Plasminogen Activator Inhibitor Type 1 Is Increased in the Arterial Wall of Type II Diabetic Subjects

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Abstract—Plasma plasminogen activator inhibitor type 1 (PAI-1) increases in diabetes, and this might contribute to decreased fibrinolysis and accelerated atherosclerosis. Increased PAI-1 levels in the vessel wall could decrease local fibrinolysis and elevate thrombus formation and the unfavorable evolution of atherosclerotic plaques. High glucose increases PAI-1 synthesis in arterial wall cells in culture, and aortic wall PAI-1 levels have been found to be elevated in diabetic animals. However, arterial wall PAI-1 levels have not been investigated in diabetic subjects. Therefore, the aim of this study was to determine the effect of diabetes on PAI-1 levels in the arterial wall. Blood samples and small tissue specimens from the mammary artery were obtained from 11 diabetic and 10 nondiabetic subjects who underwent coronary artery bypass graft surgery. PAI-1 antigen localization in the arterial wall was obtained by immunohistochemistry and was read by laser scanning confocal microscopy; plasma fibrinolytic activity was measured by lysis of fibrin plates; and PAI-1 activity was assessed by a chromogenic method. PAI-1–related immunofluorescence was increased in the arterial wall of diabetic patients, whereas plasma fibrinolysis was reduced. These data provide evidence that diabetes is associated with increased PAI-1 in the arterial wall. This might be an important factor for increased cardiovascular risk and unfavorable plaque evolution in diabetes. (Arterioscler Thromb Vasc Biol. 2001;21:1378-1382.)

Key Words: diabetes mellitus • coronary disease • fibrinolysis • arteries • plasminogen activator inhibitor type 1
PAI-1 localization in arterial wall specimens obtained from type II diabetic and matched nondiabetic subjects.

Methods

Subjects

Plasma samples and mammary artery fragments were obtained from 11 subjects with type II diabetes and 10 subjects without clinical evidence of diabetes who underwent coronary bypass graft surgery from October 1998 to December 1999 and who gave informed consent before surgery.

Characteristics of the study subjects are reported in the Table. The 2 groups of subjects (diabetic and nondiabetic) were similar with respect to the extent of vascular disease, history of myocardial infarction, clinical indications for coronary artery bypass graft surgery, and use of heparin and anesthetics during all procedures. Subjects were matched for sex, previous myocardial infarction, body mass index, and current smoking habits. Serum levels of cholesterol, triglycerides, fibrinogen, and insulin were not different between the 2 study groups. Both groups were given standard pharmacological therapy for their ischemic CVD, and of the diabetic patients, 5 were on insulin, and 6 were on sulfonylureas.

On the morning before surgery, blood samples were obtained from all subjects after a 10- to 14-hour fast. An aliquot was collected in trisodium citrate (1/10 [vol/vol]), centrifuged at 1700g for 20 minutes at 0°C to 4°C, and quickly stored at −80°C. To obtain arterial samples, a small tissue fragment was excised from the internal mammary artery and harvested during standard bypass graft surgery procedures in its distal portion before bifurcation. Samples were quickly washed in saline solution, then dumped in formalin. After subtraction of background, the fluorescence density of the immunostaining in selected images was calculated in units corresponding to 256 Gy levels of Zeiss LSM 510 software scale. The technician performing the immunostaining and the confocal microscopy analysis was blinded as to the source of the samples.

Statistical Analysis

Results are presented as mean±SD. Differences between the 2 groups were analyzed by the Student t test and χ² test when appropriate. Significance was defined as a value of P<0.05.

Results

Characteristics of Study Subjects

As shown in the Table, there was no significant difference in serum cholesterol and triglyceride levels between the 2 groups. Serum fibrinogen was also not different between the 2 groups, and serum fasting insulin concentrations were virtually identical (16.1±9.2 μU/mL in the nondiabetic subjects versus 17.7±8.2 μU/mL in the diabetic subjects, P=NS).

Total Plasma Fibrinolytic Activity and Plasma PAI-1 Activity

The mean of the lysis areas induced by plasma extracts from diabetic patients was 542±105 mm². This was significantly smaller than the mean of the lysis areas induced by plasma extracts from nondiabetic subjects (774±184 mm², P<0.05; Figure 1). Mean plasma PAI-1 activity was significantly increased in diabetic subjects (16.1±4.4 U/I/mL) compared with nondiabetic subjects (10.2±2.5 U/I/mL, P<0.001; Figure 2).

PAI-1 Localization

As shown in representative examples of arterial wall specimens after PAI-1–specific fluorescence immunostaining (Figure 3), samples from type II diabetic subjects exhibited distinctly more PAI-1–related fluorescence in the intima and in the media layers of the arterial wall (in particular, in endothelial and smooth muscle cells).
analysis allowed quantification of the fluorescence intensity of the samples: mean fluorescence intensity in samples from diabetic subjects was 155±10 arbitrary fluorescence units (AFU). This was almost 2-fold larger than the fluorescence intensity in samples from nondiabetic subjects (77±7 AFU, P<0.001; Figure 4).

**Discussion**

The results of the present study confirm that type II diabetes is associated with alterations in the fibrinolytic system and support the hypothesis that increased PAI-1 expression in the arterial wall might contribute to the development of accelerated CVD in this condition. Indeed, we documented that PAI-1–related immunofluorescence was almost 2-fold greater in the arterial walls of type II diabetic subjects compared with nondiabetic subjects. These data are consistent with those reported by Sobel et al., who found increased PAI-1 in atheroma specimens obtained from diabetic patients undergoing percutaneous transluminal coronary angioplasty. In that study, the authors indeed suggested that it was very likely that an increased PAI-1 localization in the atheroma corresponded to increased PAI-1 in the vessel wall in diabetic patients. The present report offers evidence that in type II diabetes, increased PAI-1 localization also occurs in portions of the arterial wall still free of gross atherosclerotic lesions.

For obvious ethical reasons, arterial samples could be obtained only from patients undergoing coronary artery bypass graft procedures and by using the small portion of the harvested mammary artery, which was not used for the graft, for histological preparations. Thus, by definition, all study subjects presented with severe coronary disease, and as a matter of fact, 11 of them (6 diabetic and 5 nondiabetic subjects) had a history of previous myocardial infarction. The presence of clinically relevant atherosclerotic disease has been associated with increased plasma PAI-1 antigen levels and/or activity, and increased plasma PAI-1 levels have also been observed in myocardial infarction survivors. However, in the present study, diabetic and nondiabetic subjects presented the same degree of coronary disease, and roughly the same proportion in each group had experienced a previous myocardial infarction. Yet, in the diabetic subjects, vessel wall PAI-1–related fluorescence was increased, total plasma fibrinolytic capacity was decreased, and plasma PAI-1 activity was increased. This suggests that the observed increase in PAI-1 content in the vessel wall in diabetic patients was not the result of the existing vascular disease, and it is consistent with data by Gray et al, who reported greater plasma PAI-1...
activity in diabetic compared with nondiabetic myocardial infarction survivors. Thus, our data reaffirm an independent role of diabetes in determining alterations of the fibrinolytic system.

Among the mechanisms that could lead to increased PAI-1 synthesis and, hence, decreased fibrinolysis in type II diabetes, insulin resistance and the consequent hyperinsulinemia have been those most often invoked. However, in the present study, fasting plasma insulin levels were almost identical in diabetic and nondiabetic subjects. Although it is possible that integrated postprandial insulin levels might have been greater in diabetic subjects and although diabetic subjects were probably insulin resistant (with comparable insulin levels but higher blood glucose levels), we believe that it is very likely that hyperglycemia might have contributed to increased arterial wall PAI-1 and decreased plasma fibrinolytic capacity in our diabetic subjects. This might be true, especially because plasma triglyceride levels, another factor possibly affecting PAI-1, were also not different between the 2 groups. Thus, among the main factors known to affect plasma PAI-1 levels, only free fatty acids and VLDL were not controlled for. However, because plasma triglyceride and cholesterol levels were not different between the 2 groups, it appears unlikely that FFA and VLDL levels were grossly different between the 2 groups, although these levels were not directly measured.

Indeed, there is evidence in the literature indicating that hyperglycemia can affect PAI-1. Thus, Maiello et al have shown that high glucose decreases fibrinolytic capacity in endothelial cells, and our group has shown that PAI-1 expression and release increase in human vascular smooth muscle cells when excess glucose is added to the culture medium. Furthermore, it has been shown that hyperglycemia stimulates activation of the PAI-1 gene promoter in vascular smooth muscle cells. In vivo, in the rat, we have shown that acute hyperglycemia can induce increased plasma PAI-1 activity, and in an animal model of diabetes, we have found that diabetes, in the absence of significant changes in plasma insulin, induces decreased plasma fibrinolytic activity and increased plasma PAI-1 activity. Furthermore, in the same study, we found that diabetes was associated with increased PAI-1 localization in the liver, in epididymal adipose tissue, and, most important, in the aortic wall of the study animals. As to the in vivo evidence in humans, in 2 large epidemiological studies, plasma PAI-1 levels in type II diabetic subjects increased even when corrections for insulin levels were applied. Moreover, a positive linear correlation has been demonstrated between hemoglobin A₁c levels and PAI-1 activity in type II diabetic patients, and in diabetic patients, improving blood glucose control by diet, by oral hypoglycemic drugs, or by insulin brings about a decrease in plasma PAI-1 activity. Thus, because the subjects in the 2 groups in the present study were matched for severity of CVD, for smoking habits, for pharmacological treatment, and for fasting insulin, triglyceride, and fibrinogen plasma levels, we believe that the impressive differences that we found in PAI-1 localization in the arterial wall should be, at least in part, attributed to chronic hyperglycemia in the diabetic subjects.

It should be emphasized that increased PAI-1 presence in the arterial wall could be of utmost importance for the development of CVD. Not only could decreased local fibrinolysis due to high PAI-1 lead to increased fibrin deposition and subsequent formation of a thrombus (at least in theory), but high PAI-1 levels might predispose the atherosclerotic lesions toward formation of more unstable and thus more dangerous plaques. Therefore, the presence of increased PAI-1 levels in the arterial wall might be an important factor contributing to the increased cardiovascular risk in diabetic patients. One could object on the grounds that the nondiabetic group, with a similar degree of CVD, exhibited less PAI-1 arterial wall content and, therefore, that the increased arterial wall PAI-1 content might have little to do with CVD. However, despite the fact that comparing diabetic subjects with CVD and nondiabetic subjects with CVD controls for the effect of vascular disease, per se, on PAI-1 arterial wall content, increased PAI-1 is only 1 of the main mechanisms that might be involved in the pathogenesis of CVD. Our data suggest that alterations of the fibrinolytic system might play a greater role in plaque development in diabetic subjects than in nondiabetic subjects.

In conclusion, the present study shows that in subjects with type II diabetes, not only is plasma fibrinolytic activity impaired, but PAI-1 localization is markedly increased at the level of the arterial wall. Because increased PAI-1 in the arterial wall predisposes an individual to cardiovascular events, our results, together with the previous observation of increased PAI-1 content in the atherosclerotic plaque in diabetic patients, strongly suggest that increased PAI-1 synthesis and gene expression are an important factor for increased cardiovascular risk in diabetes. Furthermore, because in the present study the main difference between diabetic and nondiabetic subjects was blood glucose levels, it is tempting to speculate that optimal blood glucose control might reduce this disproportionate elevation in PAI-1 and thus help to reduce cardiovascular risk in diabetes.

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References


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