Original Contributions

Hypertension Sustains Plaque Progression Despite Reduction of Hypercholesterolemia

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To assess the effect of hypertension on diet-induced coronary artery plaques after a return to a nonatherogenic diet, 10 cynomolgus monkeys were fed an induction regimen containing 2% cholesterol and 25% peanut oil for 6 months and then were subjected to midthoracic aortic coarctation to induce hypertension. The animals were then fed a nonatherogenic "prudent" ration for 6 additional months (hypertension-regression group). Twelve additional monkeys were fed the atherogenic diet for 6 months; six were killed (lesion-induction control group) and six were changed to the prudent diet for 6 additional months without coarctation (normotension-regression control group). At the end of the induction period, cholesterol levels averaged 744±178 mg/dl for the 22 animals and were similar for the three groups throughout the induction period. For the animals restored to the nonatherogenic diet (hypertension-regression and normotension-regression groups), serum cholesterol levels fell to 486±225 mg/dl at 1 month, to 341±162 mg/dl at 2 months, and to 234±78 mg/dl at 6 months. There was no significant difference between the hypertensive and normotensive animals. Six months after coarctation, blood pressure proximal to the coarctations for the hypertension-regression group ranged from 100/60 to 220/145 mm Hg with a mean of 166/103±36/28 mm Hg. Cross-sectional area of coronary plaques was somewhat lower for the normotension-regression control group compared with the lesion-induction control group, but the difference was not significant. Plaque area was, however, markedly greater in the hypertension-regression group than in either the lesion-induction or the normotension-regression groups (p<0.05 for each) despite progressive reduction in hyperlipidemia. Furthermore, individual mean lesion area for the hypertension-regression group correlated positively, linearly, and significantly with individual levels of mean, systolic, or diastolic pressure (p<0.001 for each). Regardless of blood pressure level or lesion area, lumen area remained normal because artery size increased with the increase in plaque area (r=0.73, p<0.02). Although hypertension sustained lesion progression under these experimental conditions, our findings do not indicate that cholesterol lowering in the presence of hypertension is necessarily without effect on coronary atherosclerosis. (Hypertension 1991;18:123-129)

Elevated blood pressure alone does not induce atherosclerosis in experimental animals.1-2 Hypertension has, however, been shown to enhance plaque formation in the presence of hyperlipidemia.3-12 Replacement of an atherogenic diet by a normal diet after an induction period may result in some reduction of plaque size13-15 and in changes in plaque composition. Little is known, however, about the effect of hypertension on established diet-induced experimental plaques when elevated levels of serum cholesterol are reduced. The present study was designed to assess the effect of hypertension, imposed at the time of reversion to a nonatherogenic regimen, on experimental diet-induced coronary artery plaques. Midthoracic aortic coarctation was used to provide a range of blood pressure elevations in the coronary arteries. Return to a nonatherogenic diet after a 6-month atherogenic diet induction period was used to produce a marked lowering of serum cholesterol values.

Methods

Twenty-two adult male cynomolgus monkeys (Macaca fascicularis), each weighing 4-7 kg (5.5±0.95), were fed an atherogenic diet containing 2% cholesterol and 25% peanut oil. The diet, which
has been in use in our laboratory for many years and has been described in detail elsewhere, results in significant aortic and coronary atherosclerosis. After 6 months on the diet, six animals were killed to assess the extent of coronary atherosclerosis induced by this regimen; these served as the lesion-induction control group. Sixteen monkeys were restored to a standard primate laboratory diet supplemented by 0.05% cholesterol and 15% corn oil, a regimen that has been considered to correspond to a "prudent" human diet designed to facilitate the arrest of further lesion formation and induce regression of established lesions. To study the effect of hypertension on coronary artery plaques previously established during the induction period, 10 of the 16 prudent diet animals underwent coarctation of the descending thoracic aorta immediately before reversion to the prudent diet and were killed 6 months later. These animals formed the hypertension-regression group. The remaining six regression diet monkeys were also killed 6 months later to establish the effects of serum cholesterol reduction alone on coronary plaques formed during the induction period; these animals formed the normotension-regression control group.

The midthoracic aortic coarctation procedure has been described in previous reports. In brief, a constricting Dacron band is placed at the midportion of the descending thoracic aorta exposed by way of a left thoracotomy. All procedures are conducted under general anesthesia and sterile conditions. Surgery and animal care follow the criteria of the National Research Council for humane care as outlined in the Guide for Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 80-23, revised 1978). During the operative procedure, blood pressure is monitored in the left brachial and left femoral arteries by catheters connected to strain gauges (Gould Inc., Cleveland, Ohio) and is recorded continuously on a strip chart. For the present study, different levels of hypertension were produced by varying the degree of constriction by the Dacron band. The constricting band was always tightened sufficiently to assure a systolic blood pressure elevation above the stenosis of at least 20 mm Hg. Blood pressures were measured again at 3 months and at 6 months, just before the animals were killed. Each animal was weighed before beginning the atherogenic diet and at monthly intervals thereafter. Blood was drawn monthly for serum lipid determinations.

The animals were killed by an overdose of sodium pentobarbital by intravenous injection, and the arterial tree was fixed in situ with 2.5% glutaraldehyde in Sorensen's buffer under conditions of controlled-pressure perfusion by way of the brachial artery catheter. Fixation in situ was continued for 30 minutes without allowing pressure to fall, using procedures described elsewhere. The heart, aorta, and major artery branches were removed and were immersed in the fixative for 24 hours.

Four samples of the left coronary artery were taken as transverse rings. One was taken midway between the origin of the vessel and the origin of the left circumflex branch; the three others were taken along the left anterior descending branch at equal intervals between its origin and midway to the apex. The samples were embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin as well as by the Gomori trichrome method for connective tissue components. Computer-assisted contour tracing was used to quantitate lumen area, lesion area, and the area encompassed by the internal elastic lamina. The latter was considered to be the potential lumen area if there were no lesion present. Percent stenosis was therefore defined as the percent of the area encompassed by the internal elastica occupied by lesion. Means of these determinations for the coronary artery sections were calculated for each animal. Means and standard deviations were then calculated for each experimental group, and multiple variant and regression analyses were performed. Differences were considered to be significant if values of p < 0.05.

Results

Blood Pressure

Brachial blood pressures of the hypertension-regression group were significantly higher than baseline values before aortic coarctation. At 6 months after coarctation, mean pressure was 166/103 ± 36/28 mm Hg for the group as a whole compared with 109/63 ± 19/12 mm Hg before coarctation. Six of the coarcted monkeys had pressure elevations greater than 40 mm Hg, whereas four had only moderate elevations of 10–40 mm Hg. In Figure 1, brachial artery blood pressures before coarctation and 6 months after coarctation are shown for the markedly hypertensive and moderately hypertensive subgroups. In each of these subgroups, mean, systolic, and diastolic blood pressures were elevated at 6 months after coarctation compared with values before coarctation. Before coarctation, monkeys in the markedly hypertensive subgroup had a mean blood pressure level of 108/61 ± 13/7 mm Hg, whereas those in the moderately hypertensive group had a mean level of 109/67 ± 26/17 mm Hg. For the subgroup with marked pressure elevation, mean, systolic, and diastolic pressure levels were all significantly greater than before coarctation (p < 0.002); mean blood pressure at 6 months after coarctation was 184/114 ± 26/22. For the monkeys with pressure elevations less than 40 mm Hg, mean blood pressure at 6 months after coarctation was 139/85 ± 31/27 mm Hg. Systolic pressures in this subgroup were significantly higher than before coarctation (p < 0.005); mean and diastolic levels were also elevated compared with pre-coarctation levels, but these differences did not reach significance.

Serum Cholesterol

Before initiation of the atherogenic diet, the mean serum cholesterol value for the 22 animals was 108 ± 20 mg/dl. During the 6-month lesion-induction
FIGURE 1. Bar graph shows brachial artery blood pressure levels in hypertension-regression monkeys before and after midthoracic aortic coarctation. Those with marked blood pressure elevations are shown on left. Those with moderate elevations are on right. Open bars represent pressures before coarctation, and cross-hatched bars represent pressures 6 months after coarctation. In each of the subgroups, systolic, diastolic, and mean blood pressures were elevated at 6 months after coarctation compared with values before coarctation. For those with marked increases in blood pressure, systolic, diastolic, and mean pressure levels were significantly higher than before coarctation. For those with moderate blood pressure increases, systolic pressures were significantly higher than before coarctation, but mean and diastolic levels were not.

*p<0.001, **p<0.002, and ***p<0.005.

Period, serum cholesterol values rose rapidly (Figure 2). At the end of the 6-month induction period, mean serum cholesterol was 736±178 mg/dl, and there were no significant differences among the three groups. During the subsequent prudent diet period of the hypertension-regression and normotension-regression groups, serum cholesterol levels declined in all animals. The rate of decline was the same for the coarcted (hypertensive) and the noncoarcted (normotensive) groups. Six months later, mean serum cholesterol had dropped to 234±78 mg/dl for the regression animals. Although higher than baseline levels, values were not significantly different for the hypertension-regression and normotension-regression groups.

Body Weight

Average body weight for each group did not change significantly between the beginning and the end of the experiment. For the lesion-induction control group, average weight was 5,323±739 g at the outset and 5,247±876 after 6 months of an atherogenic diet. For the normotension-regression control group, the values were 5,583±1,423 and 5,920±1,268 g. For the hypertension-regression group, values were 5,470±636 g at the onset and 5,344±827 g at 6 months after coarctation. There were no significant differences among the groups for either the initial or subsequent weights. Nor were there statistically significant differences in heart weight or in heart weight-to-body weight ratio among the four groups.

Effect of Hypertension on Lesions

Coronary plaque cross-sectional area and percent stenosis for each group are shown in Figure 3. Plaque area for the normotension-regression group decreased somewhat compared with the lesion-induction control group, but the change was not significant. In contrast, plaque area in the regression group with marked hypertension (0.56±0.20 mm²) was significantly greater (p<0.05) than for either the lesion-induction control group (0.28±0.19 mm²) or the normotension-regression group (0.21±0.16 mm²) despite cessation of the atherogenic diet and the fall in serum cholesterol level. Furthermore, there was a direct and linear relation between individual blood pressure levels and individual mean lesion area.
Lesion area correlates positively and linearly with mean, systolic, and diastolic pressure levels ($r=0.92$, $p<0.001$). £, Marked hypertension-regression subgroup; O, moderate hypertension-regression subgroup; A, lesion-induction control group; O, normotension-regression control group. Normotensive groups (A and O) are represented by the mean for each group. All of the points are shown in Figure 6, and mean±SD for these animals are given in the text and in Figure 3.

($r=0.92$ for mean blood pressure, $r=0.93$ for systolic, and $r=0.88$ for diastolic; $p<0.001$ for each correlation). Blood pressure is plotted against lesion area in Figure 4.

Lumen cross-sectional area was not altered significantly in relation to either blood pressure or lesion area (Figure 5). Actually, lumen area appeared to increase somewhat with lesion area. Because lumen area did not diminish, it should follow that arteries enlarged in relation to plaque deposition. Thus, when the area within the internal elastic lamina and the lesion area were compared for the hypertension-regression group (Figure 6A), a strong positive correlation was evident, with $r=0.73$ and $p<0.02$. That the increase in artery size is related to lesion area and not to hypertension or to the potentiation of lesion progression by hypertension is shown in Figure 6B. When the area encompassed by the internal elastica (i.e., the artery size) is plotted against plaque area for all 22 animals, the lesion-induction control group and the normotension-regression group included, the correspondence between plaque area and artery size remains highly significant ($r=0.60$ and $p<0.005$).

**Discussion**

Resumption of a diet low in cholesterol content after a period of high cholesterol intake produced a steady, marked reduction in serum cholesterol levels. This was associated with somewhat smaller lesion cross-sectional area in the normotension-regression group but this change, although suggestive, did not reach statistical significance by the end of the 6-month regression period. Other studies using the same species$^{13-15}$ indicate that a significant reduction in lesion area can be achieved by dietary manipulation. A longer period of exposure to the prudent diet could well have produced greater differences in our own experiments. Of particular note, however, is the fact that superimposed hypertension not only prevented regression but was associated with significant progression (i.e., enlargement of lesion cross-sectional area during the period when serum cholesterol values were diminishing toward baseline levels). Furthermore, lesion area correlated directly and significantly with individual blood pressure levels. Rela-
consistent evidence that different degrees of stenosis result consistently in different degrees of blood pressure elevation, as reflected in our blood pressure measurements. We did not include a group in which hypertension was produced without return to a cholesterol-lowering regimen. Compared with the hypertension-regression group, coarctation in the presence of a continuing atherogenic diet could have enhanced progression of coronary lesion formation. In that case our results could provide evidence that reduction in the level of hypercholesterolemia, even in the presence of hypertension has, at least, a retarding effect on atherogenesis. Thus, the results that form the basis of the present report cannot be interpreted as indicating that cholesterol lowering does not influence coronary atherosclerosis in the presence of hypertension. Studies of the effect of hypertension on the rate of progression of atherogenesis and on the evolution of individual plaques should provide further insight into the nature of the interactions between hyperlipidemia and hypertension in plaque formation and plaque composition.

The mechanism underlying the potentiation of atherogenesis by hypertension is not clear. Several investigators have suggested that factors associated with hypertension may result in vascular injuries that predispose to atherogenesis. These proposals include direct mechanical disruptive effects as well as actions of vasoactive hormones, effects of sodium flux, changes in blood rheology, and alterations in wall composition that could affect vessel wall permeability and transmural transport and the metabolism of the smooth muscle cells of the media. In our experiment, toxic injury to endothelium by angiotensin II, for example, cannot be ruled out. The aortas were stenosed proximal to the renal arteries, probably resulting in relative renal ischemia, stimulation of the renin-angiotensin system, and an associated increase in angiotensin II. The effect has been demonstrated in the rat. In our previous work with the monkey coarctation model, we have consistently found blood pressure elevations above the coarctations but also less marked but greater than normal elevations below the coarctations. We have attributed this effect to renal ischemia.

Evidence has been forthcoming from both experimental and human studies that compensatory arterial enlargement occurs when atherosclerotic plaques form such that an adequate, if not normal, lumen persists for an extended period. The compensatory enlargement phenomenon has been documented in both coronary and peripheral arteries of the cynomolgus monkey and in human coronary and carotid arteries. Compensatory enlargement was also evident in the present experiment under conditions of hypertension. Regardless of lesion area or level of blood pressure, lumen area was not diminished during the period under study due to an increase in artery size. The effect was evident in both control and hypertensive animals, even though lesion area was significantly greater in the hypertension-
regression group. The positive and significant correlation between artery size and lesion area indicates that the coronary arteries enlarged as plaque area increased. There is also evidence that the response to early plaques may actually result in an increase in lumen area. This phenomenon was suggested by trends evident in the present experiment, although the association did not reach statistical significance.

The mechanisms underlying compensatory enlargement of atherosclerotic arteries remain to be illuminated. Possible explanations have included direct effects of the plaque on the artery wall. Plaque formation may result in atrophy of the subjacent media due to interference with diffusion of nutrients from the lumen or increased plasticity due to degradation of medial matrix fibers by proteolytic enzymes elaborated by plaque cells. Alternately, increased flow rate and wall shear stress, due to reduction in lumen area by plaque encroachment, could induce enlargement by a dilational effect on the intact wall opposite the plaque. The tendency to restore wall shear stress to baseline levels by increasing lumen radius has been demonstrated in arteriovenous fistula experiments in otherwise intact arteries of dogs, monkeys, and rabbits. The degree to which hypertension, per se, may enhance or inhibit either of these possible mechanisms remains to be investigated.

Our findings suggest that blood pressure elevation in human subjects could prevent plaque regression or even sustain plaque progression despite some success in lowering of plasma cholesterol levels. Recent clinical studies have underlined an association of borderline hypertension with coronary artery disease risk factors. Further investigations that take into account the relative effects of atherosclerotic plaque progression of systolic, diastolic, mean, and intermittent elevation of blood pressure with and without success in plasma cholesterol lowering would appear to be indicated.

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