Plasminogen Activator Inhibitor–1 in Nonobese Subjects with Non–Insulin-Dependent Diabetes Mellitus

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Abstract. **Elevated plasma levels of plasminogen activator inhibitor–1 (PAI-1) have been shown to be a risk factor for the development of vascular complications in obese and hyperinsulinemic non–insulin-dependent diabetes (NIDDM) patients. To clarify whether PAI-1 also plays an essential role in the development of such complications in NIDDM patients without obesity or hyperinsulinemia, PAI-1 was analyzed in relation to blood pressure, fasting plasma levels of glucose (FPG), hemoglobin A1c (HbA1c), immunoreactive insulin (F-IRI), C-peptide (CPR), total cholesterol (TC), triglyceride (TGL), and HDL-cholesterol (HDL-C) in 77 NIDDM patients and 10 healthy control subjects. The NIDDM patients were not obese (body mass index [BMI]: <26 kg/m^2) or hyperinsulinemic, and BMI in the controls was between 19 and 24 kg/m^2. In addition, parameters of insulin secretion reserve, including ΣIRI, insulinogenic index, and CPR at 5 min after glucagon loading, were evaluated simultaneously. Plasma levels of PAI-1 were higher in the NIDDM group (9.3 ± 0.9 ng/ml) than in the controls (4.3 ± 0.7 ng/ml; $P < 0.01$). Levels of FPG and HbA1c were also elevated in the NIDDM group ($P < 0.05$ for each), but F-IRI did not differ between the two groups. However, multiple regression analysis revealed no significant correlation in the NIDDM between PAI-1 and F-IRI or the parameters of insulin secretion reserve. Regardless of the presence or absence of vascular complications, PAI-1 did not vary significantly in the NIDDM. These findings suggest that the effects of PAI-1 on the development of diabetic complications in NIDDM patients may not proceed in the same way in those with versus those without obesity or hyperinsulinemia, because no correlation was found between PAI-1 and insulin secretion reserve, while plasma levels of PAI-1 were higher in the NIDDM group than in the controls.** [P.S.E.B.M. 1996, Vol 211]

In a cascade of fibrinolytic phenomena, tissue-type plasminogen activator (t-PA) is an essential activating enzyme in fibrinolysis. Plasminogen activator inhibitor–1 (PAI-1) is a potent and specific inhibitor of t-PA. The role of PAI-1 activity in the development of vascular damage in diabetic patients has attracted much attention recently (1–5). In fact, elevated plasma levels of PAI-1 have been regarded as a risk factor for the development of diabetic proliferative retinopathy (1–3), nephropathy (2, 4, 5), and acute myocardial infarction (6). In obese, non–insulin-dependent diabetes mellitus (NIDDM) patients, plasma levels of PAI-1 have been found to correlate positively with fasting plasma levels of insulin (7–11) and triglycerides (TGL) (11), diastolic blood pressure (7), and body mass index (BMI) (2, 6–8). Results of *in vitro* studies suggested that insulin is a stimulant of PAI-1 synthesis (11). Nevertheless, it remains unclear whether the fibrinolytic processes in NIDDM patients without obesity and hyperinsulinemia proceed in the same way as those in hyperinsulinemic, obese NIDDM patients. The present study was designed to investigate whether

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PAI-1 plays an essential role in the development of diabetic angiopathy in normo- or hypo-insulinemic, nonobese NIDDM patients, as shown in hyperinsulinemic, obese NIDDM patients.

Patients and Methods

Subjects. Seventy-seven average-weight NIDDM patients (43 men and 34 women) and 10 control subjects (five men and five women) were enrolled. The subjects were diagnosed as having NIDDM according to the criteria established by the National Diabetes Data Group. BMIs of the NIDDM and control groups were 21.9 ± 0.2 kg/m^2 (mean ± SEM, range: 19.3–23.6 kg/m^2) and 22.4 ± 0.2 kg/m^2 (range: 19.6–23.8 kg/m^2), respectively. Mean age was 59.1 ± 1.2 years (range: 54–65 years) in the NIDDM group and 40.0 ± 2.7 years (range: 32–56 years) in the control group. These parameters did not differ significantly between the two groups. Thirty-seven patients were treated with oral hypoglycemic drugs and the remainder were treated only with dietary intervention. Neither group received therapeutic insulin.

Among the 77 NIDDM patients, microangiopathy was diagnosed in 43, including 26 with simple or preproliferative retinopathy as defined by Davis' criteria; a total of 20 had nephropathy defined by either microalbuminuria (14 patients) or macroalbuminuria (six patients), and 40 had neuropathy with both decreased sensitivity to vibration and complete disappearance of deep tendon reflexes. Macroangiopathy was diagnosed according to the results of electrocardiography in 20 patients, six of whom showed T-wave inversion or ST segment depression of more than 1 mm in height. The remaining 14 patients had previous histories of myocardial infarction (n = 13) or cerebral infarction (n = 1). According to the absence or presence of micro- and/or macroangiopathy, all NIDDM patients were allocated to four groups: Group I (26 patients) had no complications; Group II (n = 8) had only macroangiopathy; Group III (n = 31) had only microangiopathy; and Group IV (n = 12) had both micro- and macroangiopathies.

Clinical Measurements. The parameters measured were PAI-1, overnight fasting plasma levels of glucose (FPG) and hemoglobin A1c (HbA1c), fasting immunoreactive insulin (F-IRI) and C-peptide (CPR), and overnight fasting serum levels of total cholesterol (TC), HDL-cholesterol (HDL-C), and TGL. Subjects underwent a glucose tolerance test with oral loading of 75 g of glucose (OGTT) after overnight fasting. Plasma levels of glucose and IRI were measured at 0, 30, 60, and 120 min after the loading. Using these values, ΣIRI and insulinogenic index were calculated. Serum level of CPR was also measured at 5 min after intravenous infusion of 1 mg of glucagon as an index of insulin secretion reserve (CPR-5) (12). Blood samples were collected from the antecubital vein at 0600 hr in the morning after overnight fasting. Blood pressure was measured in the supine position by a standard sphygmomanometer. Informed consent for clinical testing was obtained from all the tested subjects.

Analytical Techniques. Plasma PAI-1 was measured by the enzyme immunosorbent assay (ELISA) method (Tint Elise; Biopool Co, Ltd., Sweden) (13), plasma glucose by the glucose oxidase method (Glucoroder-F A & T; Shino Test Co., Ltd., Japan), and HbA1c by high-performance liquid chromatography (HPLC-723GHb; Toso Co., Ltd., Japan). Plasma IRI was determined by enzyme immunoassay (Graozime Insulin; Wako Jyunyaku Co., Ltd., Japan) and CPR by radioimmunoassay (C-Peptide Kit "Daiichi"III; Daiichi radioisotope Co., Ltd., Japan). Both low-density lipoprotein cholesterol (LDL-C) and atherogenic index (AI) were calculated using the Friedewald equation: LDL-C = TC − TGL/5 − HDL-C; AI = LDL-C/HDL-C (14).

Statistical Analysis. Data were expressed as mean ± SEM. Statistical evaluation was carried out by one-way analysis of variance (ANOVA) for comparisons between the diabetic and normal control groups or among NIDDM subgroups. Multiple regression analysis was used to evaluate univariate relationships between PAI-1 and the various parameters.

Results

The control and NIDDM groups did not differ significantly with respect to age, sex or BMI. Mean values of the measured parameters in the two groups are shown in Table I. Among the humoral parameters, PAI-1, HbA1c, and FPG were significantly higher in the NIDDM group than in the controls ($P < 0.01$ for each). However, systolic and diastolic blood pressure, F-IRI, CPR, TC, TGL, HDL-C, LDL-C, and AI did not differ significantly between the two groups. NIDDM patients treated only with diet therapy and those treated with oral hypoglycemic drugs did not differ significantly with respect to BMI, systolic or diastolic blood pressure, F-IRI, CPR, TC, TGL, HDL-C, LDL-C, or AI. The correlations between PAI-1 and HbA1c, FPG, or F-IRI are shown in Table II. PAI-1 showed no significant correlation with any of these parameters in either group of subjects. The correlations between PAI-1 and the indices of insulin secretion reserve are shown in Table III. PAI-1 showed no significant correlation with any parameter of insulin secretion reserve, including ΣIRI, insulinogenic index, and CPR-5, in the NIDDM group.

Characteristics of the four NIDDM subgroups, categorized by presence or absence of micro- and/or macroangiopathy, are shown in Table IV. BMI was 22.6 ± 2.3, 21.6 ± 2.0, 22.1 ± 1.8, and 23.3 ± 2.2 kg/m^2 in these four groups, respectively, these values

Table I. Comparison of Parameters between the Normal Control and NIDDM Groups

Parameter	Normal range	Control	NIDDM
n		10	77
BMI (kg/m^2)	19–24	22.4 ± 0.2	21.9 ± 0.2
SBP (mmHg)	100–150	121.3 ± 2.1	134.0 ± 2.6
DBP (mmHg)	50–80	72.4 ± 4.3	75.3 ± 1.3
FPG (mg/dl)	80–100	92.3 ± 1.2	149.5 ± 5.3[a]
HbA1c (%)	4.8–6.3	5.8 ± 0.3	8.1 ± 0.2[a]
F-IRI (μU/ml)	5–10	6.0 ± 1.1	5.3 ± 0.5
CPR (ng/ml)	2–5	2.5 ± 0.4	2.3 ± 0.1
TC (mg/dl)	120–220	181.6 ± 4.6	203.6 ± 4.1
TGL (mg/dl)	50–150	128.3 ± 6.8	124.7 ± 8.6
HDL-C (mg/dl)	40–70	52.1 ± 3.2	46.1 ± 1.6
LDL-C (mg/dl)	70–140	121.5 ± 4.3	133.5 ± 3.7
AI	2–3.5	2.9 ± 0.2	3.2 ± 0.2
PAI-1 (ng/ml)		4.3 ± 0.7	9.3 ± 0.9[a]

Note. Values represent mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma level of glucose; HbA1c, hemoglobin A1c; F-IRI, fasting immunoreactive insulin; CPR, C-peptide; TC, total cholesterol; TGL, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AI, atherogenic index; PAI-1, plasminogen activator inhibitor–1.
[a] $P < 0.05$ vs appropriate controls.

Table II. Coefficients of Correlation between PAI-1 and Significant or Major Parameters

Parameter	PAI-1			
	Control		NIDDM	
	r value	*P* value	*r* value	*P* value
FPG	−0.034	0.684	−0.004	0.882
HbA1c	0.183	0.660	0.586	0.508
F-IRI	0.221	0.123	0.661	0.195

Note. Abbreviations are the same as in Table I.

did not differ significantly. No significant difference among the groups was found for any parameter. All PAI-1 levels in these four subgroups were higher than those in controls (4.3 ± 0.7, $P < 0.05$ for each). Table V shows the correlations between PAI-1 and the parameters related to glucose metabolism and insulin secretion in the four NIDDM subgroups. No significant correlation between PAI-1 and these parameters was found.

Discussion

The level of PAI-1 has been shown to be elevated in hyperinsulinemic NIDDM patients (7–9). Results of *in vitro* studies suggest that insulin increases PAI-1 synthesis and secretion (15, 16). High levels of PAI-1 increase venous and arterial thrombosis, which results in general endothelial dysfunction (5). There are significant, strong correlations between the elevation of PAI-1 and the prevalence of diabetic complications. Because of these findings, elevation of plasma PAI-1 level is regarded as one of the major risk factors for the development of diabetic micro- and macroangiopathy in hyperinsulinemic NIDDM patients (1, 3–5). As for NIDDM patients without obesity or hyperinsulinemia, previous studies did not measure their levels of insulin or insulin secretion reserve. In the subgroups of NIDDM patients determined by the presence or absence of vascular complications, previous studies have compared the between-group levels of PAI-1, but not the level of insulin or its secretion reserve (2–6, 9).

The present study demonstrated that the levels of PAI-1 were elevated significantly, while the insulin level was unaffected, in nonhyperinsulinemic NIDDM as in hyperinsulinemic NIDDM. A question can be raised as to why the present NIDDM patients showed an increase in PAI-1, while their plasma levels of insulin and insulin secretion reserve were not enhanced. Diastolic hypertension in hyperinsulinemic NIDDM was shown to increase plasma levels of PAI-1 (7). Hyperlipidemia, including elevations of very low density lipoprotein (VLDL) (8, 17), low-density lipoprotein (LDL) (11, 18), and apolipoprotein B (8), was also found to accelerate the synthesis and/or release of PAI-1. However, pathogenic factors believed to increase plasma PAI-1 levels did not appear to increase PAI-1 in the present study, since the plasma levels of these parameters remained within normal ranges. One possible explanation for the elevation of PAI-1 may involve hyperglycemia in the present NIDDM patients. In fact, the expression of PAI-1 mRNA in human endothelial cells was enhanced in an *in vitro* study when the glucose concentration in the medium was increased (19).

Thus, the elevation of PAI-1 may not initiate or promote diabetic vascular complications of angiopathy in nonhyperinsulinemic, average-weight NIDDM patients. This concept is supported by the present finding of no correlation between the level of PAI-1 and the parameters related to insulin secretion. To confirm this conclusion, the correlation between PAI-1 and those parameters was further examined in four subgroups of NIDDM patients, divided according to the presence or absence of vascular complications. Once again, no difference was observed in the level of PAI-1, BMI, systolic or diastolic blood pressure, F-IRI, CPR, TC, TGL, HDL-C, LDL-C, or AI among the four subgroups. No significant correlation between PAI-1 level and the parameters related to glucose metabolism and insulin secretion was found among the groups. Further, it has been reported that the level of PAI-1 does not differ in relation to micro- or macroangiopathy in the NIDDM patients. These findings suggest that the effects of PAI-1 on the development of diabetic complications in nonhyperinsulinemic, average-weight NIDDM patients might differ from those in hyperinsulinemic, obese NIDDM patients.

Table III. Coefficients of Correlation between PAI-1 and Indices of Insulin Secretion Reserve in the NIDDM Group

	ΣIRI		Insulinogenic index		CPR-5[a]	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value
PAI-1	0.098	0.802	0.196	0.613	0.211	0.238

[a] CPR-5, the value of C-peptide at 5 min after intravenous infusion of 1 mg of glucagon. Abbreviations are the same as in Table I.

Table IV. Comparison of Parameters among the Four NIDDM Subgroups Divided According to the Presence or Absence of Micro- and/or Macroangiopathy

Parameter	Group I	Group II	Group III	Group IV
n	26	8	31	12
SBP (mm Hg)	133.2 ± 3.4	131.5 ± 6.6	135.9 ± 4.6	132.5 ± 7.8
DBP (mm Hg)	74.2 ± 2.4	73.3 ± 4.1	78.8 ± 1.5	69.8 ± 4.7
FPG (mg/dl)	143.0 ± 7.3	142.0 ± 12.7	161.4 ± 9.6	137.7 ± 15.2
HbA1c (%)	7.7 ± 0.3	7.9 ± 0.5	8.4 ± 0.3	8.1 ± 0.6
F-IRI (μU/ml)	5.0 ± 0.7	9.5 ± 4.0	4.3 ± 0.5	6.6 ± 1.8
CPR (ng/ml)	2.1 ± 0.2	3.9 ± 0.7	2.0 ± 0.1	3.6 ± 0.4
TC (mg/dl)	204.9 ± 8.4	185.3 ± 7.1	210.3 ± 6.3	195.9 ± 8.5
TGL (mg/dl)	121.2 ± 17.0	134.6 ± 17.9	112.5 ± 8.2	156.6 ± 33.4
HDL-C (mg/dl)	50.3 ± 2.7	33.5 ± 1.7	51.1 ± 2.1	33.6 ± 2.8
LDL-C (mg/dl)	128.4 ± 6.9	124.8 ± 6.3	138.4 ± 6.0	136.5 ± 9.8
AI	2.7 ± 0.2	3.8 ± 0.2	2.9 ± 0.2	4.5 ± 0.6
PAI-1 (ng/ml)	10.6 ± 2.0	8.3 ± 1.2	8.7 ± 1.1	9.8 ± 2.4

Group I, without microangiopathy or macroangiopathy; Group II, with macroangiopathy but without microangiopathy; Group III, with microangiopathy but without macroangiopathy; Group IV, with both macroangiopathy and microangiopathy. Abbreviations are the same as in Table I.

Table V. Coefficients of Correlation between PAI-1 and Major Parameters in each NIDDM Subgroup

Parameter	PAI-1							
	Group I		Group II		Group III		Group IV	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value
FPG	0. 058	0.779	0.654	0.231	0.002	0.993	0.269	0.452
HbA1c	0.315	0.117	0.329	0.589	0.079	0.672	0.029	0.987
F-IRI	0.412	0.051	0.929	0.071	0.497	0.070	0.295	0.571
Σ IRI	0.385	0.615	0.798	0.412	0.137	0.826	0.277	0.723
Insulinogenic index	0.282	0.719	0.826	0.382	0.843	0.073	0.876	0.124
CPR-5	0.678	0.209	0.192	0.877	0.875	0.052	0.059	0.926

Note. Abbreviations are the same as in Table I.

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