Artery stenosis inhibits regression of diet-induced atherosclerosis


Reduction of blood pressure and serum cholesterol levels is associated with reduced risk for the development of arteriosclerotic disease. Experimental studies indicate that reduced cholesterol levels result in arrest or regression of established diet-induced arterial lesions, but the effects of blood pressure reduction on such lesions are not clear. In order to investigate the effects of blood pressure on the regression of established lesions, we induced aortic intimal disease in cynomolgus monkeys by means of an atherogenic diet, produced mediasternal aortic coartations, and restored the animals to low-cholesterol diets for 6 months. Diet control animals were neither coarctated nor restored to low-cholesterol diets. Animals with severe aortic stenosis and the regression diet had the same degree of abdominal aortic atherosclerosis and mural cholesterol content as diet control animals but esterified cholesterol and collagen content was elevated. Animals with mild coarctation and consuming the regression diet had significantly less abdominal aortic atherosclerosis than the diet control animals or the animals with severe coarctation. Although stenosis prevented the induction of lesions in previous experiments, the present study indicates that it did not reverse or delay progression of previously established lesions. The effect of pressure reduction on atherogenesis, even in the presence of reduced cholesterol levels, may depend on the extent and nature of the underlying lesions.

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Hypercholesterolemia and hypertension have been identified as clinical risk factors for the development of cardiac and peripheral vascular disease. Recent clinical data indicate that reduction of even moderate elevations of blood pressure may prevent or delay clinical manifestations of atherosclerosis. and hypertension has been shown to exert a potentiating effect on the development of diet-induced experimental lesions. However, there is little evidence that blood pressure reduction in man either arrests or reverses established lesions. Reduction of serum cholesterol levels in man may result in lesion regression, and lowering serum cholesterol levels in animals appears to reduce both the extent and the lipid content of previously established lesions. In other studies, however, while control of both hypercholesterolemia and hypertension appeared to arrest lesions, it did not reverse coronary artery lesions and was associated with persistence and complication of lesions. Utilizing thoracic coarctation in primates, we found that even small degrees of relative hypotension below the stenosis inhibited induction of lesions, while associated changes in mural composition suggested a corresponding reduction in mural metabolism. Although prevention of lesions distal to experimental coartations corresponds to the sparing of arteries distal to stenoses in man, it is not known whether this sparing results from inhibition of lesion formation or from regression of lesions already present before stenosis occurred. We have now examined the effects of coarctation and restoration to a low-cholesterol diet after lesions have already been induced. We present evidence that lesions exposed to reduced pressure levels do not regress despite marked reductions in serum cholesterol.

MATERIAL AND METHODS

Thirteen adult male cynomolgus monkeys (Macaca fascicularis), weighing 3 to 5 kg, were fed an atherogenic diet consisting of 2% cholesterol and 25%
peanut oil in standard monkey food. After 6 months on the atherogenic diet, five were put to death and served as diet control animals (group I). The remaining eight were begun on a 6 month regression diet consisting of standard primate laboratory food containing 0.05% cholesterol and 15% corn oil, designed to represent the American “prudent” diet. At the beginning of the experiment and at monthly intervals thereafter, each monkey was weighed and blood was drawn for the determination of serum total cholesterol, free cholesterol, triglycerides, cholesterol esters, phospholipids, and total lipids. Prior to institution of the regression diet the eight “regression” monkeys underwent coarctation of the descending thoracic aorta.

Animals were sedated with phencyclidine hydrochloride (Sernylan), anesthetized with intravenous pentobarbital, intubated, and ventilated with a volume ventilator. Catheters were introduced into the brachial and femoral arteries in order to measure blood pressure proximal and distal to the coarctation. The descending thoracic aorta was exposed through a left lateral thoracotomy and encircled with a 0.5 mm Dacron band halfway between the left subclavian artery and the diaphragmatic hiatus. The band was tightened while the brachial and femoral artery pressures were measured using pressure transducers and a strip chart recorder. In four animals the band was tightened so that only a mild aortic constriction was produced with less than a 20 mm Hg mean pressure gradient between the brachial and femoral arteries. These animals were considered to have a mild coarctation with minimal hemodynamic disturbance (group II). In the remaining four animals the band was tightened so that a mean aortic pressure gradient of 40 to 50 mm Hg was achieved at operation. These animals were considered to have a hemodynamically significant aortic coarctation (group III).

After 3 months the brachial and femoral arteries were cannulated again and the aortic pressure gradient was determined. Prior to sacrifice the aortic pressure gradient was measured again and each animal underwent biplane retrograde femoral aortography to determine the degree of aortic stenosis. The animals were then killed with an overdose of pentobarbital and the aorta was carefully excised. The aorta was opened and pinned onto a millimeter grid for measurement of luminal surface area. Three standard segments of aorta were sampled for study: (1) the proximal descending thoracic aorta from the left subclavian artery to 1 cm proximal to the coarctation, (2) the distal descending thoracic aorta from 1 cm distal to the coarctation to the celiac artery, and (3) the abdominal aorta from the inferior mesenteric artery to the aortic bifurcation.

The percent luminal surface area covered by atheromatous plaque in each segment was estimated by three observers and the mean of the three estimates was taken as the value for each segment. The surface area of each segment was recorded and the specimen photographed. Intima-media preparations of each sample were “de-lipidated” by the method of Folch and colleagues. Aliquots were used for total cholesterol determination in a Technicon AutoAnalyzer II. De-lipidated specimens were dried to constant weight and divided into 5 mg portions. Deoxyribonucleic acid (DNA) content was determined using the method of Burton. An extraction procedure adapted from Lansing was used to separate collagen from elastin. Elastin content of the residue was estimated using a modified Kjeldahl procedure assuming 14.8% total nitrogen content in elastin. Hydroxyproline content of the extract was determined by modification of the Neuman and Logan method, and collagen content was computed assuming a 14.4% concentration of hydroxyproline in collagen. Esterified and free cholesterol levels were determined by digitonide precipitation, and the ratio of esterified to free cholesterol for each sample was determined.
Fig. 2. Aortograms demonstrating (A) a mild (25%) aortic constriction producing no aortic pressure gradient in an animal from group II and (B) a severe (65%) aortic constriction producing a 15 mm Hg aortic pressure gradient in an animal from group III.

Table I. Atherosclerosis (values expressed as mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface atherosclerosis (%)</th>
<th>Mural cholesterol content (μg/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal thoracic</td>
<td>Distal thoracic</td>
</tr>
<tr>
<td>I. Diet control</td>
<td>56 ± 7</td>
<td>53 ± 5</td>
</tr>
<tr>
<td>II. Regression, mild stenosis</td>
<td>64 ± 16</td>
<td>44 ± 14</td>
</tr>
<tr>
<td>III. Regression, severe stenosis</td>
<td>67 ± 10</td>
<td>54 ± 15</td>
</tr>
</tbody>
</table>

*P < 0.05 compared to group III.

Elastin, collagen, DNA, and total mural cholesterol were expressed as amount per square millimeter of surface area in order to reflect changes in absolute quantity. Results were expressed as the mean ± the standard error of the mean (SEM). Statistical analysis was performed using Student's t test for unpaired data and the Wilcoxon rank sum test. Significance was assigned to a P value of less than 0.05.

RESULTS

Diet. Animals in each group maintained stable and comparable body weights during the course of the experiment. Before beginning the atherogenic diet, animals had a serum cholesterol of 123 ± 3 mg/100 ml. Institution of the atherogenic diet resulted in a prompt rise in serum lipids in all animals (Fig. 1). During the 6-month induction period there were no differences among the three experimental groups in serum total lipids, total cholesterol, free cholesterol, cholesterol esters, phospholipids, or triglycerides. After 6 months of the atherogenic diet, serum cholesterol level was 692 ± 71 mg/100 ml, and there was no difference in serum cholesterol among animal groups.

On institution of the regression diet there was a prompt reduction of hypercholesterolemia with an equivalent reduction in all lipids measured in both
coarctation groups (Fig. 1). The serum cholesterol was significantly \((P < 0.01)\) reduced compared to that measured during the diet-induction period, but did not return to prediet levels during the regression period and remained at \(224 \pm 43\) mg/100 ml. This may reflect the continued low level (0.05%) of cholesterol intake during regression or the duration of the regression period.\(^7\) At sacrifice, there was no difference in total serum cholesterol between animals in groups II (225 \(\pm 67\) mg/100 ml) and III (224 \(\pm 43\) mg/100 ml).

**Hemodynamic data.** Three months after operation none of the four animals with a mild aortic constriction (group II) had a detectable aortic pressure gradient and all had equal brachial and femoral artery pressures. The four animals with severe stenosis (group III) had a mean aortic pressure gradient of 11 \(\pm 2\) mm Hg 3 months after operation. At the time of sacrifice, animals in group II continued to have equal brachial and femoral blood pressures while each animal in group III had at least a 10 mm Hg brachial-femoral gradient. The mean aortic pressure gradient at sacrifice in group III was 14 \(\pm 2\) mm Hg.

The degree of aortic constriction was assessed from biplane aortograms in each animal. The lumen diameter at the coarctation was compared to the diameter of the thoracic aorta halfway between the left subclavian artery and the coarctation. Animals in group II had a constriction of 26% \(\pm 1\)% and no animal exceeded 30% stenosis. Diet control animals in group I, which had not undergone operation, had a 6% \(\pm 2\)% reduction in diameter at the same measurement sites due to normal taper of the aorta. In animals in group III the stenosis ranged from 64% to 70% with a mean of 67% \(\pm 2\)% (Fig. 2).

**Atherosclerosis.** The percent surface atherosclerosis in the proximal thoracic aorta of the two regression groups was not different from that of the diet control group and there was no evidence of regression of surface atherosclerosis. Similarly, the mural cholesterol content among the three groups was the same and there was no evidence of decreased mural cholesterol in the proximal thoracic aorta (Table I). There was no significant change in percent surface atherosclerosis or mural cholesterol content in the distal thoracic aorta in either regression group. In the abdominal aorta, there was a 52% reduction in percent surface atherosclerosis in animals with mild aortic stenosis compared to diet control animals. Because of the wide variance, and small number of animals, this was not statistically significant. The

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface atherosclerosis (%)</th>
<th>Mural cholesterol content (µg/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Diet control</td>
<td>1.11 (\pm 0.30)</td>
<td>0.91 (\pm 0.43)</td>
</tr>
<tr>
<td>II. Regression, mild stenosis</td>
<td>0.49 (\pm 0.08)*</td>
<td>0.34 (\pm 0.07)*</td>
</tr>
<tr>
<td>III. Regression, severe stenosis</td>
<td>0.95 (\pm 0.11)</td>
<td>1.03 (\pm 0.19)</td>
</tr>
</tbody>
</table>

\(\textit{P} < 0.05\)

**Table III. Ratio of esterified to free cholesterol content (values expressed as mean \(\pm\) SEM)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Proximal thoracic</th>
<th>Distal thoracic</th>
<th>Abdominal</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Diet control</td>
<td>0.9 (\pm 0.1)</td>
<td>1.1 (\pm 0.1)</td>
<td>1.0 (\pm 0.1)</td>
</tr>
<tr>
<td>II. Regression, mild stenosis</td>
<td>0.7 (\pm 0.1)</td>
<td>0.8 (\pm 0.2)</td>
<td>0.7 (\pm 0.1)*</td>
</tr>
<tr>
<td>III. Regression, severe stenosis</td>
<td>0.7 (\pm 0.1)</td>
<td>0.9 (\pm 0.2)</td>
<td>1.4 (\pm 0.2)*</td>
</tr>
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\(\textit{P} < 0.05\)

The difference in percent surface atherosclerosis in the abdominal aorta varied between groups II and III \((P < 0.05)\) (Table I). Similarly, there was a suggestion of a decrease in mural cholesterol content of the abdominal aorta in group II animals but this failed to reach statistical significance.

In order to correct for the wide variation in animal responsiveness to the same high-cholesterol diet,\(^{10}\) abdominal aortic atherosclerosis was expressed relative to proximal thoracic aortic atherosclerosis in each animal. When expressed in this manner, there was a 56% decrease in abdominal surface atherosclerosis \((P < 0.02)\) and a 63% reduction in mural cholesterol content \((P < 0.02)\) compared to diet control animals (Table II). Regression did not occur in animals with a severe aortic coarctation (group III) as evidenced by no change in percent surface atherosclerosis or mural cholesterol content. The ratio of esterified to free cholesterol in the abdominal aorta of animals in group II decreased while the ratio for animals in group III increased \((P < 0.02)\) (Table III).

**Wall composition.** The DNA and collagen content of the thoracic aorta proximal to a severe stenosis (group III) was increased \((P < 0.05)\) when compared to that of diet control animals, but there was no
Table IV. Arterial wall composition (values expressed as mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Collagen content (µg/mm²)</th>
<th>Elastin content (µg/mm²)</th>
<th>DNA content (µg/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal thoracic</td>
<td>Distal thoracic</td>
<td>Abdominal</td>
</tr>
<tr>
<td>I. Diet control</td>
<td>23 ± 3</td>
<td>17 ± 3</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>II. Regression, mild stenosis</td>
<td>33 ± 8</td>
<td>25 ± 6</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>III. Regression, severe stenosis</td>
<td>51 ± 11*</td>
<td>33 ± 8</td>
<td>45 ± 5*</td>
</tr>
</tbody>
</table>

*P < 0.05

change in elastin content (Table IV). In the distal thoracic aorta, there were no differences in collagen, elastin, or DNA content among the three groups. The composition of the abdominal aorta in animals with mild stenosis was not different from that in diet control animals. In animals with severe aortic stenosis, there was a 45% increase in DNA content (P < 0.02) and a 47% increase in collagen content (P < 0.02) when compared to diet control animals.

DISCUSSION

Our findings indicate that reduction of marked hypercholesterolemia to relatively low levels in cynomolgus monkeys results in a decrease in abdominal aortic atherosclerosis, but that this apparent reversal is inhibited by the presence of a midthoracic coarctation sufficient to produce relative hypotension in the distal aorta. We have shown previously that a comparable relative reduction in distal aortic pressure, instituted prior to beginning the diet, inhibited the development of abdominal aortic lesions.

Taken together, these findings suggest that the effects of blood pressure reduction on atherosclerotic lesions depend on the sequence of dietary and blood pressure modification. Thus, control of hypertension may be preventive in the early stages of atherosclerosis, but may impair reversal attempts in later stages of the disease.

Regression of atherosclerosis by reduction of hypercholesterolemia has been achieved in several animal models. Some investigators have found the cynomolgus monkey to be relatively resistant to regression and have noted a delay in the return of serum cholesterol to normal levels after institution of low-cholesterol diets. In the experiments reported here, serum cholesterol did not return to normal baseline levels during the 6-month regression period, but regression nevertheless occurred in the abdominal aorta of animals with mild aortic stenosis. This was evidenced by a decrease in the percent surface involvement, a decrease in mural cholesterol content, and a decrease in the proportion of esterified cholesterol. Hollander and associates studied cynomolgus monkeys 12 months after beginning a regression diet and found a 50% reduction in mural cholesterol and decreased esterified cholesterol in the aorta considered as a whole, but could demonstrate no decrease in the percent surface involvement or in lumen narrowing. We found a significant decrease in the abdominal aortic segment but no change in the thoracic segment. Thus, regression may occur at differing rates in different portions of the aorta much as initiation and/or progression tends to occur unevenly.

The lack of regression of surface atherosclerosis in the abdominal aorta of animals with severe aortic stenosis was accompanied by an increase in the proportion of esterified cholesterol and an increase in the collagen content despite the marked reduction in serum cholesterol. This is in contrast to the findings during regression of Hollander and co-workers and Armstrong and Megan and suggests that lesions distal to severe stenoses may have continued to progress during the low-cholesterol diet period. The increase in DNA provides additional evidence for lesion progression distal to significant stenoses despite reduction of hypercholesterolemia and suggests possible explanations for the absence of regression. When coarctation was performed prior to diet induction, reduced cellularity and reduced collagen content of the distal aorta corresponded to inhibition of lesion formation. The reduced distal pressure resulted in medial atrophy and may have produced a level of filtration and cellular metabolism which prevented lipid accumulation. The current findings suggest that once lipids gain entry to the arterial wall, cell proliferation and fibrogenesis may be maintained at abnormal levels despite the reduction in medial mechanical stress. Thus, if lipid ingress is inhibited by hypotension, the egress of lipid already present also may be inhibited and the proliferative and metabolic response to the accumulated lipid
may persist. Whether this effect continues after much longer regression periods than we have used remains to be demonstrated.

It thus appears from our data that the effect of blood pressure on atherosclerotic lesions is complex and may be different at different stages of lesion evolution. If lowered blood pressure prevents initiation and/or progression of atherosclerosis but retards regression, treatment of elevated blood pressure must indeed begin at a young age, as suggested by recent clinical studies. It is also possible that relief of proximal stenoses may be necessary when arterial disease is diffuse if regression regimens are to be effective. Conversely, it is possible that relief of stenosis by operative means, without control of risk factors, may enhance the development of disease in distal vessels no longer protected by the proximal stenosis.

The authors would like to acknowledge the expert technical assistance of Mr. Agris Slesers.

REFERENCES


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DISCUSSION

Dr. Kenneth G. Swan (Newark, N.J.). Your experimental model is comparable to the clinical condition in which unilateral common iliac arterial stenosis is treated with a femoral-femoral crossover graft. As you know the long-term patency of the latter was initially predicted to be sufficiently poor as to justify the phrase “limb salvage procedure” when describing the operation. The fact that the operation exhibited long-term beneficial effects has led many to speculate that the increased flow through the
donor vessel protected that common iliac artery from progression of disease. My question is, does the reverse hold true with regard to your experimental preparation? Put another way, does the reduction in flow, as related to the abdominal aortic stenosis experimentally induced in your monkeys, augment the development of atherosclerosis or inhibit the regression of atherosclerosis? The 14 mm Hg pressure gradient across the area of stenosis is modest. Is it associated with alterations in aortic blood flow?

Dr. Thomas F. O’Donnell, Jr. (Boston, Mass.). I congratulate Dr. Zarins for addressing a very important concept in vascular surgery: Does bypassing a stenosis or occlusion accelerate progression of atherosclerosis distal to that stenosis or occlusion? Whereas Warren and his associates (Surgery 55:135, 1964) found development of new lesions distal to untreated superficial femoral artery occlusion rare, Mozersky and his colleagues (Surgery, Gynecology & Obstetrics 135:700, 1972) observed that 47% of new lesions that developed following a femoral-popliteal reconstruction occurred distal to the original obstructing lesion. Dr. Zarins’ experimental model appears to justify what we do clinically, that is to improve pressure and flow to an arterial segment. His data show that, at least in the experimental model, these hemodynamic changes do not appear to aggravate or hasten the atherosclerotic process. I imagine the vascular surgeons sitting in the back of the room are much relieved by this observation. I would like to ask Dr. Zarins one question: What does he believe are the mechanisms in producing these changes?

Dr. Ronald Abel (Newark, N.J.) I have, simply, a problem with your conclusions and hypotheses; no problem with the experimental protocol.

It is a rare patient indeed in a clinical situation who has a serum cholesterol in the range of 600 to 1,000 mg/100 ml other than the rare patient with type 2 homozygous hypercholesterolemia.

Is this model an appropriate one, therefore, for considering the effect of dilatation of proximal lesions, or of surgical intervention of those proximal lesions? To my knowledge, there is not a clinical regimen that has been associated with regression of lesions on a statistically significant basis, despite material written for popular consumption in southern California.

So my question is, once again, related to your conclusion and hypotheses. How relevant is this model of “cheesy” atherosclerosis in primates fed cholesterol to the situation that Dr. Swan alluded to in clinical surgery?

Chairman Jonasson. Was there a difference in the sugar content of your diet during induction and regression? There is increasing evidence that hyperinsulinism may play a role in some of these atherosclerotic processes, and I wonder if your regression diet had less sugar than your induction diet.

Dr. Christopher K. Zarins (closing). In answer to Dr. Jonasson, I would like to state that the sugar contents of the induction and regression diets were the same. Even if the composition of the regression diet were different from the induction diet in some important respect, this could not account for the observation that the absence of significant stenosis did result in marked distal regression on the same regression diet. I therefore believe, Dr. Jonasson, that the data we have presented provides evidence that some mechanical factor is responsible for the inhibition of regression distal to the stenosis.

We are unable, at present, to say precisely which mechanical factor is responsible, Dr. Swan. There are at least four such factors which may be operating to prevent regression distal to the stenosis. There is a relative reduction in pressure, turbulence of flow, a decrease in pulse pressure, and possibly a decrease in volume or rate of flow. If the effects were due to turbulence, we would have expected the inhibition to be limited to the region immediately distal to the stenosis. It is instead evident throughout the abdominal aorta, far from the stenosis, where flow is likely to be stable. Total flow of course may be reduced distal to the stenosis as you suggest, Dr. O’Donnell. We have no direct evidence that it was not, but there was no functional or anatomic evidence that flow to the lower extremities or to any abdominal organ was compromised.

We would also expect that collateral flow around the thoracic aortic stenosis would have reached the abdominal aorta and restored nearly normal flow. Since the aorta distal to stenosis was narrowed, one could even argue that rate of flow could have been greater than normal.

We do have direct evidence that pressure was relatively decreased below the stenosis and that the distal aorta was atrophic. Our working hypothesis is, therefore, that reduced pressure and mural atrophy distal to the stenosis resulted in reduced metabolic activity and in a reduced rate of elimination of lipid from the lesion once these were formed. We have also shown previously that stenosis protects against distal lesion induction, probably for the same reasons. Thus we believe that both the induction and regression of lesions requires active metabolic participation by the cells of the arterial wall and that reduced pressure inhibits both of these effects.

Of course, Dr. Abel, we cannot pretend that 6 months of intense hyperlipidemia in the monkey produces the same effect as several decades of atherosclerosis in man. It is our intent, however, to utilize a standard period of atherogenesis and a standard modification of hemodynamic factors in order to observe the interactions among pressure, flow, and hyperlipidemia. Comparison with similar situations in man and the utilization of longer times of observation in monkeys will show to what extent this interesting model parallels the human disease process. The sparing of human arteries distal to stenosis does parallel our earlier findings. Our present findings would seem to support Dr. Abel’s suggestion that lesions, once formed, may be difficult to eliminate by control of hyperlipidemia along and that they are best prevented in the first place.