Pathogenesis of Carotid Bifurcation Atherosclerosis in Cynomolgus Monkeys

JAY R. KAPLAN, PH.D.,* THOMAS B. CLARKSON, D.V.M.,* STEPHEN B. MANUCK, PH.D.†

SUMMARY We report here the effect of diet and social environment on the carotid bifurcation atherosclerosis of 60 adult male cynomolgus monkeys (Macaca fascicularis) fed either an atherogenic diet (AD) or a more “prudent” diet (PD). Animals within each dietary condition lived either in unstable social groupings (which had their memberships reorganized on a regular basis) or in stable groupings. The experiment lasted 22 months, after which animals were necropsied and the extent of atherosclerosis was measured both at the carotid bifurcation and at other arterial sites.

Carotid bifurcation atherosclerosis (CBA) was significantly more extensive and severe among animals fed the AD diet. Among these animals, all of which were normotensive, lesion extent was positively associated with both plasma cholesterol concentrations and heart rate response to behavioral stress, and negatively associated with the ability to clear glucose. No experimental variables correlated with the CBA of the PD animals; these lesions, some of which were extensive, were probably pre-existing. The social conditions and individual behavior patterns of the animals had no effect on CBA in either group, despite a pronounced effect on the coronary arteries (CA) of the same animals. While the overall correlation between CBA and CA atherosclerosis was not high, animals with extensive CBA almost always had extensive CA atherosclerosis. The factors influencing CBA in the cynomolgus monkey may be similar to those affecting humans.

Methods

Animals

Study animals were 60 male, cynomolgus monkeys from Malaysia and the Philippine Islands (average age 7.5 years, estimated by dentition). These animals were assigned to one of two experiments, which differed only in diet. The experimental design and methods, and results relating to the coronary and peripheral arteries of these animals, have been described elsewhere. A brief description is given below.

Diet

Dietary manipulation involved, first, the division of animals into two groups of 30 each. One group was fed an atherogenic diet (AD), while the other group was given a more “prudent” diet (PD) (table 1). The animals were fed twice daily with a total consumption of about 120 Cal/Kg body weight/day. At the end of 22 months, all monkeys were necropsied.

Psychosocial Manipulation

Within each dietary condition, half of the animals were assigned to an unstable social condition (monkeys in 5-member social groups which were altered periodically by redistribution of monkeys among the three affected groups) while the other half was assigned to the stable social condition (monkeys in 5-member social groups of unchanging composition). The social behavior (e.g., aggression, affiliation) of each animal was also monitored twice per week with observational methods described earlier. Previously, we reported that coronary artery atherosclerosis of animals in both dietary conditions was affected by this psychosocial manipulation.

Clinical Pathological Observations

Serum Lipid Concentrations

Blood samples for the determination of TSC and HDLC concentrations were taken once per month (n = 22), while animals were anesthetized and following a 24-hour fast. TSC determinations were done using the Autoanalyzer II procedure, while HDLC concentrations were assessed by the heparin manganese precipitation technique, as described in the Lipid Research Clinics manual.
Heart Rate Responsivity to Behavioral Stress

These data represent an exploratory variable in the present study and measurements were taken only on animals in the AD condition and on only one occasion. Among these animals, however, monkeys which exhibited the largest elevations in heart rate during the threat of capture manipulation also had the greatest extent of atherosclerosis in the coronary arteries and thoracic aorta.

Necropsy and the Measurement of Atherosclerosis

At necropsy, animals were perfused with 0.1 molar sodium phosphate buffer and killed by exsanguination. After removal of surface adventitia, the carotid arteries were opened longitudinally on their anterior surface, laid flat on cardboard and placed in 10% buffered formalin for immersion fixation. Following fixation, the segments were stained with Sudan IV in isopropanol. One standard cross section was taken from each of the carotid bifurcations for microscopic evaluation. Bifurcation pads ("intimal cushions") were avoided in the selection of this section. The area occupied by intima and intimal lesion and the maximum intimal thicknesses of these sections were measured (in mm² and mm, respectively) using a Zeiss MOP III Image Analyzer. A thickness of 0.10 mm or more generally indicated the presence of plaque. Additionally, we used a zero to four scale to characterize the lesions in terms of adventitial reaction, degree of mineralization, numbers of foam cells, amount of collagen, extent of necrosis and the prominence of a fibromuscular cap.

For the common carotid arteries, gross evaluation provided estimates of the percent of total surface area affected with plaques. The vertebral, posterior communicating and basilar arteries were fixed in the same way as the carotid arteries. One section was taken from each artery; these sections were graded microscopically using a zero to three scale (0 = no lesion, 1 = fatty streak, 2 = small plaque, 3 = large plaque).

For comparison purposes, we used the coronary arteries and aortas of these same animals. The method of preparation of these arteries has been published previously. Coronary artery atherosclerosis extent for each animal was expressed as the mean intimal lesion area (in mm²) of 15 sections of perfused coronary artery. Plaque "volume" was the index of aortic atherosclerosis; it was computed (in mm³) as the total surface area covered with plaque, multiplied by the mean intimal thickness of the plaque sections.

Statistical analyses involved, primarily, group contrasts by t tests and calculation of Pearson correlation coefficients (r), except where assumptions relating to distribution characteristics (e.g., normality) were not met. In the latter cases, appropriate nonparametric analyses were used (e.g., Mann Whitney U tests and Chi-Square tests, Spearman's rho). Three of the 60 original study animals (1 PD, 2 AD) died before the end of the experiment; data from these animals were excluded.

Results

Pattern of Carotid Bifurcation Atherosclerosis (CBA)

Because CBA did not vary as a function of social grouping, data from the stable and unstable groups...
TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Atherogenic diet</th>
<th>“Prudent” diet</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>28</td>
<td>29</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSC</td>
<td>471 (83)</td>
<td>159 (22)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSC/HDLC ratio</td>
<td>13.4 (4.2)</td>
<td>2.83 (0.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>89 (7.4)</td>
<td>88 (7.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>55 (6.1)</td>
<td>54 (6.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>63.7 (6.2)</td>
<td>58.6 (5.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glucose clearance</td>
<td>2.62 (0.6)</td>
<td>2.84 (1.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

TSC = total serum cholesterol; HDLC = high density lipoprotein cholesterol; BP = blood pressure.

†”p” derived from “t” test (two tailed).

Figure 1. Mean intimal thickness and mean intimal area of left and right carotid bifurcations of animals fed atherogenic (n = 28) and “prudent” diets (n = 29).

were combined within each dietary condition. Unlike the social manipulation, the diet had a significant effect on the extent of CBA and on relationships between atherosclerosis at this site and that seen at other locations. First, CBA extent (thickness and area) was significantly greater on both the right and left sides in AD, compared to PD animals (fig. 1; all p's < 0.001, Mann-Whitney U Test). The major clinical-pathologic differences between the two dietary conditions were in their serum lipid concentrations (table 2), although fasting serum glucose concentrations also differed.

Carotid bifurcation lesion extent in the left and right sides was significantly correlated in the AD, but not the PD monkeys (AD: r = 0.74, p < 0.01; PD: r = 0.25, NS). In both groups, plaque area correlated highly with maximum plaque thickness on both the left (AD: r = 0.90, p < 0.01; PD: r = 0.91, p < 0.01) and right sides (AD: r = 0.85, p < 0.01; PD: r = 0.93, p < 0.01). In the rest of this presentation, only associations with plaque area will be shown, as these are matched closely by the thickness results.

Correlations of CBA with atherosclerosis at two other arterial sites—the coronary arteries and aorta—are summarized in Table 3. Again, the AD and PD monkeys differed in that all correlations were significant in the AD but not PD animals. Although the correlations between CBA and coronary artery atherosclerosis among the AD animals were only moderate (r's < 0.50), 8 of the 11 monkeys with significant bilateral CBA (>1.0 mm²) also had significant coronary artery atherosclerosis (plaque area and thickness above the mean). On the other hand, 4 of 17 monkeys without significant CBA also were significantly affected with coronary artery atherosclerosis.

Lesion extent in the carotid bifurcation correlated well with that in the common carotid artery in the AD (left side: r = 0.52, p < 0.01; right side: r = 0.65, p < 0.01) but not in the PD animals (r = 0, both sides, NS). Fewer animals had plaques in the common carotid artery than at the carotid bifurcation, in both the AD (17 of 28 vs 28 of 28; χ² = 13.7, p < 0.01) and PD (1 of 29 vs 19 of 29; χ² = 24.5, p < 0.01) conditions. In the other cerebral arteries (basilar, posterior communicating, vertebral), so few animals were affected with plaques (less than six, AD; less than 3, PD) that no correlations were attempted.

Histological Characteristics of Carotid Bifurcation Lesions

Carotid bifurcation lesions of PD animals contained minimal lipid and were composed of smooth muscle cells, collagen, some elastic fibers, and occasionally mineralization (fig. 2). Among the animals fed the atherogenic diet, the lesions at the carotid bifurcation...
usually contained lipid within foam cells, and athero-
necrosis was seen in some of the animals in which
cholesterol crystal clefts and mineralization were pres-
ent (fig. 3). In some of the AD animals, it appeared
that a foam cell lesion may have been induced as a
superimposed lesion on a preexisting plaque (fig. 4).

In the histological evaluation, animals given a grade
of zero or one for a characteristic were categorized as
“low” while animals given a grade of two, three or
four for a characteristic were categorized as “high.”
Table 4 contains the number of animals examined that
were categorized as being either low or high for each of
the histological characteristics. Significant dietary dif-
ferences were found in all of the characteristics except
mineralization.

Clinical Pathological Variables

Serum Lipid Concentrations

In addition to serum lipid differences between the
two dietary conditions (Table 2), there was consider-
able interindividual variability in serum lipid concen-
trations, especially among the monkeys consuming the
AD diet (e.g., the TSC/HDLC ratios among these
monkeys ranged from 3.5 to 24.0). This variability in
serum lipid concentrations was, in turn, highly corre-
lated with the CBA of the AD animals. Among the
plasma lipid variables (TSC, HDLC, TSC/HDLC ra-
tio), the overall association was strongest between
CBA and the TSC/HDLC ratio (right side: \( r = 0.55; \)
left side: \( r = 0.59; p’s < 0.01 \)). There was no correla-
tion between CBA and serum lipid variability among
the PD animals, all of which had TSC/HDLC ratios
<4.5.

Despite the marked effect on CBA of highly elevat-
ed serum lipids, extensive lesions were occasionally
found among the PD animals. Further, among those
animals (AD and PD combined) having TSC/HDLC
ratios that might be reasonably expected among hu-
mans (i.e., < 12.0), there was no association between
CBA and serum lipids. These two effects are depicted
in figure 5A. Here, the dotted line represents the TSC/
HDLC ratio thought to mark the beginning of coronary
artery atherogenicity among humans; all but one of
the animals with ratios less than 4.5 are from the PD
group while all of those with TSC/HDLC ratios above
4.5 are from the AD group. The lack of association
between serum lipids and CBA within this subset of
animals contrasts with the pattern of lesions observed
in the coronary arteries of the same animals (Figure
5B). At that site, there was almost no lesion develop-
ment unless the TSC/HDLC ratio was above 8.0.

Glucose Metabolism

Variability in glucose clearance (K) as measured in
the intravenous glucose tolerance test was significantly
associated with CBA among the AD (\( r = -0.46, \) left;
\( r = -0.40, \) right; \( p’s < 0.05 \)) but not PD animals.
This relationship was independent of the association
between CBA and serum lipids, as indicated by the
absence of any significant correlation between glucose
clearance and serum lipids. We found no relationship
between CBA and fasting values of serum glucose
concentrations.

<table>
<thead>
<tr>
<th>Diet group</th>
<th># Scoring low</th>
<th># Scoring high</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>12</td>
<td>16</td>
<td>4.30†</td>
</tr>
<tr>
<td>PD</td>
<td>20</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>18</td>
<td>10</td>
<td>3.34</td>
</tr>
<tr>
<td>PD</td>
<td>24</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>10</td>
<td>18</td>
<td>5.30†</td>
</tr>
<tr>
<td>PD</td>
<td>19</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>9</td>
<td>19</td>
<td>9.78‡</td>
</tr>
<tr>
<td>PD</td>
<td>21</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>15</td>
<td>13</td>
<td>6.69‡</td>
</tr>
<tr>
<td>PD</td>
<td>24</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>18</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>26</td>
<td>1</td>
<td>8.95‡</td>
</tr>
</tbody>
</table>

*Cell entries refer to the number of animals whose carotid bifur-
cation plaques had a particular histological characteristic to a
“low” (visual grade of 0 or 1) or “high” (visual grade of 2, 3, or 4)
degree.
†\( p < 0.05. \)
‡\( p < 0.01. \)
Blood Pressure and Heart Weight

Within groups, correlations of blood pressure (both systolic and diastolic) with CBA were not significant, except for a modest association between SBP and atherosclerosis at the right bifurcation among PD diet animals \( (r = 0.38; p < 0.05) \). Even when blood pressure was examined at different concentrations of serum lipids, there was no clear association with CBA.

Like BP, heart weight (corrected for body weight) was only slightly correlated with CBA. This association was seen on the left side among the AD animals only \( (r = 0.34, p < 0.05) \).

Heart Rate Reactivity to Behavioral Stress

Individual differences in heart rate responses to the laboratory stressor were examined only in the AD animals. This variable correlated modestly with CBA, although, like heart weight, only on the left side \( (r = 0.32, p = 0.05) \). We hypothesized that a heightened reactivity to stress might be related to CBA more appreciably if occurring together with a relative elevation in arterial blood pressure, since pressor responses associated with marked increases in heart rate would, in this case, be superimposed upon an already elevated pressure. Accordingly, we examined the left carotid data of animals which represented relative ends of the distribution of heart rate reactivity (termed high \( [n = 10] \) and low \( [n = 10] \) heart rate "reactors"), and which fell either above \( (n = 10) \) or below \( (n = 10) \) the median systolic blood pressure of all animals. Planned comparisons between group means by \( t \)-tests demonstrated that, among animals having systolic blood pressures above the median value, high heart rate reactors had significantly greater CBA than did their low heart rate reactive counterparts (high reactors: \( \bar{x} = 1.69 \) mm\(^2\); low reactors: \( \bar{x} = 0.71 \) mm\(^2\); \( t = 2.37, p < .05 \)). No similar effect was observed among monkeys falling below the median systolic blood pressure and no significant effects were noted in a parallel analysis of carotid data from the right side.

Discussion

General

Our objectives, in this study, were to characterize the normotensive cynomolgus macaque as a model for the study of CBA, and to explore atherogenesis at this site in terms of risk variables and associations with other sites. The findings suggest that the CBA of cynomolgus macaques resembles that of humans in its microscopic features (extensive collagen accumulation, foam cells, disruptions of the internal elastic membrane, mineralization and necrosis) and in the greater degree to which this site is affected relative to other arterial sites in the head and neck.

Serum Lipids, Glucose Tolerance

Perhaps the clearest findings with regard to risk variables were those relating to the effects of diet and plasma cholesterol on CBA. Not only were lesions more extensive and severe among the AD as compared to PD monkeys, but within the AD group, those animals with the highest TSC/HDLC ratios had the greatest extent of CBA. These findings with regard to serum lipid concentration seem similar to what is reported in some human studies.

However, these findings in relation to lipid and dietary factors should be qualified by two further observations. First, our groups (AD, PD) did not contain many individuals with TSCs in the 200 to 350 mg/dl range or TSC/HDLC ratios from 4.5 to 8.0, a range particularly relevant for humans. Determination of the serum lipid-CBA relationship in this group would seem important for determining with more precision the point at which serum lipids become atherogenic at this site.

Moreover, some PD animals developed considerable CBA, despite low serum lipids (fig. 5A). The lesions observed among the PD animals may have developed during the experiment as a result of the modest amount of cholesterol contained in their diet. Alternatively, these could have been pre-existing lesions...
which progressed little or not at all during the experiment. The latter interpretation may be more valid because the carotid bifurcation lesions of a group of wild-caught animals which died shortly after arriving at our institution were not significantly smaller than those of the PD animals. Further, the histological images (fig. 4) suggest that foam cell lesions superimposed on older (collagenous) lesions occurred among AD animals but not among PD animals (fig. 2). Finally, none of the experimental variables correlated with lesion extent in the PD groups; this indicates that such lesions may represent pre-existing or naturally occurring lesions.

The inverse association between clearance of glucose (K) and extent of CBA is not surprising since impaired glucose tolerance has been reported in some studies of cerebrovascular disease in human beings, and because diabetic individuals are at increased risk for stroke.17, 19 The particular mechanism for the effect in this study remains unclear, since no animals were diabetic (as indicated by weight loss and serum glucose concentrations). Nonetheless, there was considerable variability in K values.

Cardiovascular Measures and Carotid Bifurcation Atherosclerosis

It might seem surprising that there was no consistent relationship between blood pressure and CBA. This may be because blood pressures (and heart weight) in both groups were within a relatively narrow range and were similar to values reported normal for this species.20 It is thus probable that all animals were normotensive, with the restricted blood pressure variability tending to obscure any blood pressure-CBA associations. This is not to suggest that blood pressure is unimportant in the CBA of monkeys (cf Hollander et al,11 also see below); however, in the absence of marked elevations in blood pressure, other factors (serum lipids and carbohydrate metabolism) seem associated more clearly with lesion exacerbation.

The data on behaviorally-induced heart rate reactivity, although evaluated only in the AD animals, are potentially important as they indicate a relationship between high heart rate reactivity (especially in combination with SBP above the median) and increased atherosclerosis at the left carotid bifurcation. These results are consistent with our earlier finding that heart rate reactivity is associated significantly with atherosclerosis in the coronary arteries and in the thoracic portion of the aorta.8 This finding is also consistent with a recent finding by Glagov showing lowered heart rate to be associated with decreased coronary artery atherosclerosis in male cynomolgus monkeys.21 The asymmetry of the present finding may, in part, relate to flow characteristics associated with the carotid artery asymmetry of cynomolgus macaques: the left carotid artery originates directly from the aorta while the right is derived from the brachiocephalic trunk. Finally, the absence of differential heart rate reactivity in independent observations on two groups of monkeys which differed in atherosclerosis extent solely as a result of a dietary manipulation17 suggests that high heart rate reactivity was not a consequence of atherosclerosis (e.g., through baroreceptor damage).

Comparative Pathogenesis

The accurate characterization of the association between CBA and atherosclerosis at other sites is necessary to help clinicians evaluate the usefulness of noninvasively determined estimates of carotid lesions as predictors of lesion extent at other sites, particularly the coronary arteries. The findings reported here suggest that the association between CBA and lesions at other sites is much stronger among more severely affected individuals (AD) than among those with less extensive lesions (PD), and that for all individuals the right side tends to be a better predictor of other sites than the left. Approximately 75% of the individuals with extensive CBA also had extensive coronary artery atherosclerosis; this suggests that noninvasive imaging of the carotid artery may provide reasonable estimates of coronary artery atherosclerosis.

However, this result must be tempered by the knowledge that 25% of individuals with CBA of little consequence had extensive coronary artery atherosclerosis. Further, atherosclerosis at these two sites (coronary and carotid) is not altogether similar. It is clear, for example, from figures 5A and 5B, that lesions were present at the carotid bifurcation of control diet (PD) animals while these same monkeys were almost free of lesions in the coronary arteries. Thus, while there is no doubt that elevated serum lipids are atherogenic at the carotid bifurcation, significant lesions did develop in the absence of such elevations while the same was not true of the coronary arteries.

A surprising finding was the lack of a psychosocial effect on the carotid arteries despite the findings of such effects on the coronary arteries of both AD and PD animals6, 8 and similar effects on the coronary arteries of male and female cynomolgus macaques in a separate experiment.23 The absence of such a finding merely serves to emphasize, again, the differences between pathogenic processes at the carotid bifurcation and at other sites.

References

DEMENTIA is becoming a serious public health problem in our aging population. Epidemiological studies have shown that dementia is present in 4.4–8% of populations over age 65 years. Pathological investigations have revealed that of the commonest forms of dementia, about 50% of elderly demented patients suffer from senile dementia of Alzheimer’s type (SDAT), 20% from multi-infarct dementia (MID) and 10% have both diseases (MIX).\(^1\)

Differentiation between SDAT and MID is a commonly encountered diagnostic problem in the clinic. Hachinski’s ischemic score\(^2\) has proven clinically useful in distinguishing between these most common types of dementia and the validity of this scoring system has been neuropathologically established in a series of autopsied cases by Rosen, et al.\(^6\)

The present communication will attempt to correlate cognitive deficits measured in patients with MID with quantitative measurements of local cerebral blood flow measured by xenon contrast CT scanning. In the majority of cases, MID appears to be a cerebrovascular disease, and a reduction in the number of brain perfusion decreases in parallel with the severity of the cognitive disorder. The purpose of this study is to correlate these two phenomena, thereby providing an important pathophysiologic insight into the nature of cognitive deficits in dementia.

**CT-CBF Correlations of Cognitive Deficits in Multi-Infarct Dementia**

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**SUMMARY** Fifteen right-handed patients with Multi-Infarct Dementia underwent cognitive testing by the Jacobs Mini-Mental Scale (MMQ), and xenon contrast CT scanning. Local cerebral blood flow (LCBF) and local partition coefficient (LX) values were measured by stable xenon contrast CT scanning and potential methodological errors were discussed. Reduced values were graded: 0 = normal, 1 = mild, 2 = moderate, 3 = severe. Graded values were pooled and plotted on composite brain maps to display locations of abnormal Lx and LCBF values. Topographic brain maps, showing most frequent locations of reduced Lx values, confirmed the common anatomical locations of multiple cerebral infarcts to be distributed in both thalami, temporal lobes, basal ganglia, left internal capsule and right cingulate cortex. Gray matter flow values were reduced in similar cortical and subcortical regions. There were no correlations between MMQ scores and reduced LCBF values for caudate and lenticular nuclei. Direct and statistically significant correlations were found between reduced MMQ scores and LCBF values for left or right frontal cortex, left or right temporal cortex and left or right thalamus. Subgrouping MMQ tests according to functions assessed, indicated that left mid-temporal ischemia correlated with dyscalculia and memory disturbances while ischemia of both frontal lobes correlated with disorientation to time and place.

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