Does Relief of Critical Arterial Stenosis Accelerate Distal Atherosclerosis?

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ABSTRACT

Progression of atherosclerosis following balloon angioplasty, arterial bypass, or endarterectomy often limits long-term success of revascularization procedures. Therefore, the authors studied the effect of stenosis and relief of stenosis on the development of distal atherosclerosis in a primate model of diet-induced atherosclerosis. Severe stenosis (78 ± 2% diameter reduction) was produced in the midthoracic aorta in 14 cynomolgus monkeys by a constricting band. In 8 monkeys, the stenosis was left in place for three months, after which the distal aorta was revascularized by removal of the constricting band and by balloon angioplasty of the residual stenosis. The results in this group were compared with those in 6 animals whose stenoses were not reversed and with those in 10

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animals with no preexisting stenosis. All three groups received an atherogenic diet for three months with no difference in serum cholesterol (836 to 1059 mg%) during the three-month diet period. Animals with a stenosis had a significant decrease in surface atherosclerosis in the distal aorta (7 ± 6%) compared with control animals (51 ± 8%, P < 0.05). This was accompanied by marked reduction in mural cholesterol content (1.5 ± 0.4 mcg/mm² vs 6.3 ± 1.8 mcg/mm² in controls, P < 0.05) and a decrease in intimal thickness (0.04 ± 0.02 mm vs 0.07 ± 0.01 mm in controls, P < 0.05). After relief of the stenosis, the percent surface atherosclerosis (58 ± 8%), mural cholesterol (5.4 ± 1.0 mcg/mm²), and intimal thickness (0.09 ± 0.01 mm) were no different from controls. Thus, severe stenosis protects the distal vascular tree from intimal plaque formation, despite marked hypercholesterolemia. Following reversal of a stenosis, distal plaque progression does not occur at an accelerated rate but instead occurs at the same rate as without a preexisting stenosis.

Introduction

Balloon angioplasty, arterial bypass procedures, and endarterectomy are commonly performed procedures done to relieve symptomatic arterial occlusive lesions. The immediate results are often dramatic, with relief of ischemic symptoms, preservation of life or limb, or reduction in stroke incidence, depending on the tissues revascularized. Subsequent to successful intervention, however, patients may present with recurrent symptoms due to eventual progression of disease.1-3 Some investigators feel that distal vessels are subject to accelerated atherosclerosis following relief of stenoses by bypass or endarterectomy.1,2,4 Others suggest that reversal does not seem to alter the rate of atherosclerosis.5-7 The effect of relieving an arterial stenosis on the subsequent development of atherosclerotic lesions is a question of major importance when one is considering whether arterial stenoses should be corrected or relieved in only mildly or moderately symptomatic patients. We have, therefore, sought to determine the effects of reversal of high-grade arterial stenosis on the subsequent development of atherosclerotic lesions. To accomplish this, we have quantitated the degree of atherosclerosis developing subsequent to reversal of a chronic flow-limiting stenosis in cynomolgus monkeys fed an atherosclerotic diet and compared it with that developing both with and without a preexisting stenosis.

Materials and Methods

Following a thirty-day quarantine period, 24 adult male cynomolgus monkeys (Macaca fascicularis) were randomly divided into three groups. In the “stenosis” group (n = 6) a high-grade stenosis of the midthoracic aorta was created and the animals were maintained on an atherogenic diet without reversal for three months prior to termination. The “revascularization” group consisted of 8 animals that received a stenosis of the midthoracic aorta but were initially maintained on a normal diet for three months. Following this induction period, stenoses were reversed and the animals were placed on the atherogenic diet for three months. The “control” group, consisted of 10 animals that underwent a thoracotomy and a sham reversal procedure (balloon angioplasty dilatation of the midthoracic aorta) prior to being placed on an atherogenic diet.

Animals were handled and cared for according to NIH guidelines for laboratory animals.8 All animals were housed in individual cages and were allowed to eat and drink water ad lib. Baseline and terminal fasting serum lipids, serum hemoglobin and hematocrit, serum bilirubin and liver function tests, body weight, blood pressure, and fasting serum lipids were reported at three-week intervals following either reversal or sham reversal operations. Heart rate and blood pressure determinations were obtained after anesthetization of animals with ketamine (Ketalar®)
15 mg/kg given by intramuscular injection. Prior to surgical procedures, each animal received a parenteral antibiotic (250 mg Ancef® IV). All operative procedures were conducted using sterile techniques. Anesthesia for surgical procedures was provided by intramuscular injections of ketamine and intravenous administration of sodium thiopental (Surital®) in order to obtain a surgical plane of anesthesia. Proximal and distal arterial blood pressures were obtained at the time of surgery by use of indwelling brachial and femoral artery catheters connected to Statham p23db pressure transducers. Mean pressure gradients caused by the stenoses were obtained by subtracting mean femoral arterial pressure from mean brachial pressure.

Fourteen animals had a surgically created stenosis of the midthoracic aorta by a median sternotomy surgical approach. Stenoses were created by use of a technique originally described by Bomberger et al9 with, in this case, a polyethylene band tightened around the aorta via a left lateral thoracotomy approach. The band was tightened so as to create a mean aortic pressure gradient greater than or equal to 45 mmHg (critical stenosis). Following surgery, animals were allowed to recover and to mature for a period of three months (eighty-six to ninety-two days).

Revascularization and sham reversal procedures were performed as follows: One week prior to surgery, animals began receiving an atherogenic diet consisting of Purina Monkey Chow supplemented with 2% cholesterol and 25% peanut oil by weight.10 Reversal of stenoses was accomplished by use of a lateral thoracotomy approach to the thoracic aorta. The constricting polyethylene band was removed from around each aorta and the narrowed segment was dilated by means of a 2-cm-long, 7-mm-diameter angioplasty balloon advanced from a right femoral arteriotomy site. Two inflation-deflation cycles of thirty seconds duration were performed with 5 atm of pressure before removal of the balloon in its undistended state. The site of dilation was marked by placing a surgical clip adjacent to the aorta at the site. Blood pressure measurements were repeated following dilation and animals were allowed to recover. Control animals (n = 10) had not previously been subjected to vessel stenosis, and so, instead of reversal, they received a sham reversal operation consisting of thoracotomy, rib resection, and transfemoral balloon dilation of the midtho-

racic aorta. Animals from both groups were allowed to drink water ad lib the evening of surgery and were returned to their hyperlipidemic diet the following day.

Three randomly selected animals from the "revascularization" group underwent aortography one week prior to reversal of their stenoses. These images were required for comparison with the angiography performed in all the "stenosis" animals one week prior to termination. All animals that had stenoses reversed underwent biplanar aortography one week prior to the termination of the study period (Figure 1). Angiographic procedures were performed using a #9 French pigtail catheter advanced over a guidewire from a left femoral approach into the proximal descending aorta. Radio-opaque contrast medium (Renografin 60) was injected by means of a power injector (4 cc/sec × 3 sec), and rapid sequence films were taken. Magnification angiograms were used to document aortic luminal contours, to assess the adequacy of the reversal procedures, and to determine whether other associated changes in aortic contours had occurred.

Three months following either coarctation in the "stenosis" group or reversal and digitation in the "control" and "revascularization" groups, animals were exsanguinated while under anesthesia, after final blood samples had been obtained and brachial and femoral arterial pressures had been measured. The entire aorta from the aortic root to past the bifurcation of the iliac vessels was then quickly harvested and placed on a saline-soaked towel cooled from beneath by a container of ice. Each aorta was then opened longitudinally along its ventral aspect and the exposed intimal surface was photographed to document the extent and severity of the grossly visible atherosclerotic lesions.

Surgical clips were used as markers adjacent to the aorta to indicate the location of reversal and sham reversal segments. Three different regions of the aortas: the descending thoracic aorta proximal to the area of dilation (TA-1), the thoracic aorta distal to the area of dilation (TA-2), and the abdominal aorta distal to the renal arteries (Abd), were analyzed in an identical manner. Each of these segments was photographed and then sectioned in a standardized fashion for histologic and for biochemical analysis (Figure 2). The extent and severity of atherosclerotic changes of the aorta were assessed by three independent methods: (1) quantitation of surface atheroscle-
Figure 1.  
Angiogram of the descending aorta in the same animal  
A. with stenosis of the mid thoracic aorta and  
B. following reversal of stenosis.

Figure 2.  Diagram of the aorta illustrating the specific sites samples for histology and biochemical determinations.

rosis by use of computer-assisted morphometry,  
(2) measurement of intimal thickness and cross-sectional area from histologic sections, and  
(3) determination of the lipid content of the artery wall.

Sections for histology were placed in 3% glutaraldehyde and kept refrigerated until further processing. Slides for light microscopy were made from paraffin-embedded histologic samples that were sectioned to give cross sections of the aorta from each region. Separate sections 5 μm in thickness were stained with hematoxylin and eosin and Weigert-VanGieson stains. Intimal cross-sectional area, maximal intimal thickness, medial thickness, and area were determined by means of quantitative computer-assisted morphometry\textsuperscript{11} of histologic sections. Gross photographs of the aortic intimal surface were likewise projected onto a Hewlett Packard digitizer model 9874A. The specific constituents of the projected histologic or photographic image were then traced with a cursor and the data analyzed and recorded by a specially programmed Hewlett Packard system 45 model 9845A desktop computer.

Tissue pieces for biochemical analysis were placed in airtight containers and stored at \(-20\text{°C}\)
pending further analysis. Lipids were extracted from the tissue homogenates using chloroform-methanol. The total cholesterol level in the samples was then determined by the method of Ishikawa as modified by Bates and Wissler.\textsuperscript{12-14} The cholesterol content of the aortic intima was expressed in $\mu$g cholesterol per mm\textsuperscript{2} surface area of tissue segment.

The data obtained from all quantitative methods are presented as the mean ± standard error of the mean (SEM). The mean values of these data were determined to be significantly different when the Student $t$ test demonstrated a $P < 0.05$.

**Results**

**Serum Lipid Response to High Lipid Diet**

At the onset of the study, all animals had a mean total serum cholesterol level of $122 \pm 27$ mg\% and an HDL cholesterol level of $45 \pm 9$ mg\%. After 3 months of being fed a high cholesterol, high fat diet, the total serum cholesterol increased to greater than $800$ mg\% in all 3 experimental groups, while the HDL cholesterol levels decreased to less than $27$ mg\% (Table I). There were no significant differences in the terminal serum lipid values between any of the experimental groups.

**Hemodynamic Response to Stenosis and Revascularization**

Midthoracic aortic stenosis resulted in the development of significantly elevated mean blood pressures proximal to the stenosis with a pressure gradient across the coarctation. After 3 months of coarctation in the "stenosis" group, the mean blood pressure in the proximal aorta was $136 \pm 11$ mmHg with a gradient across the stenosis of $29 \pm 7$ mmHg. These values were significantly different from the terminal values in the "control" and "revascularized" groups (Table I) but not different from the three months (prior to reversal) values in the "revascularized" group. The proximal and distal mean blood pressures and the gradient in the "revascularized" group prior to obliteration of the stenoses were $130 \pm 22$ mmHg, $102 \pm 21$ mmHg and $22 \pm 5$ mmHg, respectively. Following reversal of the aortic stenosis, both the proximal and distal mean pressures decreased to $94 \pm 13$ mmHg and $93 \pm 14$ mmHg, respectively, and the terminal gradient was determined to be $2 \pm 2$ mmHg. These final values, obtained three months after stenosis reversal, were not significantly differ-

**Table I**

*Terminal Hemodynamic and Serum Lipid Values*

<table>
<thead>
<tr>
<th>Parameter Measured*</th>
<th>Control Group (n = 10)</th>
<th>Stenosis Group (n = 6)</th>
<th>Revascularized Group (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>$97 \pm 12$</td>
<td>$136 \pm 10.8^{**}$</td>
<td>$94 \pm 13$</td>
</tr>
<tr>
<td>Distal</td>
<td>$96 \pm 12$</td>
<td>$97 \pm 5.3$</td>
<td>$93 \pm 14$</td>
</tr>
<tr>
<td>Gradient</td>
<td>$0.8 \pm 2.0$</td>
<td>$29 \pm 7.0^{**}$</td>
<td>$2 \pm 2$</td>
</tr>
<tr>
<td>Total cholesterol (mg%)</td>
<td>$977 \pm 112$</td>
<td>$836 \pm 181$</td>
<td>$1059 \pm 176$</td>
</tr>
<tr>
<td>HDL cholesterol (mg%)</td>
<td>$24 \pm 3.0$</td>
<td>$26 \pm 2$</td>
<td>$19 \pm 3$</td>
</tr>
</tbody>
</table>

*All values are mean ± standard error of mean.

**Values are significantly greater at $P < 0.05$ than in the "control" and the "revascularized" groups.
ent from the values obtained in the control group (Table I).

Effect of Aortic Revascularization on Lesion Development and Aortic Cholesterol Content

Three months of high-cholesterol, high-fat diet feeding resulted in the development of grossly apparent atherosclerotic lesions in the thoracic and abdominal aorta (Figure 3). This lesion involvement was fairly homogeneous throughout the length of the aorta in the diet “control” and “revascularized” groups. The midthoracic coarctation in the “stenosis” group appeared to spare the distal thoracic aorta from lesion development.

The percent surface area of aorta occupied by lesions as quantitated from similar photographs supported this observation. The percent surface area occupied by grossly apparent lesions in the proximal aorta was similar in all three experimental groups (Table II). Distal to the stenosis, in both the thoracic and abdominal aortic segments, there was significantly less lesion involvement than in identical segments of the “control” and “revascularized” groups. Note also that lesion involvement in the distal thoracic and abdominal aorta of the revascularized group was not different from that in controls.

Reversal of stenosis also resulted in a return of the mean intimal thickness of the distal thoracic aorta to values similar to those

Figure 3. Photographs of aortas from animals fed an atherogenic diet.
A. Aorta with midthoracic stenosis. Arrow marks site of vessel stenosis. The internal surface is covered with a nearly confluent atherosclerotic lesion proximal to the stenosis. Distally, the internal surface is spared except for 2 small distal plaques.
B. Aorta three months following reversal of midthoracic stenosis. Arrow indicates site of the previous vessel stenosis. Note the internal lesions scattered throughout the thoracic and abdominal regions.
C. Aorta three months following sham reversal (no previous stenosis). Lesions are distributed in all regions of the aorta. (Arrow indicates site of balloon dilation.)
Table II
Effect of Aortic Revascularization on Lesion Development and Aortic Cholesterol Content

<table>
<thead>
<tr>
<th>Parameter Measured Aortic Segment</th>
<th>Control Group (n = 10)</th>
<th>Stenosis Group (n = 6)</th>
<th>Revascularized Group (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% surface atherosclerosis*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>53 ±6</td>
<td>36 ±12</td>
<td>56 ±7</td>
</tr>
<tr>
<td>Distal</td>
<td>51 ±8</td>
<td>7 ±6**</td>
<td>58 ±8</td>
</tr>
<tr>
<td>Abdominal</td>
<td>57 ±5</td>
<td>8 ±5**</td>
<td>55 ±8</td>
</tr>
<tr>
<td>Intimal (lesion) Thickness (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>0.08 ±0.02</td>
<td>0.11 ±0.03</td>
<td>0.16 ±0.04**</td>
</tr>
<tr>
<td>Distal</td>
<td>0.07 ±0.01</td>
<td>0.04 ±0.02**</td>
<td>0.09 ±0.01</td>
</tr>
<tr>
<td>Abdominal</td>
<td>0.05 ±0.01</td>
<td>0.05 ±0.02</td>
<td>0.06 ±0.01</td>
</tr>
<tr>
<td>Aortic wall cholesterol (µg/mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>5.8 ±1.2</td>
<td>4.3 ±0.07</td>
<td>6.9 ±1.3</td>
</tr>
<tr>
<td>Distal</td>
<td>6.3 ±1.8</td>
<td>1.5 ±0.4**</td>
<td>5.4 ±1.0</td>
</tr>
<tr>
<td>Abdominal</td>
<td>2.8 ±0.4</td>
<td>1.1 ±0.3**</td>
<td>3.0 ±0.3</td>
</tr>
</tbody>
</table>

*Values for percent surface atherosclerosis are given as the percentage of total aortic surface area occupied by gross atherosclerotic lesions as determined by computer assisted morphometry.

**Values given are significantly different from the “control” and “revascularized” groups at P < 0.05.

observed in control animals (Table II). The mean intimal thickness of the distal thoracic aorta in the “stenosis” group was 0.04 ± 0.02 mm, while in the “control” and “revascularized” groups it was 0.07 ± 0.01 mm and 0.09 ± 0.01 mm, respectively. The abdominal aortic intimal thickness was almost identical in all three experimental groups.

Effect of Aortic Revascularization on Aortic Cholesterol Content

The total cholesterol content of the thoracic and abdominal aorta underwent changes parallel to the distribution of atherosclerosis in response to revascularization. There were no differences in the proximal aortic total cholesterol content among the control, stenosis, or revascularized groups (Table II). The mural cholesterol content distal to the stenosis was, however, 1.5 ± 0.4 µg/mm², compared with 5.4 ± 1.0 µg/mm² and 6.3 ± 1.8 µg/mm² in the “revascularized” and “control” groups, respectively (P < 0.05). The abdominal aortic cholesterol content was also significantly less in the “stenosis” group than in the “control” and “revascularized” groups with values of 1.1 ± 0.3 µg/mm² and 3.0 ± 0.3 µg/mm², respectively. There were no differences in the cholesterol content of the distal thoracic or abdominal aortic segments between the “control” or “revascularized” groups.

Discussion

Clinical experience and laboratory data from animal experiments suggest that arteries distal to severe arterial occlusive lesions are relatively spared of atherosclerosis.12,9,12-15 Following arterial bypass procedures to relieve stenoses, pa-
tients often develop significant atherosclerosis distal to the bypassed lesion. Progression of distal arterial disease is thought to be an important long-term cause of lower extremity and coronary bypass graft failure.\textsuperscript{16-18} If relief of stenosis results in higher rates of distal atherosclerosis, bypass procedures in patients with minimal symptoms might be inadvisable because of the consequences of progression of distal atherosclerosis.

Little objective clinical data are available on rates of atherosclerotic progression following bypass procedures, and previous laboratory investigations have not examined this important problem. Although considerable recent interest has arisen in the development of restenosis at balloon angioplasty sites,\textsuperscript{19-22} little is known about the consequences of relieving a stenosis on the progression of atherosclerosis distal to the angioplasty site.

The current study determines the amount of atherosclerosis developing proximal and distal to a previous stenosis by using an animal model that has been shown to be similar to humans for the development of atherosclerosis.\textsuperscript{17-19}

A three-month period of diet-induced atherosclerosis was chosen to allow enough time for animals to develop easily measurable atherosclerotic lesions but was made short enough so that the physiologic consequences of reversal of stenosis might be important. Using the same atherogenic diet and coarctation procedures as in other experiments done in this laboratory allowed comparison of our data with those obtained from previous studies that have determined the degree of atherosclerotic changes occurring proximal as compared with distal to a high-grade stenosis.\textsuperscript{9,15}

Stenoses in the current experiment were of the same magnitude as have been previously shown to result in acceleration of proximal disease and distal sparing from disease.\textsuperscript{9,14,15} Reversal of these stenoses was documented by a return to normal of the brachial-femoral arterial pressure gradient and the absence of constricting lesions on angiograms following reversal. The reversal and control groups developed similar levels of hyperlipidemia in response to the high-fat diet. Animals in both groups developed enough disease to be easily definable and measurable yet not so much so as to obscure relative sparing or subtle increases in the amount of atherosclerosis at particular locations. These features made the reversal and sham reversal control groups suitable for comparison and for evaluation of the effects of reversal of a stenosis on the subsequent development of atherosclerosis.

Three different independent techniques were used to assess the degree of atherosclerotic involvement of the different regions of the aorta. Each of these techniques has been used and documented previously in this and other laboratories and together account for both lesion surface area and thickness.

Results showed that following reversal of a high-grade midthoracic stenosis of the aorta, the distal aorta was no longer protected from the development of atherosclerotic lesions. These results are in sharp contrast to the marked distal sparing from atherosclerosis of the distal vasculature of animals with flow-limiting stenosis of the aorta (Figure 3). Relief of stenosis allowed distal aortic atherosclerosis to proceed at the same rate as in sham-operated controls. Despite this increase, when compared with coarctated animals, there was no evidence to support the hypothesis that the rate of distal atherosclerosis was accelerated above levels occurring in vessels without stenosing lesions. In fact, there was no trend to suggest that this might be the case if more animals had been studied.

Proximal to the site of reversal, the results, based on the artery wall lipid content and on the severity of visible surface lesions, suggest that reversal abolished the increased rate of atherosclerosis seen proximal to stenoses. Following reversal, new proximal atherosclerotic disease developed at a reduced rate, similar to that seen in control animals without a preexisting stenosis. Although measurements from histologic sections showed an increase in intimal cross-sectional area, the thickening was hyperplastic rather than atherosclerotic. The fact that artery wall cholesterol and the amount of visible atherosclerotic lesions were not increased in the proximal region of the aorta in the reversal group is evidence that the increase in intimal area was nonatherosclerotic in origin. These results suggest that while reversal abolishes the accelerated rate of atherosclerosis occurring proximal to stenoses, following reversal, hyperlipidemia may impair the regression of nonatherosclerotic intimal thickening.

Atherosclerotic changes in patients following removal of arterial stenoses are poorly documented, despite the current high volume of surgical procedures done for severe atherosclerotic
occlusive disease. The limited information that does exist is contradictory, for some reports suggest that following reversal of stenoses, progression of atherosclerosis is increased while others maintain that rates are unchanged. The best information has been derived from patients receiving repeat angiography after aortocoronary bypass grafting procedures. These studies report an accelerated rate of atherosclerosis in coronary arteries with grafts compared with those without grafts. Although this effect is most prominent in the bypassed segment, which this study does not evaluate, some reports have claimed an accelerated rate distally as well. However, conclusions drawn based on these studies are suspect because of the following reasons: (1) only selected patients were studied following bypass; (2) evidence of disease progression was based entirely on angiograms, which are not specific for atherosclerotic lesions and often do not detect subtle atherosclerotic changes; (3) vessel occlusion, although also not specific for atherosclerosis, has been interpreted as progression atherosclerosis; and (4) the location of sites of disease progression with respect to sites of anastomoses is often poorly documented. Changes subsequent to peripheral arterial procedures are less well documented and are subject to the same criticisms. Evidence of progression of disease at these locations is based entirely on repeated clinical exam-

inations and limited serial angiograms with some findings suggesting that relief of stenosis increases the rate of distal progression and others suggesting that progression is unchanged.

Conclusion

Because this primate model of atherosclerosis demonstrates many similarities to human atherosclerotic disease, the results of this study may reflect changes occurring in patients following relief of clinically important arterial stenoses. To the extent that these results are applicable to the progression of atherosclerosis in humans, the physician and the patient should be encouraged by the findings that procedures to relieve severe arterial stenoses do not accelerate either proximal or distal atherosclerosis above levels occurring in vessels without stenoses.

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References


