Morphological Predictors of Arterial Remodeling in Coronary Atherosclerosis

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Background—Although arterial remodeling in atherosclerotic arteries affects luminal patency, the role of plaque components has not been systematically studied.

Methods and Results—Coronary segments (n=2885) were harvested from the hearts of 36 patients who died of severe coronary artery disease after perfusion fixation. Remodeling was determined by morphometric analysis of 657 sections selected as reference segments and 1318 segments with atheromatous plaques. Atherosclerotic plaques were identified as fibroatheroma, thin-cap fibroatheroma, intraplaque hemorrhage with or without rupture or erosion, or total occlusion. Plaque components consisted of calcification, lipid core, macrophage burden, and fibrosis. There was no correlation between plaque area and lumen size in proximal arteries, unlike middle and distal segments, which demonstrated a significant correlation. Marked expansion of the internal elastic lamina (IEL) occurred in plaque hemorrhages with or without and thin-cap fibroatheroma (vulnerable plaque), whereas in erosions and total occlusions there was shrinkage of the IEL. Macrophage burden, lipid core size, calcium (in fibrous plaque and lipid core), and medial atrophy were all associated with positive remodeling; fibrous areas, however, were negatively associated with remodeling.

Conclusions—Inflammation, calcification, and medial thinning are primary determinants of positive remodeling, which appears to be a feature of plaque instability. (Circulation. 2002;105:297-303.)

Key Words: remodeling ■ coronary disease ■ thrombosis ■ inflammation ■ atherosclerosis

The relationship between arterial expansion and increasing size of atherosclerotic plaque in human coronary arteries was initially studied by Glagov et al.1 Only when 40% or greater cross-sectional luminal narrowing occurs is there a decrease in actual lumen diameter because of compensatory enlargement of the internal elastic lamina (IEL). In atherosclerotic left anterior descending coronary arteries obtained at autopsy, lumen size has been shown to be independent of cross sectional area luminal narrowing, a common angiographic measure of the degree of atherosclerotic plaque.2 The effect of remodeling on the preservation of arterial lumen in moderate left main disease has been documented by serial intravascular ultrasound and intraoperative epicardial ultrasonographic measurements.3,4 Because the ultimate reduction in blood flow is as much a result of lack of IEL expansion as of increased plaque size, there currently is intense interest in the mechanisms of coronary remodeling as an intrinsic component of the atherosclerotic process.5

Although the relationship between plaque size and IEL area is firmly established, there are exceptions to the paradigm, as has been shown by post-mortem and ultrasonographic studies demonstrating negative remodeling in up to approximately 15% of human epicardial segments.6–8 The wide degree of variation in the adaptive responses to increasing intimal plaque is a consequence of a complex effect caused by risk factors, plaque composition, and vessel size on the linear relationship between IEL area and plaque size.7,9–15

Despite increasing evidence of the effect of plaque composition on arterial remodeling, to date there have been no systematic histopathologic studies correlating plaque composition and arterial remodeling. Plaque progression involves thrombosis, healing, and smooth muscle cell proliferation, all of which may affect the IEL area.16 The morphological heterogeneity of coronary artery disease has resulted in an evolving classification of the progressing atherosclerotic plaque.17–19 The purpose of this study was to evaluate the effects of plaque morphology and calcification on arterial expansion and lumen preservation in a series of hearts obtained from human autopsies.

Methods

Thirty-six hearts from patients who died of severe coronary disease were studied. There were 24 males and 12 females, aged 64±14 years (mean±SD), with a range 44 to 85 years. Hearts were perfusion fixed at physiological pressures,20 and epicardial arteries

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were removed from the heart before sectioning and radiographed. The entire coronary tree, including left main, left anterior descending to 2 cm from the apex, 3 cm of the left diagonal, left circumflex to obtuse marginal, 3 cm of the obtuse marginal, entire right or left circumflex artery to the posterior descending (depending on dominance), and the first 3 cm of posterior descending were serially sectioned at 3 to 4 mm intervals and processed for histological examination stained with Movat pentachrome, resulting in 2885 sections. The right coronary artery, left anterior descending, and left circumflex coronary arteries were divided into proximal and mid/distal regions. The proximal regions consisted of the first 3 centimeters for the right coronary, before the first diagonal branch for the left anterior descending, and the obtuse marginal for the left circumflex. Middle segments were between first and second diagonals for left anterior descending, between left obtuse marginal 1 and left obtuse marginal 2 for circumflex, and beyond 3 cm of the right to the right marginal branch. Computerized morphometry and immunohistochemical stains for CD68 (clone KP-1, macrophage marker, DAKO, dilution 1:50) were performed in 1318 segments with >40% luminal narrowing as judged subjectively, as well as 657 segments with 0% to 40% luminal narrowing to determine normalized IEL area for each segment. The 1318 segments morphometrically measured and histologically classified as fibrous plaques (n=265), fibroatheroma (with or without calcification, n=708), plaque ruptures (acute luminal thrombus with connection to lipid rich core, n=14), plaque erosion (acute luminal thrombus with intact fibrous cap, n=9), thin-cap fibroatheroma (vulnerable plaque, or plaque with thin fibrous cap with infiltration of macrophages, n=64), hemorrhage into plaque (n=29), calcified nodule (n=5), healed plaque rupture (interruption of the fibrous cap as demonstrated by Sirus red staining viewed under polarized light, without acute thrombus, n=173), and total occlusions (n=51). This classification follows previous guidelines.19 Healed plaque ruptures were defined as reported by Mann and Davies21 and previously from our laboratory.22

Morphometric measurements of the epicardial arterial sections were performed on digitized images (IPLab Spectrum image processing software, Signal Analytics Corporation). All sections (reference and atheromatous) were stained with Movat pentachrome (for elastic staining) and analyzed quantitatively by computerized planimetry for IEL area, lumen area, and plaque area. Atheromatous sections were stained with Sirius red (for collagen, adventitial thickness, and healed ruptures) and hematoxylin-eosin (for calcified area and lipid core). Remodeling was assessed by relating atheromatous segments to proximal reference segments adjusted for tapering.7 In this way, an expected IEL was calculated for each segment, and IEL expansion assessed by the formula IEL area−expected IEL area=IEL expansion into the lumen from the expected IEL; therefore, the IEL expansion is one half the plaque area. Because remodeling is a function of plaque size, IEL expansion was adjusted for plaque area by the formula (IEL area−expected IEL area)/plaque area (Figure 1).

To assess the effect of plaque composition on remodeling, plaque area, percent plaque calcified (total calcified area and fibrous or lipid core calcification), CD-68 (KP-1) percent positive area, medial thickness, and adventitial thickness were measured in each atheromatous segment. Mean medial and adventitial thickness were determined by 4-quadrant measuring and averaging the measurements per segment. An adjusted medial thickness for each segment was derived by determining the regression correlation between distance from the ostium on medial thickness in “normal arteries” (<40% cross sectional luminal narrowing) (r2=0.36, P<0.0001, slope =−0.0015). Mild medial atrophy was defined as media thickness 75% to 90% predicted, and severe media <75% predicted. Similarly, adventitial thickness was found to correlate inversely with distance from ostium (r2=0.2, P<0.0001, slope =−0.0005), and was adjusted accordingly. Calcified matrix area was determined on decalcified hematoxylin-eosin sections. The correlation between histological and radiographic assessment of calcification using this method has been previously verified.23 Calcified area was separated into calcified fibrous plaque and calcified lipid core areas based on location of the calcified matrix.

Univariate comparison of delta IEL area/plaque area among groups (plaque type, core size, extent of calcification, degree of adventitial thickness, and degree of medial atrophy) was accomplished by ANOVA means table with Fisher’s post-hoc test. Values are expressed as mean±SD. For multivariate analysis, delta IEL area/plaque area was compared with plaque components (percentage of plaque composed of fibrous tissue, percentage of plaque composed of lipid core, percentage of plaque composed of fibrocalcific plaque, percentage of plaque composed of “soft calcium” in lipid core, and percentage of plaque composed of KP-1 positive macrophages), independent of sex, distance from the coronary ostium, and patient age (multiple linear regression). Because of nonparametric distribution of measurements, log normalized values were used in multivariate analyses for percentage of lipid core, percentage of calcification, medial atrophy, and percentage of KP-1 staining.

**Results**

**Plaque Area Versus IEL and Lumen: Univariate Analysis**

There was a linear relationship between plaque area and IEL for all arteries. For the left main, proximal left anterior descending, proximal right, posterior descending, left diagonal, distal left anterior descending, distal right, proximal left circumflex, mid left anterior descending, and mid right, P values were <0.001, with r2>0.2. In the mid and distal left circumflex, the relationship between plaque area of IEL was significant to r2=0.1, P=0.03. When plaque area was plotted...
against lumen area, there was no relationship in the left main, proximal left anterior descending, and proximal right coronary arteries (Figure 2), but a significant negative relationship was observed in the mid right, proximal left circumflex, mid left anterior descending, and other distal segments combined (Figure 3).

**Effects of Plaque Type on IEL Expansion and Remodeling: Univariate Analysis**

When compared with reference segments, plaque hemorrhage with rupture demonstrated IEL expansion. There was retraction with healed rupture (Figure 4). The unadjusted degree of expansion was greater in hemorrhages with or without rupture than in other plaque types \((P < 0.0001, \text{Figure } 5A)\). Using the remodeling formula adjusting for plaque size \((\text{IEL expected IEL area})/\text{plaque area}\) (Figure 5B), hemorrhages with or without rupture showed significantly more remodeling than fibrous plaques, erosions, and total occlusions.

**Effects of Age, Sex, and Distance From Ostium on Arterial Remodeling: Univariate Analysis**

IEL area expansion adjusted for plaque size was greater in proximal segments (left main, left anterior descending before diagonal, first 3 cm of right coronary, left circumflex prior to obtuse marginal) as compared with more distal segments in both men and women (Figure 6A). There was no significant difference in remodeling in ages <65 or >65 (Figure 6B).

**Effects of Plaque Composition on Arterial Remodeling: Univariate Analysis**

When IEL area expansion adjusted for plaque size was assessed by lipid core size, there was a significant difference between none and large cores (Figure 7A, \(P < 0.0001\)). The calcified core size and calcified fibrous area were highly correlated with remodeling (figure when plaques with no calcification were compared with those with severe calcification; Figure 7, B and C).

**Media and Adventitial Thickness and Arterial Remodeling: Univariate Analysis**

Adventitial thickness was inversely associated with remodeling \((P = 0.007; \text{Figure } 8A)\). Medial atrophy (mild 75% to 90% predicted thickness, severe <75% predicted thickness) was also associated with arterial remodeling \((P = 0.002; \text{Figure } 8B)\).
ANOVA means table with Fisher’s post-hoc test). In addition, showed greater IEL expansion than all other types (P<0.0001, P<0.001). Calcified lipid core and medial atrophy were strongly negatively associated with remodeling (P<0.001, respectively). Percentage of fibrous plaque area was less strongly associated with remodeling (P=0.05, respectively).

Discussion

Effect of Vessel Size on Degree of Remodeling

This study documents a linear relationship between plaque size and IEL area for all arterial segments and demonstrates the lack of association between plaque size and lumen area for the proximal coronary arteries. These results corroborate Clarkson et al’s study of the left anterior descending coronary artery from 100 humans and 416 monkeys and a histomorphometric study on human right, left anterior descending, and left circumflex arteries. However, this study shows that, in distal segments, lumen size decreases with plaque area, suggesting that remodeling occurs much less in distal segments than in proximal segments, and the plaque size itself is an important component of luminal compromise in these vessels. In addition, the degree of remodeling, as compared with reference segments, was lesser in distal segments based on plaque area than proximal segments. These data differ from those of Sabate et al, who, in an ultrasound study, showed that the location of plaque at distal segments was an independent predictor of compensatory enlargement (odds ratio 4.6). The reason for the discrepancy between this and Sabate et al’s study is unclear, but may relate to methodological differences (autopsy versus ultrasound), definitions of remodeling used, and patients studied. Because of the strong effect of plaque composition on coronary remodeling, it is likely that variations in plaque type are more important than location in determining the response of the vascular wall to intimal disease.

Effect of Plaque Composition on Remodeling, Multivariate Analysis

By multivariate analysis, adjusting for age, sex, and distance from the coronary ostia, plaque components most strongly associated with remodeling were macrophage infiltration, percentage of fibrous calcification, and percentage of lipid core (all P<0.0001; Table). Percentage of fibrous plaque area was strongly negatively associated with remodeling (P<0.0001). Calcified lipid core and medial atrophy were less strongly associated with remodeling (P=0.002 and P=0.05, respectively).

Figure 5. Remodeling by type of plaque. A, The absolute increase in IEL area compared with reference segment is plotted by plaque type. Plaque hemorrhage including ruptured plaques showed greater IEL expansion than all other types (P<0.0001, ANOVA means table with Fisher’s post-hoc test). In addition, healed ruptures and thin-cap atheromas showed greater expansion than fibrous plaques and total occlusions (P<0.001, ANOVA means table with Fisher’s post-hoc test). B, The IEL increased adjusted for plaque area is plotted by plaque type. Plaque hemorrhage, including ruptures, showed significantly greater remodeling than fibrous plaques (P=0.003, means table with Fisher’s post-hoc test) and total occlusions (P=0.03). Fibrous plaques showed less remodeling than thin-cap atheromas and healed ruptures (P<0.01).

Figure 6. Remodeling, as assessed by IEL expansion adjusted for plaque size, was greater in proximal (left main, left anterior descending before diagonal, first 3 cm of right coronary, left circumflex prior to obtuse marginal) as compared with more distal segments in both men and women. Remodeling was greater in men than in women (P=0.01, means table with Fisher’s post-hoc test), and greater in proximal than distal segments (P<0.0001, means table with Fisher’s post-hoc test). B, There was no significant difference in remodeling in age >65 or <65, although in men there was more remodeling in the older group (P=0.08).

Effects of Plaque Composition on Remodeling, Multivariate Analysis

By multivariate analysis, adjusting for age, sex, and distance from the coronary ostia, plaque components most strongly associated with remodeling were macrophage infiltration, percentage of fibrous calcification, and percentage of lipid core (all P<0.0001; Table). Percentage of fibrous plaque area was strongly negatively associated with remodeling (P<0.0001). Calcified lipid core and medial atrophy were less strongly associated with remodeling (P=0.002 and P=0.05, respectively).
accompanied by a compensatory enlargement of the artery as seen with fibroatheromas without healing rupture sites.

The current data linking plaque hemorrhage and rupture with IEL expansion are consistent with intravascular ultrasound data that have associated positive remodeling with unstable coronary syndromes. Furthermore, the positive relationship between lipid core and IEL area has been suggested by an intravascular ultrasound study that related "hard plaques" without large lipid cores to constrictive remodeling. The finding that plaque vulnerability is associated with remodeling has also been demonstrated by the morphological study of Pasterkamp et al.

This study assessed only morphologically evident calcification, which occurs primarily in areas of apoptotic or lipid cores, first as microscopic calcium deposits, possibly of matrix vesicles derived from dying intimal smooth muscle cells. The morphological appearance of calcium in the plaque is therefore initially very small crystals, which can be seen only by special stains, and then develops into plates of calcium in the fibrotic plaque and granules of calcium in cores that are heavily infiltrated by macrophages. In this study, both forms of calcium were associated with remodeling, suggesting that the calcification process itself may be associated with remodeling independent of inflammation. The observation in this study that calcification is strongly associated with plaque expansion is contrary to intravascular ultrasound data that suggest that superficial calcium is a marker for negative remodeling, but is consistent with a different ultrasound study relating calcium arc to arterial expansion. Although this study did not show a significant positive correlation between calcification and lumen preservation, the data did not suggest that calcification played a role in plaque shrinkage. The reason for the discrepancy between the calcification data in the current study and prior clinical publications may relate to the superficial nature of calcium that is seen by intracoronary ultrasound, and the fact that underlying compensatory processes may be hidden. The pathological study of Clarkson et al also demonstrated a strong association between mineralization and plaque expansion, supporting this study and the in vivo study of Sabate et al.

The biochemical mechanisms of arterial expansion are not known. Macrophages in the atherosclerotic plaque may release matrix metalloproteinases, especially 1 and 13. It is unknown whether smooth muscle cells in the intima express...
cathepsins K and S on stimulation with IL-1β or INF-γ, causing IEL destruction. Further studies are needed to determine the balance between matrix metalloproteases and tissue inhibitors of metalloproteinases at various stages of plaque development.

Other Effects on Arterial Remodeling
Prior studies have shown a complex interrelationship between risk factors and coronary artery remodeling, and a difference between the degree of remodeling in the coronary, carotid, and renal arteries as compared with those of the lower extremity. Most clinical studies have shown that smoking is associated with negative remodeling, and there has been a suggestion that elevated cholesterol may favor adaptive remodeling. This study demonstrates that thin-capped atheromas and ruptured plaques, which are related to hypercholesterolemia, are associated with expansion of the IEL. However, a pathological study by Taylor et al. has shown an inverse relationship between plaque expansion and serum HDL levels in a small set of patients. In addition, healed plaque ruptures, which result in a modest degree of IEL expansion but a significant lumen loss, are related to serum cholesterol levels. The effect of risk factors on coronary arterial expansion is likely strongly modified by morphological variables in the plaque. In addition, cholesterol-lowering treatment may have direct effects on vascular remodeling, which may result in increased luminal patency without a decrease in plaque size.

Limitations
Because this study is an autopsy study, remodeling can only be assessed based on reference segments or by relating plaque area to lumen area, as has been done previously. Intravascular ultrasound and other imaging studies have the inherent advantage of allowing temporal assessments. However, these modalities are currently unable to provide morphological data regarding plaque composition or actual IEL in the presence of calcification.

Conclusions
This histomorphometric study has shown a strong effect of plaque components on remodeling of epicardial coronary arteries. Expansion of the IEL is associated with plaque rupture and shrinkage with plaque erosion. Arterial expansion is strongly correlated with calcification, macrophage infiltrates, and lipid core. Arterial components that result in luminal compromise are intimal collagen. These results show the complexity of the factors that influence coronary arterial patency.

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References


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