Hyperemia of the Aortic Wall in Atherosclerotic Monkeys

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SUMMARY The aortic wall is nourished by diffusion from the aortic lumen and from vasa vasorum in the adventitia and outer layers of media. Intimal proliferation in atherosclerosis might be expected to reduce the effectiveness of diffusion from the lumen and increase dependence on nourishment by vasa. This study was performed to determine whether there is increased perfusion of the aortic wall by vasa vasorum in atherosclerosis. We used microspheres to measure flow through vasa in normal and atherosclerotic cynomolgus monkeys. Blood flow to inner layers of the thoracic and abdominal aorta was less than 1 ml/min x 100 g in normal monkeys, and there was a minimal increase in atherosclerotic monkeys. Flow to the outer layers of the thoracic and abdominal aorta was 1.3 ± 0.9 and 2.2 ± 0.8 ml/min per 100 g in normal monkeys. Flow to outer layers of the thoracic and abdominal aorta was increased in atherosclerotic monkeys to 17 ± 8.9 and 31 ± 12 ml/min per 100 g (P < 0.05 vs. normal).

Thus there is increased perfusion of the atherosclerotic aorta, particularly in the outer layers. During maximal vasodilation induced by infusion of adenosine, flow through vasa was 3- to 8-fold greater in atherosclerotic than in normal monkeys. This finding suggests that proliferation of new vessels, rather than dilation of existing vessels, accounts for the increase in blood flow through vasa. We speculate that hyperemia of the aortic wall in atherosclerosis may be in part a compensatory response to increased oxygen requirements and possibly to ischemia produced by intimal proliferation and a resulting increase in diffusion distance. Circ Res 48: 669-675, 1981

ARTERIES apparently are nourished by diffusion from the lumen of the artery and from adventitial vessels (Geiringer, 1951; Wolinsky and Glagov, 1967). The thoracic aorta of large species receives additional nourishment from vasa vasorum which penetrate from the adventitia into outer layers of aortic media. These medial vasa in effect shorten the diffusion distance in the aortic wall.

Most studies of vasa vasorum have been limited to morphological observations. Recently we described a method to measure blood flow through aortic vasa vasorum (Heistad et al., 1978). We found that vasa provide considerable amounts of blood flow to the outer media of the thoracic aorta in normal dogs and minimal flow to inner layers of the aorta. Our studies also demonstrated that vasa vasorum are responsive to humoral (Heistad et al., 1978) and neural (Heistad et al., 1979) stimuli.

In normal humans and dogs, vasa vasorum in thoracic aortic media arise almost exclusively from adventitial vessels (Schlichter, 1946; Clarke, 1965). Only a few vasa originate from the lumen of the aorta (Schlichter, 1946; Woerner, 1959). In the atherosclerotic aorta, intimal proliferation extends the diffusion distance in the aorta. Thus, intimal proliferation might be expected to increase dependence on nourishment by vasa vasorum. Morphological studies suggest that vasa are more prominent in atherosclerotic than in normal aorta (Geiringer, 1951; Woerner, 1959). Vasa have been found to penetrate the intima from the lumen to supply the media of the atherosclerotic aorta. It has not been possible, however, to quantify the extent of proliferation of vasa, and it has not been clear whether there is enough proliferation of vasa to produce a detectable increase in perfusion of the aortic wall in atherosclerosis.

The primary purpose of this study was to determine whether blood flow through vasa vasorum is increased significantly in the atherosclerotic aorta. We attempted to determine whether increases in flow occur primarily in the outer layers of aortic media, in which occasional vasa normally are seen, or in the inner layers of the aorta, in which new vasa are demonstrable in atherosclerosis. We also attempted to determine whether atherosclerosis increases blood flow through vasa by dilation of existing vessels or by formation of new vessels in the aortic wall. Maximal vasodilator responses were examined in several normal and atherosclerotic monkeys during infusion of adenosine. We reasoned that, if atherosclerosis stimulates significant formation of new vessels in the aortic wall, blood flow during maximal dilator responses to adenosine should be greater in atherosclerotic than in normal monkeys.
Methods

We studied adult male cynomolgus monkeys that weighed 4–6 kg. Twelve control monkeys were fed commercial laboratory chow (Purina Monkey Chow, Ralston Purina Co.) that contains about 4% fat and is virtually free of cholesterol. In six monkeys, atherosclerosis was produced by feeding a diet containing 41% fat and 0.8% cholesterol (Armstrong et al., 1978) for 20 months. At 2-month intervals during ingestion of the normal or atherogenic diet, venous blood samples were drawn after the monkeys had been sedated with ketamine HCl 12 mg/kg, im. Total plasma cholesterol and triglycerides were determined by the method of Abell et al. (1952) as modified by the Lipid Research Clinics Program (1974) for the Auto-Analyzer II (Technicon Instruments Inc.). Triglycerides were measured by the corresponding method in the same protocol.

Measurement of Blood Flow through Vasa Vasorum

After sedation with ketamine HCl (12 mg/kg, im), anesthesia was induced with chloralose (75 mg/kg, iv). The monkeys were intubated and ventilated with room air and supplemental oxygen via a Harvard model 661 small animal respirator.

Polyethylene catheters (PE90) were inserted into both brachial arteries, one femoral artery and vein, and the splenic artery. 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, iv) and their blood was anticoagulated with heparin (500 units/kg, iv). Blood gases and pH were measured frequently with an IL 113 Blood Gas Analyzer and maintained at normal levels.

Microspheres 15 μm in diameter were injected into the left atrium to measure blood flow through vasa vasorum (Heistad et al., 1978). Injection of microspheres produced minimal changes in arterial pressure (< 5 mm Hg). Reference blood samples were withdrawn at 1.03 ml/min from the splenic artery and one brachial artery, starting 30 seconds before injection of spheres and continuing for 2 minutes after injection.

We obtained one measurement of blood flow by injecting 2–4 × 10^6 spheres labeled with 46Sc, 48Sr, 111Ce, or 125I. In three normal and three atherosclerotic monkeys, we obtained a second measurement of blood flow by injecting spheres labeled with another isotope during infusion of adenosine. These experiments were performed to determine the maximal dilator response of vasa vasorum in normal and atherosclerotic aorta. Maximal vasodilatation was produced by infusion of adenosine (5 μmol/kg per min, iv). Microspheres were injected 4–6 minutes after beginning the infusion of adenosine. Infusion of adenosine was continued for 2 minutes after injection of microspheres.

At the end of each experiment the monkey was exsanguinated. The descending thoracic aorta and abdominal aorta were removed and placed in 10% buffered formalin for 2 days. Media were separated from adventitia with the aid of a dissecting microscope, using the method of Wolinsky and Daly (1970). Histological examination indicated that only small amounts of adventitia remained. Segments of the aortic wall then were split into inner and outer layers. In normal monkeys, the weight of the inner layers (calculated as percent of total segment weight) constituted 43% of the thoracic aorta and 36% of the abdominal aorta. In atherosclerotic monkeys, the inner layers constituted 44% of the thoracic aorta samples and 45% of the abdominal aorta samples. Most tissue samples weighed 0.1–0.3 g.

The tissues and blood samples were weighed, placed in plastic tubes, and counted for 20 minutes in a 3-inch well-type γ counter, as described previously (Heistad et al., 1978, 1979). In normal monkeys, levels of radioactivity for each isotope ranged from 0 to 10 counts/min in inner layers of aorta and from 0 to 110 (usually less than 20) counts/min in outer layers. In atherosclerotic monkeys, radioactivity during control measurements ranged from 0 to 28 counts/min in inner layers of aorta and from 25 to 460 (usually 100–400) counts/min in outer layers. These values were obtained after subtraction of background radioactivity, which ranged from 8 to 30 counts/min (usually about 20 counts/min). Blood flow through vasa vasorum was calculated from the formula: 

\[ C_A \times 100 \times \text{RBF}/\text{CR} = \text{vasa vasorum blood flow (in ml/min per 100 g of aorta)}, \]

where \( C_A \) = counts per g of aortic sample, RBF = reference blood flow (rate of withdrawal of reference arterial blood samples, in ml/min), and CR = total counts in reference arterial blood. The counts in the two reference blood samples were averaged.

Results are expressed as mean ± se. Results are expressed as blood flow (in ml/min per 100 g) or as conductance (calculated from blood flow × 100/mean arterial pressure). Statistical analyses were performed by unpaired t-tests.

Autopsy Procedure

The extent of atherosclerosis was evaluated by gross examination. Light microscopic studies of preselected, lesion-prone sites were carried out in formalin fixed, processed full-thickness sections of the aorta. Electron micrographs were obtained from glutaraldehyde-fixed material taken from the inner layer.

Results

Plasma Lipids

The atherogenic diet produced a 5- to 6-fold increase in plasma cholesterol with little change in triglyceride level. After 2 months, total cholesterol was 97 ± 5 mg/dl in normal monkeys and 532 ± 24 mg/dl in atherosclerotic monkeys. Triglycerides were 24 ± 3 mg/dl in normal and 25 ± 3 mg/dl in
Atherosclerotic monkeys. These levels remained relatively constant during the duration of the study. The rise in cholesterol is produced largely by increased concentrations of low density lipoproteins (Kramsch and Hollander, 1968; Armstrong, 1976).

**Morphology**

Multivessel atherosclerosis was evident by gross examination in all experimental animals and was absent in control animals. In atherosclerotic monkeys, prominent intimal atherosclerotic changes were present in the thoracic and abdominal aorta, and medial atrophy was variable. The thickness of the thoracic and abdominal aorta was approximately 1.4 and 1.5 times greater, respectively, in atherosclerotic monkeys than in normal monkeys.

Vasa were clearly evident in the intima of atherosclerotic monkeys (Fig. 1), but none was seen in the intima of normal monkeys. Vasa were observed deep in the media of atherosclerotic monkeys (Fig. 2), but only in the outermost layers of media in normal monkeys. The frequency of medial vasa appeared to be greater in atherosclerotic monkeys. Adventitial reaction, characterized by lymphocytic infiltration, was observed in atherosclerotic monkeys, and frequency of vessels appeared to be increased.

**Blood Flow through Vasa Vasorum (Control Conditions)**

Mean arterial pressure was 74 ± 3 (mean ± SE) mm Hg in normal monkeys and 73 ± 7 in atherosclerotic monkeys. In normal monkeys, arterial blood Po$_2$ was 128 ± 6 mm Hg, Pco$_2$ was 37 ± 0.9 mm Hg, and pH was 7.37 ± 0.01. In atherosclerotic monkeys, Po$_2$ was 117 ± 7 mm Hg, Pco$_2$ was 36 ± 0.8 mm Hg, and pH was 7.36 ± 0.01.

In normal monkeys, blood flow to inner layers of the thoracic and abdominal aorta was 0.2 ± 0.1 and 0.7 ± 0.3 ml/min × 100 g. Flow to the outer layers of the thoracic and abdominal aorta was 1.3 ± 0.9 and 2.2 ± 0.8 ml/min × 100 g, respectively.

In atherosclerotic monkeys, blood flow to inner layers of the thoracic aorta was increased to 1.6 ± 0.9 (P < 0.05 vs. normal monkeys), but the increase was small when compared to the increase in outer layers. Flow to outer layers of the aorta was greatly increased in atherosclerotic monkeys to 17 ± 8.9 ml/min × 100 g (P < 0.05).

In the abdominal aorta, blood flow was 1.3 ± 0.5 ml/min × 100 g in inner layers of the aorta in atherosclerotic monkeys (P > 0.05 vs. normal monkeys) (Fig. 3). There was a marked increase in blood flow to outer layers of the aorta in atherosclerotic monkeys, to 31 ± 12 ml/min × 100 g (P < 0.05).

**Figure 1** Electron micrograph of intimal vasa channel in thoracic aorta of an atherosclerotic monkey. Vascular nature of the structure is indicated by endothelial lining cells and accompanying pericytes. Other cells contain lipid droplets.
Effects of Adenosine

Infusion of adenosine at doses that produce maximal or near maximal dilation of vasa (Marcus et al., 1977) produced hypotension. During infusion of adenosine, mean arterial pressure was 43 ± 6 mm Hg in normal and 44 ± 12 mm Hg in atherosclerotic monkeys.

In inner layers of the thoracic and abdominal aorta, blood flow decreased and conductance did not increase during infusion of adenosine in either normal or atherosclerotic monkeys. In inner layers of thoracic aorta of normal monkeys, conductance was 0.1 ± 0.1 and 0.2 ± 0.1 ml/min × g × mm Hg during control and infusion of adenosine (P > 0.05); in atherosclerotic monkeys, conductance was 0.9 ± 0.9 and 0.1 ± 0.1 during control and adenosine (P > 0.05). In inner layers of abdominal aorta of normal monkeys, conductance was 1.2 ± 1.0 and 0.7 ± 0.4 during control and adenosine (P > 0.05); in atherosclerotic monkeys, conductance was 1.5 ± 1.0 and 0.4 ± 0.4 during control and adenosine (P > 0.05). Thus, conductance is minimal in the inner layers of the aorta and it does not increase during infusion of adenosine. This finding is similar to our earlier results in normal dogs (Marcus et al., 1977; Heistad et al., 1978).

In outer layers of the thoracic aorta, conductance tended to be greater in atherosclerotic monkeys than in normal monkeys during infusion of adenosine (Fig. 4), although the values were not significantly different by unpaired t-test (P > 0.05). In the outer layers of the abdominal aorta, conductance was greater (P < 0.05) in atherosclerotic monkeys during infusion of adenosine: Conductance was 6.2 ± 2.0 in normal monkeys and 45 ± 2.8 ml/min × g × mm Hg in atherosclerotic monkeys.
Discussion

The major conclusion of this study is that there is pronounced hyperemia of the aortic wall in atherosclerotic monkeys. The increase in flow through vasa vasorum is demonstrable primarily in outer layers of the aorta. The finding that, during infusion of adenosine, flow through vasa is greater in atherosclerotic aorta than in normal aorta, suggests that new vessels, rather than dilation of existing vessels, probably account for the major fraction of the increase in flow.

In this discussion we will (1) consider the method that was used to measure blood flow through vasa vasorum, (2) examine morphological evidence of proliferation of vasa in relation to our physiological data, (3) speculate about mechanisms responsible for hyperemia in the atherosclerotic aorta, and (4) consider implications of the findings.

Method for Measurement of Blood Flow through Vasa Vasorum

We have described our method for measurement of blood flow through vasa vasorum (Heistad et al., 1978, 1979). Assumptions that are fundamental to the method appear to be valid when spheres 15 μm diameter are used to measure flow, and reproducibility of the measurements is good (Heistad et al., 1978).

One assumption of the method is that the number of capillaries and arterioles occluded by spheres is not sufficient to alter blood flow or responsiveness detectably. This assumption appears to be valid in normal dogs (Heistad et al., 1978). Our finding that dilator responses of vasa are augmented in atherosclerotic monkeys suggests that the number of vessels that are occluded by spheres is not sufficient to compromise responses of the vascular bed.

Another assumption of the method is that spheres are distributed in proportion to blood flow and that streaming of spheres is insufficient to affect measurements. This assumption appears to be valid in other organs but it has not been tested in vasa vasorum. One might speculate that intimal lesions of atherosclerosis could affect the measurements in this study. Atherosclerotic lesions, however, are rare in adventitial vessels, and limited to fatty streaks.

Because aortic samples that can be obtained in monkeys are small, the number of spheres and amount of radioactivity in each sample is limited. To compensate for the small size of the tissue samples, we injected a large number of spheres (2–4 million) and counted the samples for 20 minutes, instead of 2–5 minutes. With this approach, we have been able to reduce variance.

Because the aortic wall is thicker in atherosclerotic than in normal monkeys, and blood flow to inner and outer layers of aorta is different, the manner in which the aorta was divided is an important methodological consideration. Weights of aortic samples indicate that, in both groups of monkeys, the inner and outer layers constituted about 40 and 60% of the total, respectively. In atherosclerotic monkeys, the sample of inner layers consisted of intimal lesions and innermost layers of media; the sample of outer layers contained outer layers of media, but it also contained more of the inner (avascular) media than that in normal monkeys. Thus, if the depth of penetration of vasa from the adventitial surface were similar in atherosclerotic and normal monkeys, the manner in which the aorta was divided would tend to increase the fraction of avascular inner media in the sample of outer aorta of atherosclerotic monkeys and favor lesser flow to outer layers of the aorta in atherosclerotic monkeys. Despite this, we found that blood flow was greatly increased in outer layers of the aorta of atherosclerotic monkeys. Therefore, the manner in which the aorta was divided into inner and outer layers cannot explain our finding that blood flow is greatly increased in outer layers of the aorta in atherosclerotic monkeys. Further, it should not contribute to the finding that hyperemia in atherosclerotic monkeys is less pronounced in inner layers of aorta than in outer layers

Proliferation of Vasa in Atherosclerosis

In the normal aorta, there are virtually no vasa vasorum in the intima or inner layers of media (Geiringer, 1951; Wolinsky and Glagov, 1967). In the presence of atherosclerotic intimal lesions or thrombi, morphological studies suggest an increase in vascularization of the aorta (Geiringer, 1951; Woerner, 1959; Crawford, 1961; Higginbotham and Higginbotham, 1961; Constantinides, 1965). In atherosclerotic vessels in humans, as well as experimental atherosclerosis in rabbits, most vasa arise from adventitial vessels (Higginbotham and Higginbotham, 1961; Constantinides, 1965). Vasa also have...
been described as arising from the lumen of atherosclerotic vessels, particularly in advanced lesions. In this study, we found vasa in both the aortic media and intima. The origins of the vasa were not clearly established, but most appeared to originate from the adventitial surface and not from the aortic lumen.

The number of vasa appeared to be increased in atherosclerotic aorta in our study, but morphological quantification of the extent of proliferation of vasa has not been accomplished. Artifacts caused by microseparations, which are frequent in histological preparations of the aorta, often are difficult to distinguish from vasa vasorum, except with electron microscopy. Furthermore, it often is difficult to distinguish arterioles, venules, and possible lymphatics in the aortic wall. Thus, it has not been possible to quantify from morphological studies the extent of proliferation of vasa. We suggest that, in future studies, quantification of vasa may be facilitated by perfusion-fixation of the tissue. Microseparations will be minimized by this approach, although other limitations to quantification of vasa will remain.

We infused adenosine to determine whether the increase in blood flow in the atherosclerotic aorta reflects vasodilation or the formation of new vessels. The dose of adenosine that was infused appears to produce maximal dilation of vasa (Marcus et al., 1977), since higher doses do not produce greater increases in conductance. If the increase in blood flow that we observed in the atherosclerotic aorta under control conditions were the result of dilation of existing vessels, infusion of adenosine in normal monkeys should have increased conductance to the same level observed in atherosclerotic monkeys. This finding indicates that the capacity of vasa vasorum at maximal dilation is much greater in the atherosclerotic abdominal aorta than in the normal vessel. It is doubtful that the marked difference in capacity reflects structural or functional differences in vasa of atherosclerotic monkeys that allow them to dilate more than vasa in normal monkeys. Thus, it appears that increased blood flow in the atherosclerotic aorta cannot be accounted for by dilation of existing vessels and must be produced by proliferation of new vessels in the aortic wall.

Mechanisms of Hyperemia of Aortic Wall

We speculate that one factor that contributes to hyperemia of the aortic wall in atherosclerosis is an increase in oxygen requirements of the vessel. Several studies suggest that oxygen consumption of the aorta is increased by atherosclerosis (Chan et al., 1979). In rabbits, atherosclerosis has been reported to increase oxygen consumption of the aorta about 2.5-fold (Whereat, 1961, 1967).

Another stimulus to formation of new vessels and hyperemia of the aortic wall may be intimal proliferation with an increase in diffusion distance. We observed a 40 to 50% increase in thickness of the aortic wall in atherosclerotic monkeys. Tissue Po2 has been reported to be decreased in the aorta of atherosclerotic rabbits (Ninikoski et al., 1973). Thus, it is possible that ischemia of the aortic wall may contribute to growth of new vessels in atherosclerosis.

A third stimulus may relate to the adventitial reaction that occurs in atherosclerosis. It is possible that this response increases medial blood flow, as well as flow through adventitial vessels.

Finally, altered radial stress may contribute to increased blood flow to the atherosclerotic aorta (Doyle and Dobrin, 1973). Studies in vitro have suggested that there are some vasa channels to the inner layers of the aorta (Jaeger, 1964); these vessels may be occluded normally by compressive radial stress, which is highest at the lumen and inner aortic wall (Doyle and Dobrin, 1973). It is possible that the presence of a relatively noncompliant plaque may reduce compressive strains in the aortic wall (Doyle and Dobrin, 1973) and allow increased blood flow through vasa vasorum.

Implications of the Study

This study provides new information concerning nutrition of the aortic wall in normal and atherosclerotic primates. In normal monkeys, blood flow through vasa vasorum in the aortic media is minimal. Thus, the aorta must be nourished by diffusion of nutrients from the lumen of the vessel and from adventitial vessels. Medial vasa vasorum apparently assume a larger role in nourishment of the aorta in atherosclerosis, as blood flow through vasa vasorum in the outer layers of the aortic wall is increased considerably. The hyperemia appears to be the result, in large part, of formation of new vessels. We should point out that Buck (1959) has demonstrated that the structure of new vessels differs from that of normal vasa vasorum. Thus the effectiveness and contribution of new vasa to nourishment of the aorta is unclear.

Proliferation of vasa in the atherosclerotic aorta may have unfavorable effects. In coronary arteries, proliferation of vasa and increased propensity to intramural hemorrhage have been proposed to be one mechanism of coronary occlusion in atherosclerosis (Paterson, 1936; Gould and Ionnides, 1968). Intramural hemorrhage in the atherosclerotic aorta has received little attention, but it is possible that hemorrhage may contribute to medial calcification in atherosclerosis (Gore, 1968).

Acknowledgments

We thank Eric Larsen and Marjorie Megan for technical assistance, Anita Riggan for typing the manuscript, and Dr. Allyn Mark and Francois Abboud for critical review of the manuscript.

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doi: 10.1161/01.RES.48.5.669

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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