Effect of Atherosclerosis on Cerebral Vascular Responses to Activation of Leukocytes and Platelets in Monkeys

Frank M. Faraci, PhD; J. Antonio G. Lopez, MD; Keith Breese; Mark L. Armstrong, MD; and Donald D. Heistad, MD

The goal of this study was to test the hypothesis that atherosclerosis alters responses of cerebral arteries and the ocular circulation to the activation in vivo of leukocytes and platelets. We measured blood flow to the brain and eye using microspheres and pressure in the cerebral microvessels of normal and atherosclerotic monkeys. The intracarotid injection of $10^{-7}$ M N-formyl-L-methionyl-L-leucyl-L-phenylalanine to activate leukocytes did not alter cerebral blood flow in 11 normal or 10 atherosclerotic monkeys but increased the resistance of large cerebral arteries by 46±11% (mean±SEM) in the atherosclerotic animals. The injection of N-formyl-L-methionyl-L-leucyl-L-phenylalanine did not alter blood flow to the eye in 10 normal monkeys but decreased blood flow to the choroid by 38±9% in 11 atherosclerotic monkeys. The intracarotid injection of $3\times10^{-8}$ M prostaglandin E$_2$, a leukocyte product, produced an increase in the resistance of large cerebral arteries in five atherosclerotic but not in six normal monkeys. Prostaglandin E$_2$ reduced blood flow to the retina and choroid in the atherosclerotic monkeys by 62±22% and 65±17%, respectively. The intracarotid infusion of 25 µg/min collagen to activate platelets increased cerebral blood flow by 21±5% in 10 normal monkeys but did not alter it in 11 atherosclerotic monkeys. Collagen did not alter blood flow to the choroid in 10 normal monkeys but decreased it by 29±8% in 11 atherosclerotic monkeys. Thus, atherosclerosis potentiates the constrictor responses of large cerebral arteries and blood vessels of the eye to leukocyte activation and prostaglandin E$_2$ and impairs the cerebral vasodilator responses to platelet activation in vivo. (Stroke 1991;22:790-796)
leukocytes by the intracarotid injection of fMLP and examined the responses to PGE\textsubscript{2}, a major product released by monocytes and macrophages.

**Materials and Methods**

We studied two groups of adult cynomolgus monkeys. Normal monkeys were fed commercial chow (Purina monkey chow, Ralston Purina, Richmond, Ind.), which produced a mean±SEM plasma cholesterol concentration of 110±11 mg/dl. In a second group of monkeys, atherosclerosis was induced by feeding an atherogenic diet that contained 41% of total calories from fat and 0.8% cholesterol for 18 months. Mean±SEM plasma cholesterol concentration in this group was 693±48 mg/dl.

Each monkey was sedated with 12 mg/kg i.v. ketamine and then anesthetized with 75–100 mg/kg i.v. α-chloralose. Supplemental anesthesia was administered as needed. The trachea was cannulated, and the monkey was ventilated mechanically with room air and supplemental oxygen.

A catheter was inserted into a femoral artery and advanced into the aorta for the measurement of pressure and the sampling of arterial blood. A femoral vein was cannulated for the infusion of supplemental anesthetic. Catheters were inserted into the left atrial appendage for the injection of microspheres and into both brachial arteries for the withdrawal of reference blood samples during the injection of microspheres. Rectal temperature was monitored and maintained at 37-38°C with a heating pad. The external carotid arteries were exposed and ligated at the carotid bifurcation. Catheters were inserted into the lingual arteries for the infusion of fMLP, collagen, or PGE\textsubscript{2} at a rate of 0.2 ml/min.

After the insertion of all catheters, the animal was placed in a head holder and a craniotomy was made over the right parietal cortex as described in detail.\textsuperscript{4,5} The dura mater was incised to expose pial vessels on the cerebrum. Artificial cerebrospinal fluid (CSF) equilibrated with gases and warmed to 37°C was continually suffused over the exposed portion of the brain. For all monkeys, CSF sampled from the craniotomy had a PC\textsubscript{O}\textsubscript{2} of 38±1 mm Hg, a PO\textsubscript{2} of 62±1 mm Hg, and a pH of 7.35±0.01 (mean±SEM for all).

Blood flow was measured using radioactive microspheres 15 μm in diameter as described in detail for monkeys.\textsuperscript{4,5} At the end of the experiment, anesthetized animals were killed with intravenous KCl and the brain and eyes were removed and dissected into regional samples.

Cerebral microvascular pressure was measured using a sharpened micropipette with a 2–4 μm tip diameter coupled to a servo-null device (model 4A, Instrumentation for Physiology and Medicine, Inc., San Diego, Calif.). The tip of the micropipette was inserted into the lumen of a pial artery on the surface of the parietal cortex using a micromanipulator. Vessels were observed with a microscope coupled to a video camera and videocassette recorder. The diameter of pial arteries was measured with an electronic micrometer. Pressure was measured in pial arteries with a mean±SEM diameter of 316±25 μm in normal monkeys and 285±41 μm in atherosclerotic monkeys (p>0.05 versus normal monkeys).

Large artery resistance was calculated as (aortic pressure−pial artery pressure)/blood flow to the cerebrum. Total cerebral vascular resistance was calculated as aortic pressure/blood flow to the cerebrum.

Cerebral microvascular pressure, cerebral blood flow, and blood flow to the eye were measured under control conditions, following an intracarotid injection of fMLP, and during the intracarotid infusion of collagen and PGE\textsubscript{2}.

Three pairs of measurements were made. First, in eight normal and eight atherosclerotic monkeys, blood flow and pial artery pressure were measured under control conditions and 3 minutes following a bilateral intracarotid injection of 10\textsuperscript{−7} M fMLP. Preliminary studies in the hind limb comparing injection and infusion indicated that maximum responses to fMLP were obtained with injection of the peptide. In an additional three normal and three atherosclerotic monkeys, measurements were made following a unilateral injection of fMLP into the right carotid artery. We examined the responses to unilateral injection to test the hypothesis that constriction of one carotid artery is sufficient to affect blood flow to the brain and eye. When fMLP was injected unilaterally, cerebral microvascular pressure was measured on the ipsilateral hemisphere.

Second, in 10 normal monkeys (seven with bilateral and three with unilateral infusion) and 11 atherosclerotic monkeys (eight with bilateral and three with unilateral infusion), cerebral microvascular pressure and blood flow were again measured under control conditions and during a 10-minute infusion of collagen (25 μg/min through each catheter). Studies in the monkey hind limb indicate that infusion of collagen at 50 μg/min produces a decrease in the platelet count in venous blood.\textsuperscript{10} In the present experiments, platelet aggregates could be seen in the pial microcirculation during the infusion of collagen.

Third, in six normal and six atherosclerotic monkeys (three with bilateral and three with unilateral infusion in each group), blood flow and cerebral microvascular pressure were measured under control conditions and during a 5-minute intracarotid infusion of 3×10\textsuperscript{−9} M PGE\textsubscript{2}.

Statistical analysis was performed using paired t tests in that absolute values during interventions were compared with the preceding control value. Cerebral vascular responses were similar following unilateral and bilateral infusions and were therefore combined. A probability value of less than 0.05 was considered significant. All values are presented as mean±SEM.

**Results**

In normal monkeys, the common and internal carotid arteries were thin-walled with no gross or microscopic evidence of atherosclerotic lesions. In
Table 1. Effects of fMLP and Collagen in 11 Normal and 11 Atherosclerotic Monkeys

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal monkeys</th>
<th>Atherosclerotic monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=11)</td>
<td>fMLP (n=10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control (n=10)</td>
</tr>
<tr>
<td>Aortic pressure (mm Hg)</td>
<td>89±4</td>
<td>87±4</td>
</tr>
<tr>
<td>Aortic-pial</td>
<td>24±2</td>
<td>25±2</td>
</tr>
<tr>
<td>Cerebral blood flow (ml/min/100 g)</td>
<td>27±2</td>
<td>28±2</td>
</tr>
<tr>
<td>Vascular resistance (mm Hg/ml/min/100 g)</td>
<td>3.3±0.2</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>Total</td>
<td>29±2</td>
<td>33±2</td>
</tr>
<tr>
<td>Large arteries</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>39±1</td>
<td>39±1</td>
</tr>
<tr>
<td>Pao2 (mm Hg)</td>
<td>110±4</td>
<td>109±4</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.41±0.01</td>
<td>7.37±0.01</td>
</tr>
</tbody>
</table>

fMLP, N-formyl-L-methionyl-L-leucyl-L-phenylalanine. Values are mean±SEM.
*p<0.05 different from control by paired t test.

Atherosclerotic monkeys, lesions of the common carotid and proximal internal carotid arteries were observed that ranged from fatty streaks to fibrofatty plaques. Microscopic examination revealed diffuse lesions throughout the common carotid and proximal internal carotid arteries. We have previously examined cranial vessels in this model morphometrically and observed marked intimal proliferation of the extracranial carotid arteries.4

Under control conditions, values for aortic pressure, cerebral blood flow, and resistance of large cerebral arteries were similar in normal and atherosclerotic monkeys (Table 1). The intracarotid injection of fMLP had little effect on these variables in normal monkeys (Table 1). In contrast, fMLP produced a striking reduction in cerebral microvascular pressure in atherosclerotic monkeys (Figure 1). The injection of fMLP increased the large artery pressure gradient and the resistance of large cerebral arteries (Figure 2) in atherosclerotic animals. Constriction of the large arteries was not sufficient to reduce cerebral blood flow. Pial artery pressure returned to control levels 20–30 minutes following the injection of fMLP in atherosclerotic monkeys. Under control conditions, blood flow to the eye was similar in normal and atherosclerotic monkeys (9±1 and 7±1 ml/min/100 g, respectively, for the retina; 391±77 and 314±63 ml/min/100 g, respectively, for the choroid). The injection of fMLP had no significant effect on blood flow to the eye in normal monkeys (Figure 3) and significantly reduced blood flow to the choroid in atherosclerotic monkeys (p<0.05) (Figure 3).

In normal monkeys, the infusion of PGE2 had no significant effect on cerebral hemodynamics (Table 2). In contrast, PGE2 significantly increased the resistance of large cerebral arteries in atherosclerotic monkeys (Figure 4, Table 2). An example of the response to PGE2 is shown in Figure 5. PGE2 did not significantly alter blood flow to the eye in normal monkeys. Blood flow to the retina tended to increase
in response to PGE₂, but the response was variable (Figure 6). PGE₂ produced a marked reduction in blood flow to the retina and choroid of the eye in atherosclerotic animals ($p<0.05$ versus control for retina and choroid) (Figure 6).

In normal monkeys, the intracarotid infusion of collagen decreased the resistance of large cerebral arteries and the total cerebral vascular resistance and produced a $21\pm5\%$ increase in cerebral blood flow (Figure 7, Table 1). In contrast, collagen failed to alter the resistance of large cerebral arteries, the total cerebral vascular resistance, or cerebral blood flow in atherosclerotic monkeys (Figure 7, Table 1). In normal monkeys, collagen had no effect on blood flow to the choroid and significantly increased blood flow to the retina (Figure 8). In atherosclerotic monkeys, collagen failed to increase retinal blood flow and significantly decreased blood flow to the choroid ($p<0.05$).

**Discussion**

This is the first study to examine the effects of atherosclerosis on responses of the cerebral or ocular circulation to the activation of leukocytes and platelets. There are several major new findings. First, the activation of leukocytes in vivo with fMLP had little effect in normal monkeys but pronounced effects on the cerebral and ocular circulations of atherosclerotic monkeys. Second, PGE₂, which is produced by monocytes and macrophages, also had little effect in

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**TABLE 2. Effects of PGE₂ in Six Normal and Five Atherosclerotic Monkeys**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Normal monkeys</th>
<th>Atherosclerotic monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (mm Hg)</td>
<td>Control</td>
<td>93±2</td>
<td>92±2</td>
</tr>
<tr>
<td>Aortic</td>
<td></td>
<td>6±4</td>
<td>28±4</td>
</tr>
<tr>
<td>Aortic–pial</td>
<td></td>
<td>10±0.2</td>
<td>10±0.2</td>
</tr>
<tr>
<td>Cerebral blood flow (ml/min×100 g⁻¹)</td>
<td></td>
<td>28±2</td>
<td>30±2</td>
</tr>
<tr>
<td>Vascular resistance (mm Hg×ml⁻¹×min×100 g)</td>
<td></td>
<td>3.6±0.3</td>
<td>3.2±0.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10±0.2</td>
<td>10±0.2</td>
</tr>
<tr>
<td>Large arteries</td>
<td></td>
<td>38±1</td>
<td>...</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td></td>
<td>114±6</td>
<td>...</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td></td>
<td>7.36±0.02</td>
<td>...</td>
</tr>
<tr>
<td>Arterial pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atherosclerotic monkeys</td>
<td>PGE₂</td>
<td>89±5</td>
<td>90±7</td>
</tr>
<tr>
<td>Aortic</td>
<td></td>
<td>26±3</td>
<td>37±5</td>
</tr>
<tr>
<td>Aortic–pial</td>
<td></td>
<td>11.1±0.2</td>
<td>15±0.3*</td>
</tr>
<tr>
<td>Cerebral blood flow (ml/min×100 g⁻¹)</td>
<td></td>
<td>25±2</td>
<td>26±3</td>
</tr>
<tr>
<td>Vascular resistance (mm Hg×ml⁻¹×min×100 g)</td>
<td></td>
<td>3.6±0.5</td>
<td>3.8±0.6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10.2±0.2</td>
<td>1.5±0.3*</td>
</tr>
<tr>
<td>Large arteries</td>
<td></td>
<td>38±1</td>
<td>...</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td></td>
<td>107±6</td>
<td>...</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td></td>
<td>7.36±0.02</td>
<td>...</td>
</tr>
</tbody>
</table>

PGE₂, prostaglandin E₂, Values are mean±SEM. *$p<0.05$ different from control by paired $t$ test.
normal animals but produced pronounced vasoconstriction in atherosclerotic monkeys. Third, in vivo aggregation of platelets by the intracarotid infusion of collagen produced cerebral vasodilatation in normal monkeys but not in atherosclerotic monkeys.

The peptide fMLP produces receptor-mediated activation of polymorphonuclear leukocytes and monocytes/macrophages. The intra-arterial injection of fMLP into the perfused hind limb produces a marked decrease in the leukocyte count in venous blood. Receptors for fMLP are not present on smooth muscle cells, and fMLP has little effect on the iliac arteries of normal monkeys in vitro. These findings suggest that fMLP has little direct effect on blood vessels.

In this study, fMLP may have activated leukocytes in the blood, attached to the endothelium, or within the blood vessel wall. A recent study suggested that fMLP may activate macrophagelike cells in the wall of human arteries. The relative importance of leukocytes within or attached to the vessel wall versus circulating leukocytes is not clear. The time course of the effects of fMLP does not help differentiate between these possibilities, because the duration of the effect should be influenced by both the site of activated leukocytes as well as the mediator(s) involved.

Mediators that account for fMLP-induced constriction of cerebral arteries in atherosclerotic monkeys are not defined. Activated leukocytes release several vasoactive substances, including thromboxane A2, PGE2, leukotrienes, platelet activating factor, and oxygen-derived free radicals.

Thromboxane is a potent constrictor of cerebral arteries and arterioles in vivo. We have shown previously that atherosclerosis potentiates constrictor responses to thromboxane in cerebral and ocular blood vessels. Thus, thromboxane may contribute to leukocyte-induced constriction of atherosclerotic blood vessels following the injection of fMLP.

The effects of PGE2 on cerebral blood vessels are complex. The topical application of PGE2 has been reported to produce dilatation of pial arterioles in vivo and contraction or relaxation of large cerebral arteries in vitro. Contraction of retinal vessels in...
response to PGE$_2$ has also been observed in vitro.$^{21}$

In the present study, intravascular PGE$_2$ had little effect on cerebral blood vessels or blood flow to the eye in normal animals. In contrast, PGE$_2$ produced marked constriction of large cerebral arteries and blood vessels of the eye in atherosclerotic animals. Atherosclerosis impairs endothelium-dependent mechanisms.$^{22}$ Because the responses of large cerebral arteries to PGE$_2$ are modulated by the endothelium,$^{20}$ it is possible that impaired endothelial function contributes to the augmented constrictor responses to PGE$_2$ in atherosclerotic monkeys.

Cerebral blood vessels are responsive to several other factors released by leukocytes, including leukotrienes,$^{23}$ platelet activating factor,$^{24}$ and oxygen-derived free radicals.$^{25,26}$ It is not known whether the responses of cerebral arteries to these substances are altered by atherosclerosis. Responses to fMLP are probably complex and, because of limitations in the number of blood flow measurements that can be made with microspheres, we were not able to examine the roles of all potential mediators of vasoconstriction. The finding that atherosclerosis potentiates cerebral vasoconstrictor responses to PGE$_2$ (present study) and thromboxane$^3$ supports the hypothesis that these eicosanoids may contribute to leukocyte-induced vasoconstriction but do not exclude the possibility that other mediators may also be involved.

Examination of the effects of fMLP following indomethacin should provide insight into the role of cyclooxygenase products in mediating responses to the activation of leukocytes.

 activated platelets release substantial quantities of adenosine diphosphate (ADP), serotonin, and thromboxane.$^3$ The intravascular infusion of collagen to activate platelets decreased the resistance of large cerebral arteries and increased cerebral blood flow in normal monkeys. It is likely that ADP is the mediator of the dilator response in normal animals. The major vasoactive product released by activated platelets is ADP, which is a potent dilator of large cerebral arteries.$^{27}$

Dilatation of large cerebral arteries in response to the activation of platelets with collagen was impaired in atherosclerotic animals. Endothelium-dependent relaxation is impaired in atherosclerotic blood vessels.$^{22}$ Because the ADP-induced relaxation of large cerebral arteries is endothelium dependent,$^{27}$ we speculate that impaired dilator responses to platelet activation in vivo are related in part to this defect. Relaxation in response to aggregating platelets is impaired in basilar arteries from hypercholesterolemic pigs in vitro.$^{28}$

In preliminary studies of the perfused hind limb in normal and atherosclerotic monkeys, we observed that the infusion of collagen for 10 minutes produces transient dilatation followed by sustained constriction.$^{10}$ Vasodilatation was impaired and constriction was augmented in atherosclerotic monkeys. Based on these findings, we injected microspheres to measure blood flow near the end of a 10-minute infusion of collagen into the carotid arteries in the present study. Although we made only one measurement of blood flow, cerebral microvascular pressure was stable during the infusion of collagen, which suggests that we did not miss transient effects on the resistance of large cerebral arteries.
Mechanisms that produce enhanced constriction of atherosclerotic blood vessels are not clear. We and others have suggested that altered responses to products that are released when platelets aggregate at atherosclerotic lesions may contribute to enhanced vasoconstriction. The present study suggests that responses of cerebral and ocular blood vessels to the aggregation of platelets in vivo are altered in a direction that favors vasoconstriction during atherosclerosis.

The present study suggests that constrictor responses of cerebral blood vessels to the activation of leukocytes and to PGE₂, a major product of activated leukocytes, are enhanced markedly by atherosclerosis. We speculate that in the presence of a stenosis or partial obstruction of a cerebral artery by an embolus, a reduction in cerebral microvascular pressure in response to products released by leukocytes may contribute to cerebral ischemia during transient ischemic attacks. In the eye, a reduction in ocular perfusion in atherosclerotic monkeys can impair vision. Thus, if vasoconstriction plays a role in the pathophysiology of transient ischemic attacks and amaurosis fugax, we propose that the activation of leukocytes may contribute to these clinical states.

Acknowledgment

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References


Key Words • cerebral blood flow • retina • prostaglandins E • monkeys
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