Healed Plaque Ruptures and Sudden Coronary Death
Evidence That Subclinical Rupture Has a Role in Plaque Progression

Allen P. Burke, MD; Frank D. Kolodgie, PhD; Andrew Farb, MD; Deena K. Weber, BS; Gray T. Malcom, PhD; John Smialek, MD; Renu Virmani, MD

Background—Subclinical episodes of plaque disruption followed by healing are considered a mechanism of increased plaque burden. Detailed pathological studies of healed ruptures, however, are lacking.

Methods and Results—We identified acute and healed ruptures from 142 men who died of sudden coronary death and performed morphometric measurements of plaque burden, luminal stenosis, and smooth muscle cell phenotype. Healed ruptures were found in 61% of hearts and were associated with healed myocardial infarction, increased heart weight, dyslipidemia, and diabetes. Multiple healed rupture sites with layering were frequently found in segments with acute and healed rupture; the percent area luminal narrowing increased with increased numbers of healed sites of previous rupture. The underlying percent luminal narrowing for acute ruptures (mean 79±15%) exceeded that for healed ruptures (mean 66±14%, P=0.0001), and the area within the internal elastic lamina was significantly less in healed ruptures than in acute ruptures, when segments were grouped by distance from the ostium. Healed ruptures favored the accumulation of immature smooth muscle cells at repair sites, with a cellular proliferation index of 0.40±0.09%, significantly higher than the index at the sites of rupture (P=0.008).

Conclusions—These data provide evidence that silent plaque rupture is a form of wound healing that results in increased percent stenosis. Healed ruptures occur in arteries with less cross-sectional area luminal narrowing than acute ruptures and are a frequent finding in men who die suddenly with severe coronary atherosclerosis. (Circulation. 2001;103:934-940.)

Key Words: plaque rupture, death, sudden

Plaque rupture is the most common lesion underlying acute coronary syndromes. Pathological studies suggest, however, that some plaques may rupture silently without causing symptoms. Healed ruptures in the coronary vascular bed are readily detected microscopically by identification of breaks in the fibrous cap with a surrounding repair reaction. The matrix within the healed fibrous cap consists of a proteoglycan-rich mass or a collagen-rich scar, depending on the healing phase. The prevalence of silent ruptures in the clinical population is unknown, because imaging techniques to detect sites of previous plaque rupture are currently unavailable.

Although stenosis is easily identified by angiography, very few data exist to explain how plaques narrow. It is speculated that repeated fibrous cap rupture and thrombosis can incite plaque progression and/or tissue contraction in the absence of clinical ischemic events. Therefore, an understanding of the pathology of healed ruptures is of critical clinical importance. The purpose of this study was to determine the incidence and morphological characteristics, as well as the relationship to traditional risk factors, of healed plaque ruptures in a series of sudden cardiac death.

Methods

Case Selection
Hearts (n=142) from male patients were prospectively examined in consultation with and under the direct supervision of the Office of the Chief Medical Examiner in the State of Maryland. Criteria for entry into the study included sudden unexpected coronary death, male sex, and exclusion of noncoronary causes of death after a full forensic autopsy. Coronary deaths were defined as natural deaths without extracardiac cause of death in which ≥1 epicardial coronary artery had ≥75% cross-sectional area lumen narrowed by atherosclerotic plaque or plaque with superimposed thrombus. One hundred thirteen of these cases were published previously without data on healed plaque ruptures.

Classification of Sudden Deaths
Sudden deaths were initially classified by the presumed mechanism of death: acute thrombus due to acute plaque rupture, acute thrombus...
due to acute plaque erosion, stable plaque with healed infarct in the absence of an acute thrombus, and stable plaque (≥75% cross-sectional area luminal narrowing) without evidence of infarction. 6,8

Identification of Healed Sites
Coronary arteries were studied as previously described. 6 Any area of cross-sectional luminal narrowing grossly estimated at ≥50% was submitted for histological analysis. All acute ruptures and any section that suggested the possibility of previous rupture on the basis of Movat pentachrome staining (healed sites recognized by brilliant blue-green color) were confirmed by picrosirius red staining and polarization microscopy. 4 When viewed under polarized light, this special stain highlights collagen types III and I differentially, allowing visualization of interruptions in the fibrous cap. Healed sites consisted of breaks in collagen type I (yellow-red birefringence) overlying a necrotic core with superimposed layer of collagen type III (green birefringence) (Figure 1).

Risk Factor Analysis
Evaluation of traditional risk factors was performed on postmortem blood samples as previously described. 6 Total cholesterol, HDL cholesterol, and cigarette smoking (estimated by serum thiocyanate >90 µmol/L) were determined by serum analysis, and percent glycohemoglobin, for estimation of glucose intolerance, was determined by analysis of red blood cells. Hypertension was determined by evaluation of renal vasculature and history. 9 The association between risk factors and the presence of acute and healed plaque ruptures was determined by multivariate analysis (logistic regression with model coefficients, Statview software, SAS Institute) using age, glycohemoglobin, total cholesterol, HDL cholesterol, and body mass index as continuous variables and hypertension and cigarette smoking as nominal variables.

Morphometric Analysis of Healed and Acute Ruptures
The right, left anterior descending, and left circumflex coronary arteries were divided into proximal and middle distal regions. The proximal regions consisted of the first 3 cm for the right coronary, before the first diagonal branch for the left anterior descending, and the obtuse marginal for the left circumflex artery. Middle segments were between the first and second diagonals for the left anterior descending, between left obtuse marginal 1 and left obtuse marginal 2 for the circumflex, and beyond 3 cm of the right coronary artery to the right marginal branch. The arteries were grouped by distance from the ostium to compare internal elastic lamina (IEL) areas between acute and healed ruptures at proximal, middle, and distal regions. The areas within the external elastic lamina, IEL, and lumen were measured on coronary sections by use of imaging software (IP Laboratory, Scanalytics, Inc). In the case of multiple ruptures in a single section, only the most superficial rupture site with the acute thrombus or healed rupture repair site was measured. The percent stenosis was derived from the formula (1 − lumen area/I E L area) × 100. In cases with acute plaque rupture, the area of the thrombus was not included for the calculation of percent stenosis.
Immunohistochemistry

Cell populations were identified by use of mouse monoclonal antibodies against human muscle α-actin (1A4, Sigma Chemical Co., dilution 1:4000), macrophages (KP-1, Dako, dilution 1:50), and the T-cell marker (CD45RO, Dako, dilution 1:400). Smooth muscle maturation markers included calponin (Dako, 1:200) and smoothelin (Monosan, 1:10). To identify proliferating cells, tissue sections were stained with the monoclonal MIB-1 antibody (Ki-67, ImmunoTech, 1:500 dilution).

Analysis of immunohistochemistry for cell type and markers of smooth muscle cell (SMC) maturation were performed with color-image software (Bioquant). The most recent or superficial healed smooth muscle cell (SMC) maturation were performed with color-image software (Bioquant). The most recent or superficial healed smooth muscle cell (SMC) maturation were performed with color-image software (Bioquant). The most recent or superficial healed smooth muscle cell (SMC) maturation were performed with color-image software (Bioquant).

The mean cell proliferation index in the healed rupture sites was calculated.

Results

Patient Demographics and Presumed Mechanism of Death

The mean age of the 142 men was 51±11 years. There were 38 blacks and 104 whites. One hundred thirty men died without a history of heart disease. There was a history of heart disease in 12 cases. These consisted of recent angioplasty for unstable angina (n=1), congestive heart failure (n=2), left ventricular hypertrophy by ECG (n=1), and a vague history of “heart problems” that were not further clarified (n=8). Thirteen hypertensive patients were on antihypertensive medications, including ACE inhibitors (n=5), calcium channel blockers (n=4), β-blockers (n=4), and diuretics (n=5). Ten patients were being treated for diabetes with insulin or oral hypoglycemic medication, and 3 patients were taking statin drugs.

The mechanism of death was presumed to be acute plaque rupture with acute thrombus in 44 men (mean age 49±10 years), acute plaque erosion with acute thrombus in 23 men (mean age 45±8 years), stable plaque with healed infarct in 41 men (mean age 53±11 years, P=0.004 versus eroded plaque, Student’s t test), and stable plaque without myocardial infarct in 34 men (mean age 54±12 years, P=0.003 versus eroded plaque and P=0.04 versus plaque rupture).

Healed Ruptures and Culprit Plaque and Healed Infarcts

There were a total of 189 healed rupture sites, 22 of which (12%) resulted in total coronary occlusion. One or more healed ruptures not underlying an acute rupture site were present in 86 of 142 hearts (61% of total). Healed ruptures were especially frequent in hearts with acute plaque rupture (75% of cases) and hearts with stable plaque and healed myocardial infarction (80% of cases, Table 1). In hearts with stable culprit plaques, those with healed myocardial infarcts were more likely to have ≥1 healed ruptures than those without healed myocardial infarcts (80% versus 53%, P=0.01, Fisher’s exact test), and the mean number of healed ruptures was greater (2.0±1.4 versus 1.1±1.4, P=0.016, Table 1, Student’s t test).

Evidence of Multiple Healed Previous Rupture Sites in Acute Rupture and at Healed Rupture Sites

Of the 44 acute ruptures, Sirius red staining demonstrated evidence of healed previous rupture in 33. Of these, 9 showed

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>n</th>
<th>With Healed Rupture (%)</th>
<th>No. of Healed Ruptures per Heart, Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute thrombus with plaque rupture</td>
<td>44</td>
<td>33 (75)</td>
<td>1.5±1.2*</td>
</tr>
<tr>
<td>Acute thrombus with plaque erosion</td>
<td>23</td>
<td>2 (9)</td>
<td>0.2±0.6</td>
</tr>
<tr>
<td>Stable plaque with healed myocardial infarction</td>
<td>41</td>
<td>33 (80)</td>
<td>2.0±1.4†</td>
</tr>
<tr>
<td>Stable plaque without healed myocardial infarction</td>
<td>34</td>
<td>18 (53)</td>
<td>1.1±1.4</td>
</tr>
</tbody>
</table>

*Significantly more than acute erosion, P<0.0001.
†Significantly more than acute erosion, P<0.0001 and stable plaque without healed infarct, P=0.016.

1 healed previous rupture site, 9 showed 2 healed previous rupture sites, 9 showed 3 healed previous rupture sites, and 6 showed 4 healed previous rupture sites. Only 11 acute rupture sites overlay large necrotic cores filled with hemorrhage and little collagen deposition. Acute ruptures occurring at sites of ≥3 healed previous rupture sites demonstrated greater underlying luminal narrowing (94±4% versus those without healed previous rupture (74±12%, P=0.001, Student’s t test). Of the 189 healed ruptures, 57 (30%) demonstrated only 1 healed rupture site, 64 (34%) showed 2 (Figure 2), 60 (32%) 3, and 8 (4%) 4.

Morphometric Comparison of Acute and Healed Plaque Ruptures

The mean percent cross-sectional area luminal narrowing increased with the number of healed rupture sites (Figure 3) and was consistently greater in acute versus healed ruptures. Acute ruptures occurred in arteries with a larger IEL compared with healed ruptures in all 3 locations: proximal, middle, and distal segments (Figure 4).

Cell Proliferation and Muscle Differentiation

The mean cell proliferation index in the healed rupture sites was 0.40±0.09%, compared with 0.16±0.04% in areas of acute rupture (P=0.008, Student’s t test) (Figure 5). Evidence of cell proliferation was found in healed rupture sites in 76% of cases studied, but in only 1 case of acute rupture. The mean percent area of α-actin was 19.9±3.7% in areas of healed rupture, versus 2.1±1.3% for calponin (Figure 6). The mean % α-actin area/ % calponin staining in areas of healed rupture was 15.7±8.4%. There was no evidence of smoothelin expression in healed rupture sites, despite staining within the media as an internal control.

Healed Ruptures and Risk Factors

Elevated total cholesterol and total/HDL cholesterol were associated with the presence of 1 or 2 healed ruptures, and elevated cholesterol, total/HDL cholesterol, glycohemoglobin, advanced age, and increased body mass index were associated with ≥3 healed ruptures (Table 2).
Healed Ruptures and Heart Weight

Heart weight was increased in men who died with healed infarcts and stable plaque (mean 530±116 g) compared with plaque erosion (464±105 g, P=0.03) or stable plaque without healed infarction (460±98, P=0.008, Student’s t test). As the numbers of healed ruptures increased, so did heart weight (468±116 g, no healed ruptures; 498±118 g, 1 to 2 healed ruptures; 558±114 g, >2 healed ruptures; none versus 1 to 2 healed ruptures, P=0.03, and none versus >2 healed ruptures, P=0.002). When hypertensive men were excluded, there was a greater association between heart weight and healed ruptures (431±73 g, no healed ruptures; 493±113 g,}

Figure 2. Multiple healed rupture sites. A, Cross section of epicardial artery (Movat pentachrome) with thick fibrous cap overlying hemorrhagic necrotic core. Sirius red stain (B) of a section ~0.1 mm distal shows dense collagen overlying first healed rupture (1) and a second necrotic core (2). With polarization (C), more recent rupture site is identified as a strand of collagen (arrow). More recent collagen is green, near lumen, and older collagen more orange/yellow, toward base of plaque.

Figure 3. With increased numbers of healed rupture sites, there is an increase in mean percent luminal narrowing in both acute and healed rupture (stable plaque) sites. A, At sites of healed rupture (stable plaque) without acute rupture, percent luminal narrowing increased with increasing numbers of healed rupture sites (P=0.0001, ANOVA, Fisher’s protected least significant difference post hoc test). B, Likewise, at sites of acute rupture, percent luminal narrowing increased with increasing numbers of previous healed rupture sites (P=0.007, ANOVA, Fisher’s protected least significant difference test). Thrombus was not measured to derive percent narrowing.

Figure 4. When grouped by distance from ostium, acute ruptures occurred in arteries with greater IEL area compared with healed ruptures. This figure compares IEL area in acute and healed ruptures by distance from coronary ostium (proximal, middle, and distal, as defined in Methods). Ruptures were grouped by site to adjust for obvious effect of distance from ostium. Difference in IEL between acute rupture and healed plaque rupture was significant in all groups: distal (P=0.006), mid (P=0.03), and proximal (P=0.02) arteries.
A retrospective analysis of the mechanisms of progressive luminal narrowing based on morphology at a single time point has its limitations. Nevertheless, the present study supports the hypothesis of plaque rupture as a mechanism of increased luminal narrowing. First, evidence of previous plaque rupture was common in arteries with acute rupture. Indeed, an acute rupture overlying a single, large, lipid-rich pool without evidence of previous collagen layering was the exception in these culprit plaques. Second, acute ruptures overlying healed ruptures were more narrowed than de novo ruptures, suggesting that repetitive injury may cause plaque enlargement. Third, there was a low but significantly increased rate of cell proliferation in the SMC-rich regions of healed rupture sites, providing evidence of further plaque expansion.

Although the current paradigms of coronary atherosclerosis emphasize the importance of SMCs and matrix as contributing factors to plaque growth, the mechanism(s) of arterial narrowing are most likely complex, involving more than an overall increase in plaque burden. Wound contraction as a mechanism of arterial narrowing beyond that of plaque burden alone is supported by a number of animal studies. Fibrin is involved in the progression of luminal narrowing. Sequential injury in the rabbit is associated with wound contraction associated with the interaction between SMCs and extensive fibrin deposition. These experimental data, along with our study, suggest that selective components of the coagulation cascade may be potential therapeutic targets for the progressive luminal loss associated with episodic ruptures.

In the present study, healed repair sites were characterized by a predominance of SMCs, most likely derived from both migration and proliferation. The relatively low rate of proliferating SMCs in the healing rupture sites is compatible with reports of cell proliferation in native plaques removed by atherectomy. The relatively high rate of cell proliferation in the SMC-rich regions of healing sites compared with the acute plaque rupture may reflect increased vascularity and wound healing, as suggested by previous topographical studies. The weak expression of calponin in the neointimal SMCs suggests that initially the SMCs are proliferating, with few contractile features. Calponin has been demonstrated to be expressed primarily in differentiated SMCs and is involved in the regulation of contraction. The complete absence of smoothelin expression within the plaque intima corroborates the lack of fully differentiated contractile SMCs in areas of plaque repair.

The present study demonstrates an association between diabetes (glucose intolerance) and healed plaque ruptures, as well as an association between obesity and healed plaque ruptures. The fact that the 3 risk factors (dyslipidemia, glucose intolerance, and obesity) would all contribute to an increase in healed plaque ruptures is not unexpected, because of the interrelationship between diabetes, obesity, hypertension, hypertriglyceridemia, and decreased HDL cholesterol. Interestingly, however, we did not find an association between acute plaque ruptures and glucose intolerance. The association between acute ruptures and diabetes may be more difficult to detect because fatal plaque rupture is a single

Figure 5. Cellular proliferation in healed rupture sites. A, Hematoxylin-eosin-stained section of healed rupture site outlined by arrows. Lipid core is above healed rupture site. * indicates junction of healed site with relatively normal neointima. Adjoining section was stained for Ki-67, a proliferation marker (B); box in A represents healed site. Scattered SMCs with nuclear staining (arrows) are seen in brown; nonreactive nuclei appear blue.

Discussion

Plaque rupture occurs in atherosclerotic plaques with a thin fibrous cap overlying a necrotic core that is rich in lipids, and it is the most important mechanism of plaque instability that leads to coronary thrombosis. Angiographic studies have firmly established the association of thrombosis as an important mechanism of unstable angina and acute myocardial infarction. Not all severely narrowed segments identified by angiography, however, have evidence of previous acute thrombosis, and a few angiographic studies that have demonstrated plaque progression in the short term have suggested that thrombosis is the cause. Autopsy studies have suggested that coronary thrombosis may occur in the absence of cardiac symptoms, resulting in progression of atherosclerosis. Our observations in sudden unexpected death demonstrate that patients who died with acute plaque rupture and those with healed myocardial infarction had the highest frequency of healed plaque ruptures (75% and 80%, respectively), whereas those who died with plaque erosions had the fewest (9%) and those who died with stable plaques had an intermediate incidence (53%).

1 to 2 healed ruptures; 554±112 g, >2 healed ruptures; P=0.0001, none versus >2 healed ruptures).
event, as opposed to multiple events in the case of healed plaque ruptures. In a previous study with noncoronary deaths used as controls, we demonstrated an association between diabetes and sudden coronary death with stable plaque in women, although healed ruptures were not investigated in that study.21

Because of a lack of an animal model of plaque rupture, we are restricted to autopsy tissue to explain the morphological changes that occur in unstable plaques. Nevertheless, on the basis of our static observations, the data indicate that at the very least, repeated plaque ruptures that heal are frequent in men who died suddenly. The data further suggest that silent ruptures result in significant increase in plaque burden and negative remodeling. Thus, in many cases, fatal ruptures may represent the final stage of an ongoing process of arterial wound healing.

Acknowledgment

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References


Figure 6. Healed rupture site, SMC immunophenotype. A, Movat pentachrome stain demonstrates matrix-rich area of healed rupture site (arrows). B, α-Actin stain, with weaker staining for calponin (C).

TABLE 2. Multivariate Analysis (Logistic Regression), Association of Risk Factors and Healed Ruptures

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Healed Ruptures, P</th>
<th>Acute Rupture, P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.003</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>0.01</td>
<td>0.0003</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Glycohemoglobin</td>
<td>0.48</td>
<td>0.02</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0.76§</td>
<td>0.58§</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.91</td>
<td>0.46</td>
</tr>
<tr>
<td>Age</td>
<td>0.16</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Comparison with 0 healed ruptures.
†Vs cases without acute rupture.
‡Analysis removing total and HDL cholesterol as separate variables.
§Negative association.


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