Clinical Investigation and Reports

Smooth Muscle Cell Abundance and Fibroblast Growth Factors in Coronary Lesions of Patients With Nonfatal Unstable Angina

A Clue to the Mechanism of Transformation From the Stable to the Unstable Clinical State

Moshe Y. Flugelman, MD; Renu Virmani, MD; Rosaly Correa, MD; Zu-Xi Yu, MD; Andrew Farb, MD; Martin B. Leon, MD; Amir Elami, MD; Ya-Min Fu, MD; Ward Casscells, MD; Stephen E. Epstein, MD

Background. The mechanisms responsible for the transformation of stable angina to unstable angina, a major cause of morbidity and mortality, are commonly believed to be plaque rupture and thrombosis. We determined whether additional mechanisms are operative by analyzing the histopathology and immunohistopathology of coronary plaques retrieved by directional atherectomy of patients with unstable angina in whom no intraluminal thrombus was demonstrated by angiography.

Methods and Results. The histological findings of atherectomy specimens from 34 patients with unstable angina were compared with those of 24 patients with postangioplasty restenosis, whose lesions are known to be composed of smooth muscle cells (SMCs), and 10 patients with stable angina, whose lesions contain relatively few SMCs. We also studied the expression of acidic and basic fibroblast growth factors (aFGF and bFGF), whose role in the vascular response to injury has been established. Specimens from unstable angina resembled those from postangioplasty restenosis in regard to SMC abundance (scale, 0 to 3; 1.4±0.9 versus 1.7±0.9; P=NS), and both differed from those of stable angina. Thrombus and/or hemorrhage occurred in only 34% of patients with unstable angina (compared with 8% of restenosis patients and in none of stable angina patients). Active lesions (defined as lesions containing one or more of the following: thrombus, hemorrhage, abundant and disorganized SMCs in the presence of loose connective tissue, or inflammatory infiltrate) were observed in 56% of the unstable angina patients and in 50% of the restenosis patients but in none of the stable angina patients. The expression of aFGF and bFGF was detected in 80% to 100% of unstable angina (n=11) and restenosis (n=10) specimens but in only 1 of 5 stable angina specimens.

Conclusions. Microscopic evidence of thrombosis and plaque rupture occurred in only one third of unstable angina patients, selected because they had no angiographic evidence of intracoronary thrombus. Moreover, their lesions resembled those of restenosis patients in regard to SMC abundance, lesion activity, and the expression of aFGF and bFGF. Our findings therefore suggest that an alternative mechanism to plaque rupture and thrombus formation may be operative in the precipitation of unstable angina; namely, in a subset of patients, SMC proliferation may lead to gradual plaque expansion and thereby to luminal narrowing and unstable angina. Our data also suggest a role for aFGF and bFGF in this process. (Circulation. 1993;88:2493-2500.)

KEY WORDS • smooth muscle cells • angina • growth factors

Unstable angina pectoris is a major cause of morbidity and mortality leading to 750,000 hospitalizations annually in the United States alone. Thrombosis and primary plaque rupture have been implicated as the mechanisms responsible for the transformation of asymptomatic stable coronary lesions to symptomatic unstable lesions; however, definitive histopathological evidence has been available only in a subgroup of patients with fatal unstable angina pectoris. It is therefore possible that other mechanisms may also contribute to the precipitation of unstable angina.

One such mechanism was suggested by studies on the pathogenesis of postangioplasty restenosis, a condition that shares the rapid but usually not precipitate development of clinical signs of increasing coronary obstruction. Because smooth muscle cell proliferation has been shown to be a primary causal mechanism in the restenosis process, in the present investigation we...
examined the hypothesis that a similar mechanism is responsible for the development of nonfatal unstable angina pectoris.

To estimate the importance of smooth muscle cell proliferation in the development of this clinical syndrome, we compared atherectomy specimens of lesions of unstable angina patients with those of restenosis patients, whose relatively cellular lesions are known to be composed predominantly of smooth muscle cells, and with those of stable angina patients, whose lesions are composed of dense collagen and contain relatively few smooth muscle cells in the fibrous cap. As an integral part of this concept, we also sought to determine the relative abundance in these lesions of both acidic and basic fibroblast growth factors (FGF), as both are important mediators of smooth muscle cell proliferation and migration.

Methods

Patients

Atherectomy specimens from 70 consecutive patients undergoing directional coronary atherectomy were analyzed. The patients were referred to a tertiary referral center (Washington Hospital Center) for angiographic diagnosis and therapy. Patients with evidence of significant coronary narrowing (>60% narrowing of a major epicardial artery) and lesion anatomy favorable for directional atherectomy were included in the study. Patients with total coronary occlusion and those with unequivocal angiographic diagnosis of intracoronary thrombus underwent different revascularization procedures and thus were not part of the current investigation. Three patients were excluded from the study because their atherectomy specimens contained only media or were too small to be informative. A fourth patient was excluded from the study because a consensus in regard to the pathological findings could not be reached. Thus, the study consisted of a total of 66 patients.

Patients were classified according to their admission diagnosis into one of the three following groups: (1) unstable angina pectoris (32 patients), defined as one of three clinical syndromes: angina pectoris occurring at rest (17 patients), recent onset angina pectoris (<2 months' duration) (10 patients), and accelerated angina pectoris (5 patients); (2) postangioplasty restenosis (24 patients), defined by atherectomy being performed at least 1 week after angioplasty (mean, 4 months; median, 2 months; range, 1 week to 19 months), and (3) stable angina pectoris (10 patients).

Tissue Preparation

Atherectomy specimens were fixed at the time of the procedure in 10% buffered formalin. Tissue was dehydrated in graded series of alcohol and embedded in paraffin block. Serial sections were stained for hematoxylin and eosin, Movat's pentachrome, Mallory's phosphotungstic acid hematoxylin (PTAH), and Masson's trichrome stains. Serial unstained sections were used for immunohistochemistry.

Immunohistochemistry

In 26 atherectomy specimens, immunohistochemistry was performed using polyclonal antibodies against acidic and basic FGFs. Anti-basic FGF (Ab) IgG was a kind gift from Dr A. Baird, La Jolla, Calif (concentration used, 2.0 µg/mL), and the antiacidic FGF (Ab) IgG was a kind gift from Dr J. Sasse, Tampa, Fla (concentration used, 2.5 µg/mL). Both antibodies have been described previously. Specimens were incubated with the primary antibody overnight at 4°C. Incubation with biotinylated secondary antibody was carried out at room temperature, followed by incubation with avidin and biotinylated horseradish peroxidase complex (ABC method, Vector Labs). The sections were counterstained with methyl green. Two controls were used: (1) nonimmune rabbit serum and (2) antibodies preadsorbed with acidic or with basic recombinant human FGF. Due to the small amount of tissue retrieved by coronary atherectomy, the specificity of the antibodies to human acidic and basic FGFs was assessed by Western blotting of protein extracts from four human

### Table 1. Demographic and Angiographic Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, Mean (Range)</th>
<th>Sex</th>
<th>Vessels Diseased, No.</th>
<th>Atherectomy Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable AP (n=10)</td>
<td>66 y (53-77)</td>
<td>Male, 10 Patients</td>
<td>1, 5 Patients</td>
<td>LAD, 5 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female, 0 Patients</td>
<td>2, 2 Patients</td>
<td>Cx, 2 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3, 3 Patients</td>
<td>RCA, 2 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other, 1 Patient</td>
</tr>
<tr>
<td>Unstable AP (n=32)</td>
<td>61 y (41-76)</td>
<td>Male, 27 Patients</td>
<td>1, 15 Patients</td>
<td>LAD, 20 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female, 5 Patients</td>
<td>2, 12 Patients</td>
<td>Cx, 6 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3, 5 Patients</td>
<td>RCA, 3 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other, 3 Patients</td>
</tr>
<tr>
<td>Restenosis (n=24)</td>
<td>59 y (34-80)</td>
<td>Male, 20 Patients</td>
<td>1, 10 Patients</td>
<td>LAD, 13 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female, 4 Patients</td>
<td>2, 10 Patients</td>
<td>Cx, 2 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3, 4 Patients</td>
<td>RCA, 7 Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other, 2 Patients</td>
</tr>
</tbody>
</table>

AP indicates angina pectoris; LAD, left anterior descending coronary artery; Cx, circumflex coronary artery; and RCA, right coronary artery.
coronary arteries. The four arteries were excised from the hearts of patients undergoing heart transplantation. The underlying cause of transplantation was ischemic cardiopathy (2 patients) and dilated cardiomyopathy (2 patients). The arteries were frozen in liquid nitrogen after adjacent tissue was trimmed, and 200 mg of arterial segments was homogenized and proteins were extracted from the homogenate. The extracted proteins were incubated with heparin-Sepharose beads for 18 hours at 4°C. At the end of the incubation, the beads

<table>
<thead>
<tr>
<th>Group</th>
<th>Thrombus and/or Hemorrhage</th>
<th>Active Lesions</th>
<th>SMC Predominance Scale 0 to 3 (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable AP (n=10)</td>
<td>0</td>
<td>0</td>
<td>0.7±0.6</td>
</tr>
<tr>
<td>Unstable AP (n=32)</td>
<td>11 (34%)</td>
<td>18 (56%)</td>
<td>1.4±0.9</td>
</tr>
<tr>
<td>Post-PTCA restenosis (n=24)</td>
<td>2 (8%)</td>
<td>12 (50%)</td>
<td>1.7±0.9</td>
</tr>
</tbody>
</table>

SMC indicates smooth muscle cell; AP, angina pectoris; and PTCA, percutaneous transluminal coronary angioplasty.
were washed in 0.6M NaCl and then boiled. The proteins removed from the beads were run in a polyacrylamide gel, with size markers and human recombinant acidic and basic FGF (UBI, Lake Placid, NY) as positive controls in separate lanes. After blotting the samples to nitrocellulose, the blots were hybridized with the antibodies used for immunohistochemistry and developed with anti-rabbit IgG labeled with alkaline phosphatase.

**Histochemical and Immunohistochemical Analysis**

The stained specimens were analyzed by three independent observers blinded to the patient’s clinical diagnosis. The specimens were analyzed for (1) the presence or absence of thrombus and hemorrhage, (2) the presence of smooth muscle cells, based on cell morphology and PTAH staining (graded 0 to 3; 0, absence of smooth muscle cells; 3, predominance of smooth muscle cells in the specimen), and (3) lesion activity, where active lesions were defined as containing one or more of the following: thrombus, hemorrhage, abundant and disorganized smooth muscle cells in the presence of loose connective tissue, or inflammatory infiltrate. The immunohistochemical sections were classified as positive for the presence of acidic or basic FGFs when the cell cytoplasm stained brown and negative when no peroxidase reaction was noted. Because we previously found a good correlation between PTAH staining and immunohistochemistry for α-smooth muscle cell actin for the identification of smooth muscle cells in atherectomy specimens, we used PTAH staining in the present investigation to assess predominance of smooth muscle cells.37

**Statistical Analysis**

To compare the rating of smooth muscle cell predominance, we used the Mann-Whitney test. For dichotomous variables, we used Fischer’s exact or χ² tests.

**Results**

The demographic and angiographic data of patients are summarized in Table 1. The three observers agreed in 89% of cases in regard to plaque hemorrhage, 78% of cases in regard to the presence of thrombus, and in 88% of cases with regard to lesion activity. In cases of disagreement, the opinion of the majority was used in the analysis. For smooth muscle predominance, the arithmetical average was used in the analysis.

Typical lesions of patients with stable angina pectoris, unstable angina pectoris, and postangioplasty restenosis stained with hematoxylin eosin are shown in Fig 1, and the histological findings in the three groups of patients are summarized in Table 2. Analysis of the atherectomy specimens of patients with unstable angina pectoris demonstrated that while only a minority (34%) of the specimens had evidence of thrombus or hemorrhage, the prevalence of this finding was still significantly higher than in the specimens of patients with restenosis (8%) (P < .03) or of those with stable angina (0%). Active lesions were observed in about half of both the unstable angina (56%) and in restenosis patients (50%) but in none of the stable angina patients. Smooth muscle cells predominated in the specimens of both patients with restenosis and those with unstable angina (1.7 ± 0.9 versus 1.4 ± 0.9, P = NS), whereas the lesions of patients with stable angina showed far fewer smooth muscle cells (0.7 ± 0.6).

Western blot analysis (Fig 2) demonstrated that the antibodies used in the immunohistochemical analysis recognized acidic and basic FGFs, as indicated by the positive immunoreaction with heparin binding proteins extracted from human coronary arteries; these proteins were of the identical molecular weight as human recombinant acidic and basic FGFs.

Typical immunohistochemical findings of unstable angina patients using antibodies directed against acidic
FGF and basic FGF are demonstrated in Figs 3A and 3B, respectively. Analysis of the immunohistochemical staining showed that immunoreactivity for acidic and basic FGFs was observed in most patients with unstable angina and restenosis and in only 1 out of 5 in the stable angina group (20%) (Figs 4A and 4B).

Discussion

Previous studies designed to investigate the mechanisms responsible for the development of unstable angina pectoris have concluded that the clinical syndrome is caused by plaque rupture, hemorrhage, and thrombus formation. This conclusion derives from studies using post mortem analyses, coronary angiography, and coronary angioscopy, which convincingly proved the validity of this causal linkage. The presence of thrombus and the contribution of dynamic changes of vascular tone (as suggested by experimental observations of cyclic flow variations) undoubtedly explains the clinical course of many patients with unstable angina pectoris. However, many of these studies demonstrated that a sizeable percentage of patients with unstable angina do not have plaque rupture or thrombus that can be identified, at least at the time of the studies. Moreover, only a minority of patients with unstable angina pectoris will respond favorably to thrombolytic therapy. Hence, it would appear that plaque rupture and thrombus formation are not the only mechanisms leading to the precipitation of unstable angina.

In the present investigation, almost two thirds of our patients exhibited no evidence of thrombus on analysis of
tissue derived from atherectomy. This figure underestimates the prevalence of thrombus in unstable angina because only patients who had no evidence of intraluminal thrombus on angiography were entered into the study. The fact remains, however, that there is still a significant number of patients with unstable angina, in this and other studies, who have no angiographic or pathological evidence of intracoronary thrombus.18-23,28,29

It must be pointed out that by the time of atherectomy in this subgroup of patients, it is possible the original plaque dissection had healed, and any thrombus originally present had lysed or organized. Hence, plaque rupture and thrombus formation cannot be definitively ruled out as the common cause of all episodes of unstable angina pectoris. Moreover, the size of atherectomy specimens is small, and it can be argued that the apparent lack of thrombus was due to sampling error. The stable angina group is rather small and serves mostly to amplify the similarities between the groups of unstable and restenosis patients.

Although our study cannot refute such possibilities, the results do provide an alternative mechanism to plaque rupture, hemorrhage, and thrombus formation in the precipitation of unstable angina in a subset of patients. Thus, in the majority of the specimens obtained from patients with unstable angina, the bulk of the lesions consisted of cells in a loose extracellular matrix (predominantly glycosaminoglycans); moreover, smooth muscle cells were the dominant cell type. Such findings rendered these specimens indistinguishable from those of patients with restenosis. This observation is conceptually important because human and animal studies have provided evidence that arterial injury induces smooth muscle proliferation and migration with the production of loose connective tissue and that this mechanism contributes to postangioplasty restenosis. The fact that the histological characteristics of the lesions of patients undergoing atherectomy for unstable angina pectoris are indistinguishable from those of patients with restenosis strongly suggests that the mechanism responsible for both may be the same: Smooth muscle proliferation and the associated secretion of glycosaminoglycans increase the mass of the atheroma, which thereby exacerbates the coronary obstruction and precipitates an ischemic syndrome.

Our hypothesis is further supported by the finding that the expression of both acidic and basic FGFs are prominent in the lesions derived from unstable angina patients when compared with the expression of these peptides in patients with stable angina. The lesions of patients with stable angina were also relatively acellular (we must emphasize, however, that our stable angina group is too small to make such comparisons definitive).

Just as the histological appearance of the unstable angina lesion was similar to that of the restenosis lesion, so was the immunohistochemical appearance; both displayed high levels of expression of acidic and basic FGFs. Acidic and basic FGF have been found to stimulate proliferation and migration in many cell types, including smooth muscle cells, both in vitro and in vivo.10,30-32 The presence of the growth peptides should be regarded as an indicator to the activity of the lesions...
and should not carry any implications regarding their role in the triggering events of unstable transformation.

We wish to emphasize that our findings do not negate the prevailing concept that unstable angina occurs as a result of plaque rupture and thrombus formation. We believe that these mechanisms undoubtedly account for the precipitation of unstable angina in many patients. This concept is supported by the findings of histological evidence of thrombus and/or hemorrhage in a significant number of patients, even in our selected group of patients. Our findings do not negate the possibility that changes in vascular tone contribute to the development of unstable angina (as a primary cause or by triggering the development of plaque rupture or thrombus formation). On the other hand, our data support the concept that the precipitation of unstable angina cannot be ascribed to this mechanism alone. Rather, it appears that its pathophysiology is more complex and that one of the additional contributing causes is smooth muscle cell proliferation, a process that may be amplified, at least in part, by acidic and basic FGFs.

This conceptualization, even if correct, does not identify the primary precipitating stimulus leading to overexpression of acidic and basic FGFs and to smooth muscle cell proliferation. We can at this time only speculate as to the possible triggering event. Thus, it is possible that hemorrhage into a plaque, minor fibrous cap tears and dissection, microthrombi with dynamic changes of vascular tone, or other mitogenic stimuli lead to the expression of multiple growth factors, including acidic and basic FGFs, which in turn initiate a cascade of events in which the dominant component is smooth muscle cell proliferation (Fig 5). This also may be associated with migration of smooth muscle cells from the underlying media into the plaque and the synthesis and secretion by smooth muscle cells of extracellular matrix, processes leading to expansion of the original plaque. Given the complexity of the process, it is also possible that the expansion and resulting conformational changes caused by this proliferative mechanism may make the plaque more vulnerable to ulceration and secondary thrombus formation and that in some patients, both of these mechanisms contribute to the precipitation of unstable angina.

Conclusions

We believe that the development of unstable angina is precipitated by plaque rupture and thrombus formation in many individuals, but in others it may be caused by excessive smooth muscle cell proliferation. Although we cannot yet identify the mechanisms that trigger smooth muscle cell proliferation in patients whose clinical situation changes from a stable to an unstable anginal pattern, our findings will, we hope, lead to future studies designed to elucidate the responsible mechanisms. Such information, once obtained, will undoubtedly improve our approach to the treatment and perhaps to the prevention of the development of unstable angina pectoris.

References


Smooth muscle cell abundance and fibroblast growth factors in coronary lesions of patients with nonfatal unstable angina. A clue to the mechanism of transformation from the stable to the unstable clinical state.

M Y Flugelman, R Virmani, R Correa, Z X Yu, A Farb, M B Leon, A Elami, Y M Fu, W Casscells and S E Epstein

_Circulation_. 1993;88:2493-2500
doi: 10.1161/01.CIR.88.6.2493

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/88/6/2493