Local Effects of Stenoses: Increased Flow Velocity Inhibits Atherogenesis

Christopher K. Zarins, M.D., Richard A. Bomberger, M.D., and Seymour Glagov, M.D.

SUMMARY Thoracic aortic coarctations were produced in cynomolgus monkeys by one of three methods: circumferential banding to produce a symmetric channel with a rigid wall, lateral plication to provide an asymmetric channel with splinting on one side, and lateral plication plus banding to provide a rigid asymmetric channel. The degree of luminal constriction was 58 ± 12%, with no significant difference among groups. After 3–12 months on an atherogenic diet, the coarctation channels were remarkably free of lesions compared with the aorta immediately proximal to the coarctation (p < 0.001). Banding resulted in sharp circumferential termination of the lesions just proximal to the constriction. Lateral plication resulted in an oblique termination of proximal disease with sparing opposite the plication. Lesions distal to coarctations occurred in a pattern related to the configuration of the coarctation channel and tended to form immediately below the plication. Sparing in and immediately beyond the constriction was independent of the rigidity of the aortic wall or of previous disruptive endothelial or medial injury associated with the operative procedures. Endothelium was preserved within the coarctation channel and over all lesions proximal and distal to the constriction. The findings suggest that flow separation and instability tend to favor atherogenesis, whereas increased flow velocity per se may exert a protective effect.

ENDOTHELIAL DISRUPTIONS and changes in endothelial permeability have been associated with excessive shear stresses due to elevated velocity of blood flow and have been implicated in the initiation and progression of atherosclerosis. In previous experimental studies, we found that the endothelium was intact in zones of presumed high shear stress and that diet-induced lesions did not occur preferentially in high shear areas about the aortic ostia of major arteries. When endothelial disruption was evident, it was present only over advanced multilayered lesions regardless of location. To define further the relationship between endothelial integrity, atherosclerotic plaque localization and alterations in flow velocities and instabilities, we studied intimal lesions around aortic coarctations in hyperlipidemic cynomolgus monkeys.

Materials and Methods

Coarctation of the middle portion of the descending thoracic aorta was produced as previously described in 12 male cynomolgus monkeys that weighed 3–5 kg. The monkeys were sedated with phenylephrine hydrochloride, anesthetized with pentobarbital, intubated and ventilated with a volume ventilator. The middle portion of the descending thoracic aorta was exposed through a left anterolateral thoracotomy and upper and lower extremity pressures were monitored continuously by means of left brachial and left femoral artery catheters.

Four monkeys were subjected to circumferential banding to provide a symmetric stenosis with firm external support; constriction was produced in this group by wrapping the aorta with a 1-cm-wide Dacron band. In four monkeys, an asymmetrically narrowed aortic segment 1 cm long was produced by plicating and suturing together the left sides of the anterior and posterior walls of the aorta using Dacron pledges to hold the sutures. In the remaining four, the aorta was plicated as described above and the narrowed segment was wrapped with a 1-cm Dacron band to provide an asymmetric channel with firm external support. In each case the degree of stenosis was increased by tightening the circumferential band or placing additional plication sutures until the difference between brachial and femoral arterial pressure was 40–50 mm Hg.

The monkeys were fed an atherogenic diet containing 2% cholesterol and 25% peanut oil for 3–12 months. Serum cholesterol and body weight were determined at monthly intervals. Before sacrifice, each monkey underwent biplane retrograde femoral aortography for evaluation of the degree of aortic constriction. Brachial and femoral artery pressures were again measured and the aortic pressure gradient was determined. Two monkeys from each group were killed 6 months after beginning the atherogenic diet; one from each group was killed after 3 months and another from each group was killed after 12 months. Serum cholesterol at sacrifice was 630 ± 268 mg% (SD), with no significant differences between groups.

After the final determination of the pressure gradient across the coarctation, a catheter was placed in the opposite femoral artery for infusion of fixative. Fixation was then carried out by perfusion with 3% buffered gluteraldehyde at 37°C while blood was drained through a central venous catheter and the intra-arterial pressure was monitored. The rate of infusion was controlled to maintain a femoral artery pressure at 90–100 mm Hg during the 30-minute fixation period. The aortas were then excised and fixation was continued by immersion for an additional 24 hours in gluteraldehyde.

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II-221
hours. Each aorta was then opened by means of two longitudinal incisions (to avoid distortion due to bending) and examined. The percentages of surface involvement above the stenosis, below the stenosis and in the stenotic channel were estimated by three observers and the aortas and stenoses were photographed for further evaluation. The percentage of surface area covered by lesions was estimated separately for the 2-cm segments of aorta immediately above and immediately below the zone of constriction and in a 1-cm segment in the middle of the stenotic channel. The configuration of lesions immediately above and below the coarctation and the location of lesions within the coarctation channels were recorded. Samples of lesions above and below the stenotic zone and within the stenotic channel were then removed for transmission and scanning electron microscopy. Tissue processing and viewing were carried out as described in detail elsewhere. Differences in the extent of surface atherosclerosis between the coarctation channel and adjacent aorta were assessed using the paired t test; \( p < 0.05 \) was considered significant.

**Results**

After the surgical procedure, with the chest closed and the animal breathing spontaneously, the mean aortic pressure gradient was 15–70 mm Hg (mean \( \pm \) sp 41 \( \pm \) 16 mm Hg). There was no significant difference between the three coarctation groups in operative aortic pressure gradient: banding, 40 \( \pm \) 11 mm Hg; plication, 27 \( \pm \) 12 mm Hg; plication plus banding, 48 \( \pm \) 11 mm Hg. At sacrifice, the aortic pressure gradients had decreased to 6 \( \pm \) 5 mm Hg, with a range of 0–15 mm Hg, and were similar in all three groups. Aortography before sacrifice revealed luminal constrictions of 35–75% (mean 58 \( \pm \) 12%). There were no significant differences between the three groups in degree of stenosis at sacrifice. The contour of the stenotic segment varied, depending on the method used to produce the narrowing (fig. 1). Banding resulted in a symmetrical funnel of entry and exit in relation to the stenotic channel, whereas plication produced asymmetric zones of transition, straight on the right and curved on the left. Plication plus banding resulted in an asymmetric channel, but the limits of the constriction were determined by the band in some instances. Poststenotic dilatation was seen occasionally.

**Extent of Lesion Deposition**

The extent of surface atherosclerosis was affected by the coarctation channel in each group (table 1). There was extensive deposition of plaque in the aorta proximal to the coarctation in each group, with no significant difference between groups. The mean extent of surface atherosclerosis in the proximal aorta was 80 \( \pm \) 25% in all monkeys. In each monkey, surface atherosclerosis was markedly reduced in the

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**Figure 1.** Typical aortograms showing luminal constriction and configuration of the various coarctation channels. (A) Circumferential banding resulted in symmetric stenosis; in this instance, there was a 70% stenosis with poststenotic dilatation and a mean aortic pressure gradient of 15 mm Hg. (B) Lateral plication resulted in an asymmetric channel; in this instance there was a 50% stenosis and no persisting pressure gradient. (C) Plication plus banding resulted in an asymmetric channel with rigid support; in this instance, there was a 65% stenosis with an aortic pressure gradient of 10 mm Hg.
**Table 1. Extent of Surface Atherosclerosis**

<table>
<thead>
<tr>
<th></th>
<th>Banding (n = 4)</th>
<th>Plication (n = 4)</th>
<th>Banding and plication (n = 4)</th>
<th>All monkeys (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal to coarctation</td>
<td>95 ± 6</td>
<td>70 ± 35</td>
<td>74 ± 24</td>
<td>80 ± 25</td>
</tr>
<tr>
<td>Coarctation channel</td>
<td>9 ± 14⁺</td>
<td>5 ± 7⁺</td>
<td>10 ± 8⁺</td>
<td>8 ± 10⁺</td>
</tr>
<tr>
<td>Distal to coarctation</td>
<td>21 ± 27⁺</td>
<td>28 ± 28</td>
<td>10 ± 14⁺</td>
<td>20 ± 23⁺</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*⁺p < 0.05 compared with proximal.

†p < 0.001 compared with proximal.

Coarctation channel compared with the proximal aorta. Surface atherosclerosis in the channel was significantly decreased in each group, with no difference between groups. When all 12 monkeys are considered, there was a 90% reduction in surface atherosclerosis in the coarctation channel compared with the proximal aorta (p < 0.001). In the aorta immediately distal to the coarctation, surface atherosclerosis was reduced (table 1), but lesions were present at variable distances and locations beyond the coarctation and were related to the configuration of the coarctation channel and degree of stenosis. There was no difference between the three groups in extent of distal atherosclerosis and there was no statistical difference in the extent of atherosclerosis between the coarctation channel and immediately distal aorta. Four of the 12 monkeys had minimal atherosclerosis in both the coarctation channel and distal aorta, and in only one monkey was the channel lesion more extensive than the immediately distal lesion. The effects of different degrees of stenosis on more distant distal lesions in the aorta has been presented elsewhere.

**Pattern of Lesion Deposition**

The pattern of intimal plaque distribution about the coarctation channel was dependent on the contour of the luminal surface. The symmetric configuration associated with banding resulted in an abrupt horizontal termination of the confluent intimal foam cell lesions just proximal to the narrowing. Lesions were very few or absent within the coarct channel but were prominent again in the distal aortic segment. Distal lesions were sparser than those in the proximal segment and the pattern of deposition was irregular (fig. 2). Plications that produced asymmetric narrowings

![Images A, B, C]

**Figure 2. Typical examples of plaque localization about the coarctation channels.** (A) Circumferential banding resulted in a sharp margin of lesion demarcation just proximal to the constricting band. The coarctation channel and immediately distal aorta are free of gross lesions. (B) Lateral plication resulted in an oblique plaque margin proximal to the coarctation. The coarctation channel is free of lesions. Plaques are present immediately distal to the plication. (C) Plication and banding resulted, in this instance, in circumferential cessation of lesions proximal to the coarctation with no lesion in the coarctation channel. Lesions are present immediately distal to the plication.
were associated with oblique proximal lesion edges and asymmetric extensions of the proximal lesions toward the constriction channel on the side of plication. The narrowed channel opposite the plication was free of lesions, but lesions resumed just distal to the plication in the area of presumed flow separation, i.e., distal to the channel on the plication side. The surface opposite and just distal to plication was spared (fig. 2). Even with early lesions, after only 3 months of the atherogenic diet, the unique pattern of localization relative to the plicated channels could be seen. Plication and banding resulted in localization patterns with features of both types of coarctation. When the bands produced a constriction in addition to that produced by the plication, the proximal pattern was that associated with banding. Distal to the coarctation, the pattern associated with plication prevailed.

Ostia of intercostal arteries were evident within several of the coarctation channels in all three types of stenosis. Despite the absence of lesions of the surrounding channel surface, small lesions were frequently evident at the proximal rims of the ostia in a pattern similar to that seen elsewhere in the aorta (fig. 3). Loops of suture material used to produce plications could be seen projecting into the lumen on the sutured side in plicated specimens. Small lesions parallel and distal to the sutures were occasionally present and did not extend beyond the immediate vicinity of the sutures.

**Light Microscopy**

Light microscopy of the coarctation channel revealed a thickened intima containing foam cells in
the aorta proximal to the coarctation, while the center of the channel had a thin intimal layer. Distal to the coarctation, the thickened intima was again visible (fig. 4).

Endothelial Integrity

Endothelial surfaces were intact and uninterrupted throughout when examined by scanning electron microscopy (fig. 5), i.e., over the confluent proximal lesions, the sparser distal lesions, and within the spared coarctation channels. Endothelium was also intact and continuous over the sharp transitions between lesions and spared areas. Transmission electron microscopy revealed that both proximal and distal lesions consisted of multiple layers of closely packed spherical foam cells with overlying attenuated intact endothelium conforming to the irregular contours of the immediately subjacent lipid-filled cells (fig. 6). Within the coarctation channels, isolated vacuolated cells or small groups of two or three cell profiles were often found in the intima in a single layer. These cells were generally elongated and not in contact with the overlying intact endothelium. In contrast to the endothelial surfaces over the large proximal or distal lesions, luminal surfaces in the coarctation channels were always regular and smooth.

Discussion

Although lesions about the stenoses were distributed in a manner suggesting a close relationship to patterns of flow, the zone of highest flow velocity, the coarctation channel itself, was spared. In contrast, zones of flow separation or flow instability, particularly at the inlet or outlet of asymmetric stenoses, were areas where lesions developed. The potentiating effect of such disturbances is further emphasized by the finding that within otherwise lesion-free coarctation channels, ostia of vessel branches showed patterns of plaque deposition similar to those seen elsewhere about ostia.9 The sparing of the intimal surface around the sutures used to plication provides some
evidence that even severe medial distortion and previous endothelial injury did not favor lesion formation in the high flow velocity region. Endothelium was intact throughout and both regular and flat within the channel. Alteration in wall compliance was probably not a factor, as channels reinforced by banding and unsupported stenoses opposite unbanded plications were equally spared. Histologic sections failed to reveal adventitial fibrosis that could have provided increased tissue support opposite these plications.

Fry demonstrated endothelial injury after applying shear stresses in the range of 400 dyn/cm² to the arterial luminal surface in dogs. This level is probably higher than that found normally in the arterial tree. Reidy and Bowyer suggested that endothelial damage occurs in areas of high shear stress when they identified spindle-shaped endothelial cells at aortic branch flow dividers in specimens fixed in situ but opened and pinned on flat surfaces. Using careful perfusion fixation techniques and avoiding artifacts due to bending or distortion of the aortic wall after perfusion fixation, we showed that the endothelium at ostial flow dividers is normally intact. Similarly, in the present study, the endothelium proximal and distal to, as well as within, coarctation channels was intact and showed no evidence of disruption. Although it is conceivable
that endothelial damage could occur at very high flow velocities, it does not appear to occur with levels of shear stress attained in our experimental model, i.e., in narrow, asymmetric aortic channels. On the contrary, elevated flow velocities in such channels might instead induce adaptive changes in endothelial adhesion and subendothelial matrix composition that could enhance resistance to detachment or to atherogenic in-sudations. That disturbances such as flow separation, stagnation or instability, rather than increased flow velocity per se, are factors in lesion development is also supported by recent findings in our laboratory that moderate stenoses not associated with pressure gradients may enhance distal atherogenesis, whereas critical stenoses associated with significant distal pressure reduction have an inhibitory effect.\(^\text{10}\) Enhancement of atherogenesis distal to stenosis increased with increasing distance from the narrowing. Four of the 12 monkeys in the present study had no lesions either within the stenotic channel or immediately distal to the stenosis, although all four had plaques beyond the 2-cm distal reference zone. The conditions for lesion induction were apparently reestablished only at a distance from the constriction, providing further evidence for a relationship to the flow fields associated with the stenoses.

The mechanism by which increased flow velocity and decreased pressure may inhibit atherogenesis is unclear. Reduced convection of circulating substances into the arterial wall would be expected with decreased pressure, whereas diffusion into the wall is probably reduced in the presence of the narrowed boundary layer associated with high flow velocity. Increased pressure and flow stagnation would be expected to have the opposite effect and increase entry into the wall of circulating materials. The data presented in this report suggest that studies that relate instabilities of the flow field and flow separations and stagnations to endothelial functional integrity are likely to be more illuminating than those that relate endothelial damage to increased flow velocities. Precise correlations of lesion distribution with such flow profile patterns should also help to identify the significant injurious mechanical factors.

References