Coronary Composition and Macrophage Infiltration in Atherectomy Specimens From Patients With Diabetes Mellitus

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Background—Lipid-rich, inflamed atherosclerotic lesions are associated with plaque rupture and thrombosis, which are the most important causes of death in patients with diabetes mellitus. This study was designed to quantify lipid composition and macrophage infiltration in the coronary lesions of patients with diabetes mellitus.

Methods and Results—A total of 47 coronary atherectomy specimens from patients with diabetes mellitus were examined and compared with 48 atherectomy specimens from patients without diabetes. Plaque composition was characterized by trichrome staining. Macrophage infiltration was characterized by immunostaining. Clinical and demographic data were similar in both groups. The percentage of total area occupied by lipid-rich atheroma was larger in specimens from patients with diabetes (7±2%) than in specimens from patients without diabetes (2±1%; P=0.01), and the percentage of total area occupied by macrophages was larger in specimens from patients with diabetes (22±3%) than in specimens from patients without diabetes (12±1%; P=0.003). The incidence of thrombus was also higher in specimens from patients with diabetes than in specimens from patients without diabetes (62% versus 40%; P=0.04). Plaque composition, macrophage infiltration, and thrombus were similar in lesions from diabetic patients treated with insulin compared with lesions from patients treated with sulfonylureas or diet.

Conclusions—Coronary tissue from patients with diabetes exhibits a larger content of lipid-rich atheroma, macrophage infiltration, and subsequent thrombosis than tissue from patients without diabetes. These differences suggest an increased vulnerability for coronary thrombosis in patients with diabetes mellitus. (Circulation. 2000;102:2180-2184.)

Key Words: atherosclerosis • coronary disease • diabetes mellitus • plaque

Atherosclerosis is the major cause of premature death in patients with either insulin-dependent or non–insulin-dependent diabetes; it accounts for ≈80% of all deaths and 75% of all hospitalizations in these patients.1 Diabetic patients with ischemic heart disease have a poor outcome after myocardial infarction compared with nondiabetic patients.2 This outcome is even worse in diabetic patients treated with sulfonylureas.3 Furthermore, diabetic patients have an increased incidence of restenosis after percutaneous transluminal coronary angioplasty compared with nondiabetic patients.4

Plaque composition may play a role in the plaque disruption and thrombosis that leads to acute coronary events.5-7 Lesions with a large lipid core and increased macrophage infiltration may have a higher risk for disruption than sclerotic plaques.7-9 In addition, macrophage infiltration is also a predictor for restenosis after coronary intervention.10 Despite extensive autopsy studies, histopathologic analyses of lipid composition and macrophage infiltration in coronary lesions from patients with diabetes mellitus are limited. This study was designed to identify differences in plaque composition and macrophage infiltration in coronary lesions from patients with diabetes mellitus.

Methods

Definitions and Patient Population
From October 1990 to November 1994, 82 consecutive directional coronary atherectomy (DCA) procedures were performed for symptomatic coronary artery disease in patients with diabetes mellitus at the cardiac catheterization laboratory of Massachusetts General Hospital. Of these procedures, 47 were performed in culprit coronary lesions from patients with acute ischemic events; these patients comprise the study population. Forty-eight specimens from the culprit lesions of patients without diabetes mellitus who were matched to the study population by clinical syndrome comprise the control group. Lesions from patients treated for restenosis, saphenous vein graft disease, stable angina, or congestive heart failure were excluded. Diabetes mellitus was defined as a fasting blood
glucose level >140 mg/dL in ≥2 different values. All diabetic patients were known diabetics (mean, 8±4 years) at the time of the intervention. There were 13 insulin-dependant and 34 non–insulin-dependant diabetic patients. Of the 34 non–insulin-dependent diabetics, 14 were treated with sulfonylureas and 20 were treated with diet. To further evaluate if plaque composition was different in these 3 groups of diabetic patients, a subgroup analysis was performed.

Atherectomy Specimens
Multiple pieces of tissue were obtained from each lesion and immediately immersion-fixed in 10% buffered formalin. Tissue was routinely processed for paraffin embedding. Sections were serially cut at 5 μm, mounted on lysine-coated slides, and stained with hematoxylin and eosin and with the trichrome method.

Morphometry
Total and segmental areas for each of the plaque components were quantified by planimetry for each specimen. The trichrome connective tissue stain was used to identify and quantify specimen composition, as previously reported. Briefly, collagen-rich fibrocellular tissue is composed of tissue with few cells and densely stained collagen (Figure 1A); hypercellular tissue is composed of a loose connective tissue matrix containing numerous stellate cells (Figure 1B); lipid-rich atheromatous gruel is composed of acellular debris with cholesterol clefts (Figure 1C); and thrombus, which is defined as fibrin deposition, plaque hemorrhage, or both, stains red (Figure 1D). Media was recognized by the presence of internal elastic lamina and more orderly arranged smooth muscle cells and connective tissue matrix and was excluded. Each specimen area was outlined at a low magnification (×40) and quantified by manual planimetry. Total area in square millimeters and the percentage of the total area are reported.

Immunocytochemistry
Human macrophage antibody staining was performed using 5-μm-thick sections that were deparaffinized and rehydrated with PBS. Macrophages were identified using anti-human pan-macrophage anti-CD-68 and KP-1 (M814 DAKO) at a concentration of 7.6 μg/mL. Sections were blocked with normal goat serum and 3% H2O2 in water, washed in PBS, and incubated with the primary antibody for 1 hour at 37°C. They were then washed in PBS, and primary antibodies were detected with a biotin-streptavidin–amplified detection system (SuperSensitive Kit, Biogenex) that was developed with diaminobenzidine. Sections were dehydrated, cover-slipped, and examined. Positive control slides (spleen for KP-1), nonimmune negative controls, and processing controls were performed. Each stained sample was outlined manually, and the areas occupied by stained macrophages were measured. All measurements were performed in a blind fashion.

Statistical Analysis
Results are expressed as mean±SEM, and P<0.05 was considered statistically significant. To compare 2 discrete variables (clinical and demographic data in diabetics versus nondiabetics), a 2-tailed Fisher test was used, and to compare 3 discrete variables (clinical and demographic data in the 3 diabetic groups), a χ2 test was performed. A 2-tailed Student’s t test was used to compare Gaussian samples. To compare data that are not compatible with a normal frequency distribution, a 2-tailed Student’s t test was performed with a logarithmic transformation of each individual value (morphometric data), and to compare data that are not compatible with a normal frequency distribution after logarithmic transformation (thrombus data), a robust test was used to compare medians (Rs1 software 6.0, Brooks Animation).

Results
Clinical and Demographic Data
The clinical and demographic characteristics of the study and control populations were similar (Table 1). No significant differences existed between the 2 groups in age, sex, or clinical syndrome at the time of atherectomy. The incidence of risk factors for coronary artery disease was similar as well. The number of vessels containing a >50% lesion was greater in the diabetic group (Table 1). Three subgroups of diabetic...
patients were identified; they were (1) patients under insulin therapy, (2) patients under oral sulfonylurea therapy, and (3) patients under diet therapy. The clinical and demographic characteristics of the patients in these subgroups were similar (Table 2).

Morphometry Data
Total plaque area was similar in specimens from diabetic and nondiabetic patients (Table 3). The percentage of total area occupied by lipid-rich atheroma was larger in specimens from patients with diabetes than in specimens from patients without diabetes (P = 0.01). The area occupied by thrombus was larger in specimens from patients with diabetes than in specimens from patients without diabetes (P = 0.03; Table 3). The incidence of coronary thrombus was also higher in specimens from patients with diabetes (29 of 47) than in specimens from patients without diabetes (19 of 48; 62% versus 40%, respectively; P = 0.04). The percentage of total area occupied by macrophages is shown in Figure 2. Macrophage infiltration was greater in specimens from patients with diabetes than in specimens from patients without diabetes (22 ± 6% versus 12 ± 6%; P = 0.003). Figure 3 provides an example of macrophage regions in the atherectomy tissue of patients with and without diabetes mellitus. The plaque composition of lesions from patients with diabetes mellitus is shown in Table 4. No differences existed in lipid-rich atheroma, collagen-rich fibrocellular tissue, hypercellular tissue, or macrophage areas in specimens from diabetic patients treated with sulfonylureas compared with specimens from diabetic patients treated with insulin or diet alone. The incidence of thrombus was 69% in the insulin group (9 of 13), 64% in the sulfonylurea group (9 of 14), and 55% in the diet-alone group (11 of 20; P = NS).

Discussion
This study demonstrates a significant increase in lipid pool content and macrophage infiltration in coronary specimens from patients with diabetes mellitus. In addition, coronary thrombosis was found more frequently in the coronary specimens from patients with diabetes mellitus.

### Table 1. Clinical Data of Patients With Diabetes Mellitus and Patients Without Diabetes

<table>
<thead>
<tr>
<th></th>
<th>Diabetes (n=47)</th>
<th>No Diabetes (n=48)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63 ± 10</td>
<td>60 ± 10</td>
<td>0.16</td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>17/30</td>
<td>13/35</td>
<td>0.23</td>
</tr>
<tr>
<td>Symptoms, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstable angina</td>
<td>30 (63)</td>
<td>35 (69)</td>
<td>0.38</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>17 (33)</td>
<td>13 (27)</td>
<td>0.38</td>
</tr>
<tr>
<td>Coronary risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>28 (60)</td>
<td>32 (66)</td>
<td>0.52</td>
</tr>
<tr>
<td>Hypertension</td>
<td>37 (79)</td>
<td>33 (69)</td>
<td>0.35</td>
</tr>
<tr>
<td>Smoker</td>
<td>24 (51)</td>
<td>25 (52)</td>
<td>1.0</td>
</tr>
<tr>
<td>Family history</td>
<td>27 (58)</td>
<td>28 (58)</td>
<td>1.0</td>
</tr>
<tr>
<td>No. of vessels*</td>
<td>1.8 ± 0.8</td>
<td>1.3 ± 0.6</td>
<td>0.004</td>
</tr>
<tr>
<td>Time interval,† d</td>
<td>11 ± 7</td>
<td>11 ± 8</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Values are mean ± SEM or n (%).
*Coronary artery disease >50% by angiography.
†Time interval between symptoms and atherectomy.

### Table 2. Clinical Data From Diabetic Patients Treated With Insulin, Sulphonylureas, or Diet

<table>
<thead>
<tr>
<th></th>
<th>Insulin (n=13)</th>
<th>Sulphonylureas (n=14)</th>
<th>Diet (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59 ± 11</td>
<td>65 ± 9</td>
<td>63 ± 11</td>
<td>0.22</td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>4/9</td>
<td>7/14</td>
<td>6/20</td>
<td>0.29</td>
</tr>
<tr>
<td>Symptoms, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstable angina</td>
<td>10 (77)</td>
<td>7 (50)</td>
<td>13 (65)</td>
<td>0.23</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>3 (23)</td>
<td>7 (50)</td>
<td>7 (35)</td>
<td>0.24</td>
</tr>
<tr>
<td>Coronary risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>6 (46)</td>
<td>10 (71)</td>
<td>12 (60)</td>
<td>0.25</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10 (77)</td>
<td>10 (71)</td>
<td>17 (85)</td>
<td>0.71</td>
</tr>
<tr>
<td>Smoker</td>
<td>8 (61)</td>
<td>4 (29)</td>
<td>12 (60)</td>
<td>0.25</td>
</tr>
<tr>
<td>Family history</td>
<td>7 (54)</td>
<td>7 (50)</td>
<td>13 (65)</td>
<td>0.75</td>
</tr>
<tr>
<td>No. of vessels*</td>
<td>1.7 ± 0.8</td>
<td>2.1 ± 0.7</td>
<td>1.7 ± 0.8</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values are mean ± SEM or n (%).
*Coronary artery disease >50% by angiography.
The common factor precipitating acute coronary events is thrombosis. Thrombosis occurs in disrupted or eroded plaques. Plaque disruption (fibrous cap rupture) is more frequently seen in men and occurs in plaques with a large lipid core, increased macrophage infiltration, and decreased collagen and smooth muscle cell content. Thrombosis (loss of endothelial cells) is more frequently seen in premenopausal women with sudden cardiac death and occurs in plaques with abundant smooth muscle cells, which are usually in clusters. In this study, the incidence of coronary thrombosis was higher in specimens from patients with diabetes mellitus. This finding is in agreement with those of Silva et al., who found an increased incidence of angiographically documented coronary thrombosis in diabetic patients with unstable angina. It is also in agreement with recent clinical studies showing a significant benefit of aggressive antithrombotic therapy with platelet IIb/IIIa receptor antagonists in diabetic patients undergoing percutaneous revascularization.

The increased thrombotic predisposition of diabetic tissue may be multifactorial. First, a larger amount of lipid-rich atheroma was found in lesions from patients with diabetes. Previous studies identified the lipid core as the major thrombogenic substrate of the atherosclerotic plaque. Second, increased areas of macrophage infiltration were found in the lesions from patients with diabetes. Previous studies showed that culprit lesions from patients with acute coronary syndromes have an increased macrophage content when compared with lesions from patients with chronic stable angina. The present study included only culprit lesions from patients with either unstable angina or myocardial infarction; thus, the increased macrophage infiltration observed in the diabetic population represents an increase over the increase seen in lesions from patients with acute coronary syndromes. Finally, macrophages colocalize with tissue factor in coronary lesions from patients with unstable angina, and tissue factor is considered the primary initiator of thrombogenesis and thrombin generation in vivo.

The use of sulfonylurea drugs reportedly increases cardiovascular mortality in patients with diabetes mellitus. Sulfonylureas may impair ischemic preconditioning and prevent coronary vasodilation in response to ischemia. In addition, sulfonylureas may have atherogenic properties. In an attempt to identify differences in plaque composition between diabetic patients treated with sulfonylurea agents and those treated with insulin or diet, a subgroup analysis was performed. No significant differences were observed in this analysis, which suggests that sulfonylurea treatment may not be involved in changing the coronary plaque composition of patients with diabetes mellitus. However, this analysis was performed on a small number of lesions in each group, which diminishes its power to identify potential differences. Further studies should be performed to confirm these findings. Finally, atherosclerotic lesions from patients with diabetes mellitus exhibit an increased amount of advanced glycosylation end products. Specific receptors for proteins modified by these products have been identified in macrophages, and these receptors may play a role in arterial wall collagen turnover in patients with diabetes. However, the role of advanced glycosylation end products in plaque disruption is unclear and remains to be elucidated.

Limitations
Macrophage infiltration could be evaluated by cell counting and not by quantified planimetry. However, comparisons of macrophage areas are properly evaluated by quantified planimetry. In addition, DCA can extract only 33% of the plaque in primary lesions, so histological analysis may not reflect total plaque composition. Furthermore, DCA has been almost totally replaced by coronary stenting in clinical practice. This explains why the sample collection for this study was performed 6 years ago. Nevertheless, DCA is a unique technique to obtain human coronary tissue in vivo.

Conclusions
Coronary tissue from patients with diabetes exhibits a larger content of lipid-rich atheroma, macrophage infiltration, and...
TABLE 4. Total, Segmental, and Percent Areas of Plaque Components and Macrophages in Coronary Lesions From Diabetic Patients Treated With Insulin, Sulphonylureas, or Diet

<table>
<thead>
<tr>
<th>Areas</th>
<th>Insulin (n=15)</th>
<th>Sulphonylureas (n=16)</th>
<th>Diet (n=21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total area</td>
<td>5.09±1.46 (100)</td>
<td>3.65±0.6 (100)</td>
<td>4.69±0.7 (100)</td>
<td>0.35</td>
</tr>
<tr>
<td>Atheroma</td>
<td>0.38±0.15 (8±4)</td>
<td>0.18±0.9 (5±3)</td>
<td>0.32±0.14 (7±4)</td>
<td>0.60</td>
</tr>
<tr>
<td>Collagen</td>
<td>3.82±1.4 (72±8)</td>
<td>2.91±0.5 (80±6)</td>
<td>3.6±0.6 (79±5)</td>
<td>0.30</td>
</tr>
<tr>
<td>Hypercellular</td>
<td>0.73±0.3 (17±7)</td>
<td>0.37±0.1 (11±3)</td>
<td>0.47±0.2 (9±3)</td>
<td>0.33</td>
</tr>
<tr>
<td>Thrombus</td>
<td>0.17±0.05 (3±1)</td>
<td>0.19±0.07 (4±1)</td>
<td>0.26±0.01 (5±1)</td>
<td>0.85</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.84±0.3 (19±9)</td>
<td>0.97±0.5 (17±4)</td>
<td>1.52±0.4 (25±8)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Measurements are in mm². Values are mean±SEM (%).

subsequent thrombosis than tissue from patients without diabetes. These differences suggest an increased vulnerability for plaque disruption and thrombosis in patients with diabetes mellitus.

Acknowledgments

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References

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