Occurrence of Stroke in a Nonhuman Primate Model of Cerebrovascular Disease

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A relation between hypertension, atherosclerosis, and stroke is well documented in humans. We report a similar relation in two hypertensive cynomolgus monkeys with severe cerebral atherosclerosis. In our primate model hypertension is induced by surgical coarctation of the aorta. These monkeys, when fed an atherogenic diet, develop severe cerebrovascular atherosclerosis. In this setting two monkeys developed spontaneous cerebral hemispheric strokes that occurred during treatment of hypertension. Since the strokes were topographically related to severe atherosclerotic narrowing of cerebral arteries and occurred without evidence of either thrombosis or embolization, they are presumed to be related to disturbances of blood flow. In both humans and animals cerebral perfusion is autoregulated to a constant flow over a wide range of mean arterial blood pressures. In hypertension both the upper and lower limits of autoregulation are increased. With treatment of hypertension readaptation to more normal levels is reported to be inconsistent and slow to develop. It is therefore postulated that the strokes in these two monkeys were due to hypoperfusion as a result of the combination of pharmacologic reduction in blood pressure and severe occlusive atherosclerosis.

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While a relation between hypertension, atherosclerosis, and stroke is well documented in humans, this relation has yet to be established in nonhuman primates. In cynomolgus monkeys with hypertension induced by surgically coarcting the thoracic aorta, Hollander et al reported severe cerebrovascular atherosclerosis when the monkeys were fed an atherogenic diet. We report two cases of spontaneous stroke in this monkey model of cerebrovascular atherosclerosis. These monkeys were part of a group of 16 similarly treated animals on a pharmacologic regimen to reduce elevated blood pressure. Within this group, these two monkeys showed severe cerebrovascular atherosclerosis and the most marked reduction in blood pressure, suggesting that antihypertensive medication played a role in their pathogenesis.

Materials and Methods

Both brains were embedded whole in celloidin and cut in gapless serial sections at a thickness of 35 μm. Every twentieth section was stained with Nissl’s stain for myelinated fibers. The large arteries at the base of the brain and the extracranial internal carotid arteries were removed at the time of autopsy and visually inspected at a magnification of 2× for atherosclerotic plaques, thrombi, and emboli. Representative sections from predetermined sites (all areas labeled in Figure 4) and all grossly visible atherosclerotic plaques were embedded in polyethylene glycol with 4-μm-thick sections stained with oil red O, Verhoeff-van Gieson’s stain, and toluidine blue. Morphometric measurements were performed on the histologic cross sections with a Zeiss MOP III Image Analyzer (Thornwood, New York). Lesion size or intimal area was determined by digitizing the area between the internal elastic membrane and the luminal surface of the artery. The percent stenosis (shown in Figure 4) is the maximum narrowing observed in all arteries showing atherosclerosis and was calculated from the ratio of lesion area to the area enclosed by the internal elastic membrane.

Both monkeys were wild-caught adult males of Philippine origin that were in captivity for about 6 months before inception of the study. They ate well and were normally active until the time of their stroke. Before the experimental diet, the monkeys were maintained on Purina monkey chow (Richmond, Indiana). The atherogenic diet consisted of a mixture of Purina monkey chow and banana mash to which 2% cholesterol and 10% butter by weight were added. Hypertension was produced by surgically coarcting the aorta as described previously. A small segment of thoracic aorta, approximately 1 cm long, at about the level of the fifth intercostal space was stenosed by suturing the walls together so that luminal diameter was decreased to about 2.5 mm. A supporting band of umbilical tape was then drawn around the coarted segment and sutured without further constriction of the vessel. The coarctation of the aorta resulted in a decrease in luminal area of about 75-80% as indicated by the autopsy findings.

Before coarctation of the aorta, baseline observations were made including estimation of food consumption, measurements of body wt, blood pressure, electrocardiography (ECG), serum cholesterol,
triglycerides, high density lipoprotein (HDL) cholesterol, electrolytes, blood urea nitrogen, creatinine, and calcium as previously described. Food consumption was estimated daily, and measurements of body wt, plasma lipids, and other functions were performed at 2-month intervals throughout the experimental period. The moneys consumed their daily food allocation and maintained their body wt until they developed complicating stroke. Measurements of blood pressure, drawing of venous blood, and ECG were performed under 10 mg/kg ketamine i.m. sedation following an 18–24-hour fast. Blood pressure in the brachial artery was monitored indirectly by the ultrasonic cuff method with the use of the Arteriosonde (Roche, Boston, Massachusetts). These findings were confirmed by simultaneous direct measurements of the intra-arterial pressure in the brachial artery and thoracic aorta above the coarctation with an indwelling catheter and strain gauge transducers attached to a Beckman dynograph recorder (Fullerton, California). Blood pressure and relevant blood chemistries of the two monkeys, H-633 and H-593, are shown in Figure 1 and Table 1, respectively. In both monkeys blood pressure increased significantly following coarctation and remained elevated until treatment with antihypertensive drugs. During feeding of the high-cholesterol atherogenic diet, total plasma cholesterol increased significantly, while HDL cholesterol decreased without a change in triglycerides; plasma lipids were not significantly altered by antihypertensive drug treatment. The other blood tests, which include glucose, blood urea nitrogen, creatinine, sodium, potassium, calcium, and hematocrit, showed no significant change throughout the study. The baseline values and plasma lipid responses to the atherogenic diet were not significantly different from values in normotensive animals.

Results
Monkey H-633 had a baseline body wt of 5.3 kg and blood pressure of 91/59 mm Hg. Following coarctation of the thoracic aorta, postoperative blood pressure in the arteries above the coarctation was 184/109 mm Hg, with subsequent blood pressure measurements of 180–185/104–110 mm Hg. ECG showed voltage changes due to hypertension. Two months after coarctation Monkey H-633 was started on a hypercholesterolemic diet. Twelve months after coarctation the intra-arterial blood pressure was 192/117 mm above the coarctation and 97/87 mm Hg below it. Monkey H-633 was treated with an antihypertensive drug regimen consisting of 35 mg hydrochlorthiazide, 10 mg prazosin, and 100 mg captopril with steady decline of blood pressure. Eight months after the initiation of antihypertensive treatment Monkey H-633 was found unable to move the right side of its body; blood pressure was 90/50 mm Hg. Monkey H-633 was found dead the next day. At autopsy the body weighed 4.3 kg and the heart 23 g, with a heart wt:body wt ratio of 5.3 g/kg. The respective normal values (mean ± SD) for heart wt and heart wt:body wt ratio are 18.7 ± 4.5 g and 3.1 ± 0.5 g/kg. The brain weighed 61 g; normal brain wt is 65.2 ± 6.1 g. The lungs were congested and the heart was enlarged, with severe coronary artery atherosclerosis. There was no evidence of coronary thrombosis, myocardial infarction, or mural thrombus.

Examination of histologic sections of the brain revealed a recent massive left hemisphere infarct (Figure 2A–2C). The histologic features of the infarct were

**Figure 1.** Cuff (••••••••) and intra-arterial (••••••••) blood pressures in monkeys H-633 (left) and H-593 (right). In both monkeys strokes occurred at lowest blood pressure while receiving antihypertensive treatment.
Twelve months after surgery the intra-arterial blood pressure was 160/100 mm Hg. Following coarctation of the thoracic aorta the blood pressure was 172/85 mm Hg above the coarctation and 115/85 mm Hg below it. Monkey H-593 was treated with an antihypertensive drug regimen consisting of 35 mg hydrochlorothiazide, 10 mg prazosin, and 100 mg captopril. Six months after the initiation of antihypertensive treatment Monkey H-593 was observed one morning favoring its left arm; neurologic evaluation indicated that if the right arm was restrained he could accurately but awkwardly reach with his left arm, with poor motor control of the left hand. There was also evidence of left-sided neglect. Two days after the stroke the intra-arterial blood pressure was 95/50 mm Hg above the coarctation and 80/65 mm Hg below it. The hemiplegia became progressively more severe, and Monkey H-593 was killed 2 weeks after its onset.

ECG 1 day before death showed high voltage changes consistent with left ventricular hypertrophy without complicating myocardial ischemia or infarction. At autopsy the body weighed 4.4 kg and the heart 19 g, indicating that if the right arm was restrained he could accurately but awkwardly reach with his left arm, with poor motor control of the left hand. There was also evidence of left-sided neglect. Two days after the stroke the intra-arterial blood pressure was 95/50 mm Hg above the coarctation and 80/65 mm Hg below it. The hemiplegia became progressively more severe, and Monkey H-593 was killed 2 weeks after its onset.

Table 1. Clinical Data for Two Monkeys as Nonhuman Primate Model of Cerebrovascular Disease

<table>
<thead>
<tr>
<th>Blood pressure (mm Hg)</th>
<th>Baseline 633</th>
<th>Baseline 593</th>
<th>Coarctation + atherogenic diet 633</th>
<th>Coarctation + atherogenic diet + antihypertensive therapy 633</th>
<th>Coarctation + atherogenic diet + antihypertensive therapy 593</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>94±5</td>
<td>96±5</td>
<td>181±8*</td>
<td>176±5*</td>
<td>112±12</td>
</tr>
<tr>
<td>Diastolic</td>
<td>63±3</td>
<td>70±4</td>
<td>110±5*</td>
<td>104±5*</td>
<td>63±4</td>
</tr>
<tr>
<td>Body wt (kg)</td>
<td>5.3±0.2</td>
<td>5.6±0.1</td>
<td>5.2±0.2</td>
<td>5.9±0.4</td>
<td>4.8±0.4</td>
</tr>
<tr>
<td>Plasma lipids (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>138±10</td>
<td>111±3</td>
<td>820±141*</td>
<td>613±68*</td>
<td>710±96*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>88±3</td>
<td>77±14</td>
<td>31±5*</td>
<td>38±6*</td>
<td>31±4*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>33±3</td>
<td>38±10</td>
<td>31±5</td>
<td>40±12</td>
<td>33±4</td>
</tr>
<tr>
<td>Blood tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>64±8</td>
<td>60±10</td>
<td>64±10</td>
<td>62±8</td>
<td>60±4</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>13±3</td>
<td>12±3</td>
<td>14±2</td>
<td>11±3</td>
<td>16±4</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9±0.1</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Sodium (meq/l)</td>
<td>144±2</td>
<td>145±4</td>
<td>146±3</td>
<td>145±2</td>
<td>142±3</td>
</tr>
<tr>
<td>Potassium (meq/l)</td>
<td>4.0±0.4</td>
<td>4.0±0.3</td>
<td>3.8±0.4</td>
<td>3.8±0.2</td>
<td>3.9±0.3</td>
</tr>
<tr>
<td>Calcium (meq/l)</td>
<td>9.4±0.5</td>
<td>9.3±0.2</td>
<td>9.2±0.4</td>
<td>9.2±0.2</td>
<td>9.4±0.2</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36.0±0.5</td>
<td>36.5±1.0</td>
<td>34.8±1.4</td>
<td>36.0±0.7</td>
<td>36.0±0.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD. HDL, high density lipoproteins.
*p<0.01 compared with baseline values.

loss of Nissl staining, poor demarcation of anatomic features, universal ischemic nerve cell change, and mild hypertrophy of astroglial cell nuclei. At the posterior pole of the hemisphere (Figure 2A) all areas were involved. At a more rostral level (Figure 2B) virtually the entire cerebral cortex was infarcted together with the amygdala and basol ganglia; the medial part of the thalamus, cingulate gyrus, and adjacent interhemispheric cortex were spared. At a still more rostral level (Figure 2C) the distribution was similar with, however, sparing of the ipsilateral septum. The extent of atherosclerotic narrowing of the major cerebral arteries is shown in Figures 3A–3C and 4 and was most marked ipsilateral to the recent hemispheric infarct. The ipsilateral extracranial internal carotid artery was narrowed by 74%, the middle cerebral artery by 72%, and the proximal posterior cerebral artery by 63%. There was also complete occlusion of the ipsilateral posterior communicating artery and severe atherosclerosis at several levels in the basilar artery.

Monkey H-593 had a baseline body wt of 5.6 kg and blood pressure of 92/60 mm Hg. Following coarctation of the thoracic aorta the blood pressure was 160/100 mm Hg in the arteries above the coarctation, with measurements over the next 3 months of 175–180/98–119 mm Hg. Three months after coarctation Monkey H-593 was started on a hypercholesterolemic diet. Twelve months after surgery the intra-arterial blood pressure was 172/85 mm Hg above the coarctation and 115/85 mm Hg below it. Monkey H-593 was treated with an antihypertensive drug regimen consisting of 35 mg hydrochlorothiazide, 10 mg prazosin, and 100 mg captopril. Six months after the initiation of antihypertensive treatment Monkey H-593 was observed one
immediately surrounded the older lesion with sparing of the base of the occipital lobe and the cortex of the calcarine fissure. At more rostral levels (Figure 2E and 2F) its distribution was similar to that noted in Monkey H-633 except that the ipsilateral interhemispheric cortex and septum and contralateral cingulate cortex were also involved. The extent of atherosclerotic narrowing of the major cerebral arteries is shown in Figures 3D–3F and 4. Atherosclerosis was most marked ipsilateral to the infarcts with 92% narrowing of the extracranial internal carotid artery, 52% and 55% narrowing, respectively, of the proximal anterior and middle cerebral arteries, and 82% reduction in the lumen of the proximal posterior cerebral artery.

None of the infarcts in these two monkeys was hemorrhagic. Macroscopic and microscopic examination of the large arteries at the base of the brain and the extracranial internal carotid artery and microscopic survey of the pial and penetrating arteries in the whole brain serial sections showed no evidence of thrombosis or emboli in either monkey.

**Discussion**

Both monkeys had recent massive cerebral hemispheric infarcts involving the anterior, middle, and posterior cerebral artery territories, severe cerebrovascular atherosclerosis that was most marked ipsilateral to the infarct, and pharmacologic reduction of elevated blood pressure. One had an older infarct in the anterior cerebral artery-middle cerebral artery border zone that corresponded to a clinically documented hemiparesis. Careful search for cerebrovascular thrombosis or emboli or a cardiac source of emboli failed to provide evidence for vascular occlusion as the etiology of these strokes. Evidence favoring a nonocclusive disturbance of blood flow includes the presence of a border zone infarct in one monkey and in both monkeys the involvement of three different major circumferential arteries, the close relation of the infarcts to atherosclerotic narrowing of cerebral blood vessels, and the close relation between the marked pharmacologic reduction of blood pressure and the strokes. Further, of 16 similarly treated monkeys, the two that developed strokes had severe cerebral atherosclerosis and the most marked pharmacologic reduction in blood pressure.

In humans, nonhuman primates, and rodents, cerebral blood flow is autoregulated over a wide range of mean arterial blood pressures. At the lower limit of autoregulation cerebral blood flow decreases, and at its upper limit it increases. With arterial hypertension,
both the upper and lower limits of autoregulation are set at higher mean arterial blood pressures.\textsuperscript{5–12} With treatment of established hypertension in humans, readjustment of autoregulation toward normal levels has been reported but is inconsistent and slow to develop. Thus Strandgaard\textsuperscript{8} noted that in nine effectively treated hypertensives only some patients autoregulated to a lower level. In a second group of four patients with severe uncontrolled hypertension who were studied 8–12 months after treatment only one had seemingly shifted his cerebral blood flow autoregulation toward normal.\textsuperscript{7} As a result of elevation of the lower limit of autoregulation and the uncertainty of effects of the treatment of hypertension on autoregulation, patients with hypertension have been noted to be susceptible to hypotension. Graham\textsuperscript{13} reported cerebral artery border zone infarction in a hypertensive patient as a result of lowering the blood pressure to the normal range. Jackson et al\textsuperscript{14} reported six hypertensive patients who were admitted to the hospital in a coma following rapid reduction of blood pressure to normotensive values; one had residual deficit. Ruff et al\textsuperscript{15} noted, in a study of 132 patients with transient ischemic attacks, that in 7 patients the ischemic attacks occurred during hypotension; all 7 had hypertension, and 4 of these 7 had significant carotid stenosis. Rosenfeld et al\textsuperscript{16} noted in 208 consecutive ischemic strokes that 16 could be attributed totally or partially to hypotension, usually iatrogenic; 12 of these 16 patients were known to be hypertensive prior to the strokes. A similar sensitivity to hypotension has also been noted in the treatment of hypertensive encephalopathy by Ledingham and Rajagopalan.\textsuperscript{17}

Another reported complication of hypertension is aberrant autoregulation. Griffith et al\textsuperscript{18} studied cerebral blood flow in 10 hypertensive patients during inhalation of 5% carbon dioxide and noted that in five patients blood flow was decreased rather than increased. Meyer et al\textsuperscript{19} noted that hypertensive patients with strokes were more likely to show decreased cerebral blood flow on a tilt table than normotensive individuals. This "dysautoregulation" was noted to occur more frequently with subcortical and brainstem infarcts.

Other possible mechanisms of a further reduction in blood pressure include unobserved cardiac arrhythmia and drug-related orthostatic hypotension. The latter has been reported as a complication of prazosin.\textsuperscript{20} Additional factors contributing to cerebral ischemia, such as dehydration, electrolyte imbalance, nitrogen retention, and decreases in blood volume, were not revealed by the blood analyses. However, reduced cardiac output in one of the two monkeys, H-633, may have played a role since this monkey was found to have congestive heart failure associated with severe coronary artery atherosclerosis at autopsy.

Observations of the adverse effects of treatment of hypertension and hypotension in humans and the apparent frequent failure of readjustment of the lower limit of autoregulation with treatment of hypertension suggest that pharmacologic reduction in blood pressure might have played a role in the pathogenesis of the hemispheric strokes in these two monkeys. It is also possible that the dysautoregulation of cerebral blood flow noted by Meyer et al\textsuperscript{19} could have been a factor in the monkey with the prior cortical and subcortical infarct.

The other determinant factor in these infarcts appears to be the topography of atherosclerosis. In both
Brains location of the infarcts was closely related to the extent of atherosclerotic narrowing of the extracranial and intracranial cerebral arteries. The border zone infarct in Monkey H-593 was associated with severe atherosclerosis in the ipsilateral extracranial internal carotid and proximal anterior and middle cerebral arteries, while the hemispheric infarct was associated with these narrowed vessels and a severely involved ipsilateral posterior cerebral artery. Monkey H-633 had severe atherosclerosis ipsilateral to the recent infarct involving the extracranial internal carotid, middle cerebral, and posterior cerebral arteries with complete occlusion of the ipsilateral posterior communicating artery and severe atherosclerosis at several levels of the basilar artery. Furthermore, the extent of involvement of the anterior cerebral artery territory in the two monkeys was also closely correlated with the distribution of atherosclerotic plaques. In cynomolgus monkeys the proximal anterior cerebral arteries unite into a single, unpaired (azygous) anterior cerebral artery (referred to as the main anterior cerebral artery in Figure 4) that supplies both cerebral hemispheres. In Monkey H-633 the extent of luminal narrowing in these anterior cerebral arteries was less than that in Monkey H-593. Correspondingly, in Monkey H-633 there was sparing of part of the ipsilateral anterior cerebral artery territory and in Monkey H-593 involvement of the anterior cerebral artery territories in both cerebral hemispheres.

Thus, these two recent hemispheric strokes appear to be related to the topography of severe atherosclerotic narrowing of the cerebral arteries and to pharmacologic reduction of elevated blood pressure. Other possible mechanisms include a further reduction in blood pressure due to an unobserved cardiac arrhythmia or drug-induced postural hypotension. Whatever the mechanism, the occurrence of strokes in both humans and monkeys in this setting underscores the potential hazard of excessive treatment of hypertension in the presence of severe occlusive atherosclerosis.

Figure 4. Distribution of percent stenosis of cerebral arteries narrowed by atherosclerosis in monkeys H-633 (A) and H-593 (B). MAC, main anterior cerebral artery; PAC, proximal anterior cerebral artery; ICA, extracranial internal carotid artery; MCA, middle cerebral artery; PCom, posterior communicating artery; PC, posterior cerebral artery; SCA, superior cerebellar artery; B, basilar artery; AICA, anterior inferior cerebellar artery; PICA, posterior inferior cerebellar artery; V, vertebral artery.

References
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