>
<td>13.1</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>DOA</td>
<td>150</td>
<td>8.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* $U_{\text{max}}$ = maximum flow velocity; Re = Reynolds number; ACA = anterior cerebral artery; PACA = parent ACA; DACA = daughter ACA; DOA = olfactory artery; Q = flow rate.
† Measurements obtained at points a, b, and c in Figs. 4 and 6.
Cerebral blood flow patterns in aneurysms in rats

Fig. 6. Diagrams of the streams in a bifurcation with a shallow invagination (left) and a bifurcation with a small aneurysm (right). The numbers and small arrows indicate the flow velocities of the particles (mm/sec). ACA = anterior cerebral artery; P_{ACA} = parent ACA; D_{ACA} = daughter ACA; DOA = olfactory artery. The maximum velocity of the particles flowing in the parent ACA is shown under the large arrow; a, b, and c indicate the points where the diameter of each vessel shown in Table 1 was measured.

7). The wall shear stress at each point was 0.74, 0.34, 0.88, and 0.61 Pa, respectively. The shear stress was highest at the distal end of the aneurysm.

Discussion

Laboratory Preparation

In this animal model, aneurysms develop most frequently on the ACA, at the origin of the OA on the side opposite the carotid ligation.12,14 We have shown that these induced aneurysms are very similar morphologically and histopathologically to human cases.9 We have also revealed the developmental sequence of cerebral aneurysms and the process of involvement of the apical intimal pad into the aneurysmal wall.12,14 Based on this bifurcation of the animal model, flow characteristics can be analyzed in the preaneurysmal, early aneurysmal, and fully developed aneurysmal states.

Intimal Pad

At the ACA bifurcation, the apical intimal pad is located just distal to the apex on the side of the daughter ACA. During early aneurysmal development, this intimal pad was gradually incorporated into the aneurysmal wall and disappeared.12 These observations suggest the importance of the apical intimal pad in the development of aneurysms.

Stagnant Flow and Endothelial Injury

One of the unique findings of the present study is that small latex particles tend to accumulate at the region just distal to the apical intimal pad at the site of early aneurysm formation.14 Among the particles of various sizes used in the present study, only smaller particles were found to accumulate. This phenomenon corresponds to stagnation of flow. Scanning electron microscopic study has shown that degeneration of endothelial cells occurs at this site even in this preaneurysmal state.15

Although the present findings were obtained in a steady (nonpulsatile) flow state, and the flow velocity might be different from that in a vital state, it is sug-

Fig. 7. Drawing showing the wall shear stress at and just distal to a shallow invagination. The arrows indicate the vectors of wall shear stresses. The length of the scale (uppermost arrow) represents a wall shear stress of 0.5 Pa. Wall shear stress was: 1 = 0.74 Pa; 2 = 0.34 Pa; 3 = 0.88 Pa; and 4 = 0.61 Pa (see text). Note that the wall shear stress was highest at the distal end of the invagination.
suggested that there is a significant relationship between the focal endothelial injury and the stagnation that occurred. Stagnation of the blood may cause anoxic injury to endothelial cells. Adhesion and accumulation of platelets or leukocytes may also cause damage to intimal tissue. The contributions of platelets or leukocytes to the development or enlargement of these aneurysms should be more clearly elucidated.

Flow Patterns

At the bifurcation with early aneurysmal change, some flow streams crossed the main stream in the daughter ACA. This might indicate the presence of a disturbed flow such as a vortex of the types shown in the glass model studies. At the stage of shallow invagination and small aneurysm formation, a marked decrease in flow velocity was observed in the particles, with a direction of flow toward the fundus at the proximal end of the dome. According to Bernoulli’s theorem, a marked pressure gradient is present at the proximal end of the dome, between the intra- and extra-aneurysmal lumina. The wall of the aneurysm near the proximal end is considered to be exposed to high pressure. This focal high pressure may be responsible for further injury to the aneurysmal wall or direct enlargement of the aneurysm.

In the present study, the velocity of the particles passing along the aneurysmal wall was very low, about 10% to 15% of the maximum axial velocity in the branches. Particles running along the wall were also among the smaller particles present. The tendency of small particles to stagnate may be responsible for mural thrombus formation, which may cause metabolic disorders of the aneurysmal wall.

Wall Stress

The wall shear stress (or tangential force induced by the flow in a vessel), which is one of the important hemodynamic forces affecting the wall, was estimated roughly at and near the aneurysmal wall in the case of shallow invagination. This showed that the wall shear stress was highest at the distal end of the invagination. Wall shear stress is known to cause injury to endothelial cells, and this finding is further evidence that hemodynamic stresses affect aneurysmal development.

References


Manuscript received February 2, 1990. Accepted in final form August 6, 1990.

Address reprint requests to: Haruoiko Kikuchi, M.D., Department of Neurosurgery, Kyoto University Medical School and Hospital, Sakyo-ku, Kyoto 606, Japan.