Blood Flow through Vasa Vasorum of Coronary Arteries in Atherosclerotic Monkeys

Donald D. Heistad and Mark L. Armstrong

Morphologic studies and postmortem arteriograms provide qualitative evidence that atherosclerosis induces neovascularization in coronary arteries. In this study we obtained the first measurements of blood flow through vasa vasorum in atherosclerotic coronary arteries. We measured blood flow with microspheres to intima media and to adventitia in normal and atherosclerotic cynomolgus monkeys. Blood flow to adventitia was not altered by atherosclerosis. The blood flow to intima media, however, was increased more than fivefold by atherosclerosis: flow was $3 \pm 1$ in normal animals and $16 \pm 5$ in atherosclerotic monkeys ($p < 0.05$). Blood flow was threefold greater in atherosclerotic than in normal coronary arteries during adenosine-induced vasodilation. These results suggest that proliferation of new vessels, not dilatation of existing vessels, accounts for the increase in flow through vasa in intima media. Histologic studies indicated that vasa vasorum to intima media in atherosclerotic coronary arteries originate from adventitial vasa. We conclude that: 1) there is a large increase in blood flow through vasa vasorum to intima media, but not to adventitia, in atherosclerotic coronary arteries; and 2) increased flow is produced primarily by proliferation of new vessels that originate from adventitial vasa.


The nourishment of arteries is accomplished by diffusion from the lumen of arteries and from vasa vasorum. Most arteries have a rather extensive network of adventitial, but not medial, vasa. In the thoracic aorta of large species, branches of adventitial vasa penetrate into the media and provide an important source of nourishment.

Vasa vasorum are seen only rarely in media of normal coronary arteries in dogs and humans, but they are seen much more commonly in atherosclerotic coronary arteries. Proliferation of vasa in the intima and media, with an increased propensity to intramural hemorrhage, has been proposed as one mechanism of coronary occlusion in atherosclerosis. In addition, postmortem angiograms in humans suggest that there is proliferation of vasa in atherosclerotic coronary arteries, and it has been suggested that the vessels may be exposed to high concentrations of circulating vasoconstrictor substances.

We have developed a method that provides the first measurements of blood flow through vasa vasorum. We found that in normal dogs vasa provide substantial amounts of blood flow to the media of the thoracic aorta, but only minimal flow to media of coronary arteries. In atherosclerotic monkeys, we found a pronounced increase in blood flow through vasa vasorum in the intima-media of the aorta.

In this study, we have obtained the first measurements of blood flow through vasa vasorum in atherosclerotic coronary arteries. Our goal was to determine whether there is an increase in blood flow through vasa vasorum to the intima media and to the adventitia of coronary arteries in atherosclerotic monkeys. We have suggested that medial vasa are more effective than adventitial vasa in the nourishment of blood vessels. Thus, it was important to separately determine the flow through vasa to intima media and to adventitia.

In addition, we attempted to determine whether atherosclerosis increases the flow through vasa vasorum by formation of new vessels or by dilatation of existing vessels. Blood flow was measured during vasodilatation produced by adenosine. Our premise was that if atherosclerosis stimulates formation of new vessels in the aortic wall, blood flow through coronary vasa during dilatation should be greater in atherosclerotic than in normal monkeys.

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Methods

Adult male cynomolgus monkeys were studied. Control monkeys (n = 10) were fed Purina Monkey Chow (Ralston Purina Company) that contains approximately 4% fat and is virtually free of cholesterol. The control monkeys weighed 5.3 ± 0.4 kg (mean ± SE). Atherosclerosis was induced by feeding eight monkeys a diet with 41% fat and 0.8% cholesterol for 17 ± 1 months. The atherosclerotic monkeys weighed 5.4 ± 0.3 kg. Venous blood samples were drawn at 2-month intervals after the monkeys had been sedated with ketamine HCl 12 mg/kg given intramuscularly (i.m.). The total plasma cholesterol and triglyceride levels were determined by the methods of the Lipid Research Clinics protocol. The study conformed to the guiding principles of the American Physiological Society for animal research.

Measurement of Blood Flow through Vasa Vasorum

The monkeys were sedated with ketamine HCl (12 mg/kg, i.m.) and anesthetized with chloralose (75 mg/kg) intravenously (i.v.). The monkeys were intubated and ventilated with room air and supplemental oxygen. Polyethylene catheters were inserted into a brachial artery for measurement of arterial pressure, into a brachial and common carotid artery for withdrawal of reference blood samples, and into a femoral vein for infusion of drugs. A thoracotomy was performed and a catheter was placed in the left atrium for injection of microspheres. The monkeys were paralyzed with decamethonium bromide (0.5 mg/kg, i.v.) and given heparin (500 units/kg, i.v.). Blood gases and pH were measured frequently and maintained at normal levels.

Microspheres with a mean diameter of 15 μm were injected into the left atrium to measure the blood flow through vasa vasorum. Because blood flow through a tissue is calculated by determining the number of microspheres that lodge in capillaries of the tissue, the method measures capillary blood flow and not flow through an artery. Reference arterial blood samples were withdrawn at 1.03 ml/min starting 20 seconds before injection of microspheres and continuing for 2 minutes after injection. We injected 2 to 4 × 10⁶ spheres to measure blood flow under control conditions. The blood flow was also measured by injection of microspheres labelled with another isotope during infusion of adenosine. Our goal was to compare the dilator response of vasa vasorum in normal and atherosclerotic coronary arteries. We infused adenosine (5 μM/kg per minute i.v.) at a dose that appears to produce maximal dilatation of vasa vasorum. In half of the monkeys, blood flow was measured under control conditions and then during infusion of adenosine; in half of the monkeys, the order was reversed.

The monkeys were sacrificed with KCl (i.v.) at the end of each experiment. The proximal 2 to 3 cm of left anterior descending, circumflex, and right coronary arteries were removed and placed in 10% buffered formalin for 2 days. The intima and media were stripped from the adventitia, with the aid of a dissecting microscope, by using the methods of Wolinsky and Daly. A critical aspect of this approach is that samples of intima media that are virtually uncontaminated by adventitia are obtained. Histological examination of the intima media indicated minimal contamination (0 to 5%) with adventitia, and minimal contamination of adventitial samples with media. Samples of intima media, adventitia, and blood were weighed, placed in plastic tubes, and counted in a gamma counter, as described previously. The radioactivity in counts per minute (cpm) was 258 ± 68 and 39 ± 18 in the adventitia and media of normal monkeys, and 1167 ± 378 and 1929 ± 1127 in the adventitia and intima media of atherosclerotic monkeys. Blood flow through vasa vasorum was calculated from the equation:

\[
\text{flow (ml/min per 100 g vessel)} = \frac{\text{counts/g of coronary artery} \times 100 \times \text{rate of withdrawal of reference arterial blood samples, in ml/min}}{\text{total counts in reference arterial blood}}
\]

An average value for counts in the two blood samples was calculated. Samples of left ventricle also were obtained, and the blood flow was calculated during control conditions and infusion of adenosine.

The results are expressed as mean ± SE. Vascular conductance was calculated from the equation:

\[
\text{conductance} = \frac{\text{blood flow (in ml/min/g vessel)}}{\text{mean arterial pressure}}
\]

We compared the values in normal and atherosclerotic monkeys by unpaired t tests.

Morphologic Examination

The coronary arteries were examined grossly for the extent of atherosclerosis. Sections of formalin-fixed coronary arteries were examined with light microscopy. To visualize vasa vasorum in coronary arteries, we infused colloidal carbon in two normal and three atherosclerotic monkeys. After the monkeys were sacrificed, the proximal aorta was isolated by ligation of the ascending aorta and by inflation of a balloon in the left ventricle. Approximately 30 ml of colloidal carbon was infused into the proximal aorta. The coronary arteries were removed, fixed in formalin, dehydrated by graded alcohols, and made translucent by clearing with methyl salicylate and benzyl benzate. Very thick sections, approximately 1 mm in thickness, were cut and examined microscopically.

Results

Plasma Lipids

Plasma cholesterol increased five- to sixfold within 2 months after institution of the atherogenic diet and changed little thereafter. Triglycerides were not elevated by the atherogenic diet. At the terminal measurement, the total cholesterol level was 86 ± 7 mg/dl in normal monkeys and 600 ± 36 mg/dl in atherosclerotic monkeys. Triglycerides were 43 ± 10 mg/dl in normal and 39 ± 9 mg/dl in atherosclerotic monkeys. The increase in cholesterol is produced primarily by an increase in concentration of low density lipoproteins.
Figure 1. Thick section (approximately 1 mm) of the coronary artery wall from two atherosclerotic monkeys. Colloidal carbon was infused, and the vessels were made translucent by the Spalteholz method. Arrows indicate the junction of adventitia and intima media, identified in sections stained with hematoxylin-eosin. A. There is an extensive network of adventitial vasa vasorum, and vasa penetrate into the intima media. B. Although the atherosclerotic lesion is less severe, vasa vasorum nevertheless penetrates into the intima media.
Morphology

In normal monkeys, the coronary arteries were thin-walled, without gross or microscopic evidence of atherosclerotic lesions. In monkeys that were fed the atherogenic diet, the epicardial coronary arteries were thickened and elongated. Microscopic examination showed diffuse lesions throughout the epicardial arteries. The lesions ranged from fatty streaks to fibrofatty plaques, and the mean thickness of the lesions was greater than the medial thickness. Focal necrosis was frequent, and there was an occasional calcification of the lesions. Early atherosclerotic lesions were observed in occasional small (< 200 µm) epicardial arteries and rarely in adventitial vasa.

In thick sections that were prepared for examination of vasa vasorum by the injection of colloidal carbon and by making the tissue translucent, opacified vasa were moderately dense in the adventitia of coronary arteries in both normal and atherosclerotic monkeys. No vasa were seen in the media of normal coronary arteries, but in atherosclerotic arteries, we often observed vasa branching from adventitial vessels into media (Figure 1). Intimal vasa were not seen.

Blood Flow through Vasa Vasorum

Under control conditions, the mean arterial pressure was 68 ± 7 mm Hg in normal monkeys and 69 ± 3 mm Hg in atherosclerotic monkeys. In normal monkeys, arterial blood pO₂ was 103 ± 9 mm Hg, pCO₂ was 33 ± 1 mm Hg, and pH was 7.41 ± 0.02. In atherosclerotic monkeys, pO₂ was 106 ± 14 mm Hg, pCO₂ was 33 ± 2 mm Hg, and pH was 7.40 ± 0.02.

In normal monkeys, there was minimal blood flow through the vasa vasorum to the intima media of the coronary arteries (Figure 2) and substantial levels of flow to the adventitia (Figure 3). Thus, in normal monkeys, as in normal dogs, the media of coronary arteries must be nourished primarily by diffusion from the lumen of the artery and from the adventitial vasa.

The blood flow and conductance of the vasa vasorum in the intima media were increased five- to sixfold in atherosclerotic coronary arteries (Figure 2). The blood flow and conductance to adventitia in coronary arteries were not altered by atherosclerosis (Figure 3).

The infusion of adenosine was efficacious as a vasodilator: the myocardial conductance was 2.1 ± 0.2 and 7.5 ± 0.6 ml/min per g per mm Hg respectively, during control and adenosine infusion in normal monkeys, and 2.0 ± 0.3 and 6.8 ± 1.4 respectively, during control and adenosine infusion in atherosclerotic monkeys. The mean arterial pressure during adenosine infusion was 38 ± 4 mm Hg in normal monkeys and 40 ± 3 mm Hg in atherosclerotic monkeys. During the infusion of adenosine, the blood flow and conductance of vasa vasorum in the intima media were threefold greater in the coronary arteries of atherosclerotic monkeys than in normal monkeys (Figure 4). The blood flow and conductance to adventitia were similar in normal and atherosclerotic coronary arteries during the adenosine infusion (Figure 5).
The number of microspheres that lodge in the capillaries of a tissue is proportional to the blood flow to that tissue. We have previously used microspheres to measure the blood flow in normal arteries and in the atherosclerotic aorta, under control conditions and during the infusion of adenosine. The assumptions of the method appear to be valid when spheres 15 μm in diameter are used to measure the blood flow through vasa. Because only small sized samples of coronary arteries can be obtained, the number of spheres and the radioactivity in the tissue samples are relatively small. When the number of microspheres and the radioactivity in a tissue sample are small, the absolute values are not compromised but the variance is increased. We therefore injected a large number of microspheres (2 to 4 million) to compensate for the small size of tissue samples. Using this approach, we were able to reduce the variance.

The number of microspheres that lodge in the capillaries of a tissue is proportional to the blood flow to that tissue. Thus, adventitial blood flow indicates flow to the capillary bed of the adventitia, and does not reflect the blood flow through adventitial arteries and arterioles to the underlying media.

Discussion

The major new findings of this study are: 1) there is minimal blood flow through the vasa vasorum to the media of coronary arteries in normal monkeys, but substantial flow to the adventitial vasa; 2) there is a marked increase in blood flow through vasa to the intima media of atherosclerotic coronary arteries, but no increase in flow to the adventitial vasa; 3) during vasodilation, the blood flow through the vasa vasorum to the intima media is threefold greater in atherosclerotic than in normal coronary arteries. This finding suggests that formation of new vessels, not dilatation of existing vessels, accounts for the increase in blood flow through vasa in atherosclerotic coronary arteries.

Methods for Measurement of Flow

We have previously used microspheres to measure the blood flow in normal arteries and in the atherosclerotic aorta, under control conditions and during the infusion of adenosine. The assumptions of the method appear to be valid when spheres 15 μm in diameter are used to measure the blood flow through vasa. Because only small sized samples of coronary arteries can be obtained, the number of spheres and the radioactivity in the tissue samples are relatively small. When the number of microspheres and the radioactivity in a tissue sample are small, the absolute values are not compromised but the variance is increased. We therefore injected a large number of microspheres (2 to 4 million) to compensate for the small size of tissue samples. Using this approach, we were able to reduce the variance.

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Evidence for Proliferation of Vasa

Morphologic studies of the aorta and coronary arteries indicate that there is proliferation of vasa vasorum in atherosclerotic vessels. Postmortem arteriograms also indicate that there is an extensive network of vasa in atherosclerotic coronary arteries in humans. Our study confirms and extends previous studies that suggest that proliferation of vasa occurs in atherosclerotic coronary arteries.

Adenosine was infused in this study to determine whether blood flow was increased in the intima media of atherosclerotic coronary arteries by dilatation of existing vessels or by formation of new vessels. We reasoned that if resting blood flow is increased in atherosclerotic coronary arteries as the result of dilatation of existing vessels, infusion of adenosine in normal monkeys should increase conductance to the level observed in atherosclerotic monkeys. We found, however, that blood flow and conductance of vasa during infusions of adenosine remained much higher in atherosclerotic coronary arteries than in normal arteries. This finding indicates that increased blood flow in the intima media of atherosclerotic coronary arteries is not produced primarily by dilatation of existing vasa and must reflect proliferation of new vessels in intima media of the coronary arteries.

In contrast to the hyperemia of intima media, there was no increase in blood flow to the adventitia in atherosclerotic monkeys, under control conditions or during infusion of adenosine. Thus, the stimulus for angiogenesis in atherosclerotic coronary arteries apparently affects the intima media, but not the adventitia.

Stimulus for Vasa Formation

We have speculated previously that several factors may contribute to hyperemia of atherosclerotic vessels. Stimuli to neovascularization and hyperemia in atherosclerotic vessels may include increases in vascular oxygen consumption, intimal proliferation with increases in diffusion distance of substrate, and alterations in radial stress.

A recent study suggests that the efficacy of an angiogenesis factor depends on pO2. The finding may be relevant to neovascularization in atherosclerotic arteries. Tissue pO2 has been reported to decrease in the aorta of atherosclerotic rabbits, presumably because wall thickness and diffusion distances are increased by atherosclerosis. Thus, production of an angiogenesis factor in atherosclerotic vessels, with reduction of pO2 in the wall of the vessel, may be synergistic in the stimulation of growth of vasa vasorum. A synergistic effect of an angiogenesis factor and a reduction in pO2 in the vessel wall may also explain the preferential increase in blood flow to the intima media, but not to the adventitia, of atherosclerotic coronary arteries.

Implications of Findings

In normal monkeys, there is minimal blood flow through vasa vasorum to the intima media of coronary arteries. Thus, coronary arteries in monkeys, as well as dogs, must be nourished by diffusion from the lumen of the artery and...
from adventitial vasa. The pronounced increase in blood flow to the intima media in atherosclerotic coronary arteries suggests that vasa in intima media play an increased role in nourishment of these vessels.

The structure of new vasa differs from that of normal vasa, and it appears that new vessels are relatively fragile. It has been suggested that a rupture of vasa may produce intimal hemorrhage and coronary occlusion. Others have suggested that intimal dissection from the lumen, not rupture of vasa, may account for intimal hemorrhages. Our findings do not address this question, but it seems reasonable to speculate that proliferation of thinned vasa vasorum in the intima media may predispose to intimal or subintimal hemorrhage. It has also been proposed that new vasa may deliver increased amounts of vasoactive substances to the wall of the coronary arteries, and thereby predispose them to vascular spasm. Our findings are compatible with this hypothesis.

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