Elevated Plasma Level of Plasminogen Activator Inhibitor-1 (PAI-1) in Patients with Relapsing-Remitting Multiple Sclerosis

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ONODERA, H., NAKASHIMA, I., FUJIHARA, K., NAGATA, T. and ITOYAMA, Y. *Elevated Plasma Level of Plasminogen Activator Inhibitor-1 (PAI-1) in Patients with Relapsing-Remitting Multiple Sclerosis.* Tohoku J. Exp. Med., 1999, **189** (4), 259-265 —— Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system and one of the earliest changes in inflammatory focus involves the activation of vascular endothelial cells. We determined the plasma level of plasminogen activator inhibitor-1 (PAI-1), a key regulator of fibrinolysis and cell migration, in patients with MS. The level of plasma PAI-1 was significantly higher in active MS cases when compared to stable MS and controls. Plasma concentrations of tissue plasminogen activator, transforming growth factor β-1, and lipoprotein-a remained normal in spite of disease activity. These results suggested that PAI-1 plasma levels are associated with MS disease activity and is a good marker for MS relapse. —— fibrinolysis; multiple sclerosis; plasminogen activator inhibitor-1; relapse © 1999 Tohoku Univeersity Medical Press

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. One of the earliest and pivotal changes in inflammatory focus involves the activation of vascular endothelial cells and fibrin deposition (Wakefield et al. 1994). Endothelial cell activation antigens are expressed on isolated central nervous system microvessels in MS patients (Washington et al. 1994). However, endothelial injury and fibrinolytic processes involved in MS have received little attention in comparison with studies investigating the role of adhesion molecules. Plasminogen activator inhibitor-1 (PAI-1) is a potent inhibitor of fibrinolysis that functions in the regulation of the plasmin-based pericellular proteolytic cascade (Stoop et al. 1997). PAI-1 also serves to regulate cell migration by binding to matrix proteins such as vitronectin and heparin. PAI-1 is synthesized in endothelial cells and its release may be stimulated by the onset of inflammation. Transforming growth factor-β (TGF-β), which has been

reported to modify the disease activity of MS, strongly enhances PAI-1 production (Riccio et al. 1988). In order to determine whether the plasma level of PAI-1 changes in response to MS disease activity, we measured the plasma concentrations of PAI-1, tissue plasminogen activator (tPA), and other factors such as lipoprotein-a [Lp(a)] and TGF-β1 that may have an effect on the plasma PAI-1 level in MS patients.

Materials and Methods

Eligible patients between the ages of 19 and 52 years (2 men and 13 women, average age, 33 years) were positively diagnosed as having clinical relapsing-remitting multiple sclerosis (RRMS). During the observation period, 12 patients experienced relapses. Patients were grouped into the following two categories: (1) active RRMS stage ($n = 12$) currently experiencing exacerbation of the disease (defined as the appearance of new symptoms or significant aggravation of old symptoms for at least 24 hours without any fever), and (2) stable MS ($n = 12$) or RRMS patients with no exacerbation of symptoms for at least 6 months. Disability was measured according to the expanded disability status scale. None of the patients received continuous immunosuppressive therapy. Although other disease processes may increase the level of PAI-1, none of the patients showed any laboratory evidence of diabetes mellitus, liver, renal, or metabolic disorders. T2-weighted MRI revealed two or more lesions in the central nervous system of all patients in this study. The cerebrospinal fluid (CSF) was examined in acute MS patients for cell counts, protein (the Biuret method), and IgG index (IgG determined by TIA method). For active MS patients, blood was drawn within 2 weeks after the onset of a clinical relapse and prior to the initiation of treatment. Blood and CSF samples were collected without venous stasis using a wide-bore needle from MS patients or healthy controls ($n = 10$) in the morning. Samples were cold-centrifuged, immediately separated, and stored in aliquots at $-70°C$. Plasma PAI-1 (Biopool, Ventura, CA, USA), tPA (Biopool), and TGF-β1 (R & D systems, Minneapolis, MN USA) levels were quantitated using ELISA. Lp(a) (Shionogi, Osaka) concentration was quantitated using TIA. Samples were assayed in duplicate according to the protocols of manufactures. Overall differences between the groups were analyzed with the Mann-Whitney's test.

Results

As shown in Table 1, the plasma PAI-1 level in patients with active MS was 6 times higher than the control ($p < 0.01$). In addition, PAI-1 levels in active MS patients were significantly higher than those for stable MS ($p < 0.01$). Plasma PAI-1 levels in stable MS patients were not significantly different from those of the control group. Further comparison of plasma PAI-1 was made longitudinally. In 4 patients, temporal profiles of PAI-1 level were monitored and 3 of them showed a more than 2-fold increase in PAI-1 levels compared to the values

TABLE 1. *Plasma PAI-1 and tPA levels in patients with active and stable MS, and in controls*

	Control	Active MS	Stable MS
PAI-1 (ng/ml)	11.2 ± 2.1	70.3 ± 13.5[a,b]	20.5 ± 5.4
	$n = 10$	$n = 12$	$n = 12$
tPA (ng/ml)	4.2 ± 0.58	3.8 ± 0.71	4.0 ± 0.67
	$n = 10$	$n = 8$	$n = 8$
TGF-β1 (ng/ml)	7.1 ± 0.62	9.4 ± 1.75	10.8 ± 1.73
	$n = 7$	$n = 7$	$n = 7$
Lp(a) (μg/ml)	236 ± 53.6	194 ± 36.5	144 ± 34.7
	$n = 7$	$n = 5$	$n = 8$

All values are mean$\pm$s.e.m.   Significant difference $(p < 0.01)$; [a], between controls and active MS; [b], between active and stable MS.

measured 1 to 2 months after their relapses (Fig. 1).   In one RRMS patient, a normal PAI-1 value (4.8 ng/ml) was observed 1 month before the relapse followed by a high PAI-1 level at the time of relapse (104 ng/ml).   Interestingly, 14 days after the relapse, the patient's PAI-1 level returned to normal (2.4 ng/ml) despite a worsening of symptoms.   Thus PAI-1 concentrations may promptly increase at the active disease stage.

Plasma levels of tPA in both the active and stable MS groups were not different from those in the control group.   There was no correlation between PAI-1 and tPA levels in active RRMS patients (r, Fig. 2A).   In addition, TGF-β1 levels in both the active and stable MS groups were not different from the control (data not shown).   No correlation between PAI-1 and TGF-β1 concentrations was observed in the active MS group ($r = 0.33$).   Plasma Lp(a) levels in
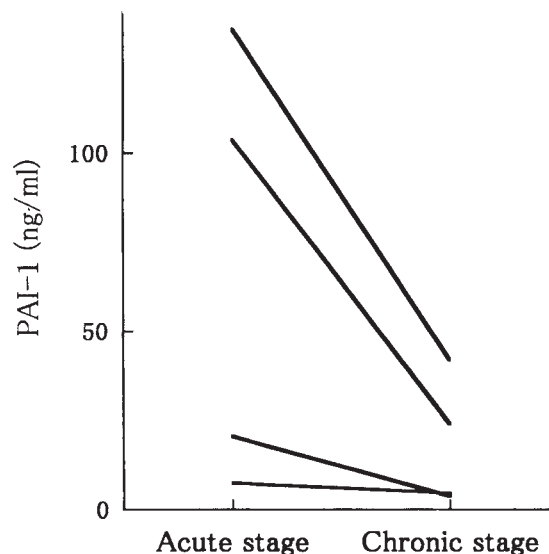

Fig. 1.   A longitudinal comparison of plasma PAI-1 levels was made in 4 patients with RRMS.   PAI-1 levels were compared to the values measured immediately after relapses (acute stage) and 1 to 2 months after relapses (chronic stage).
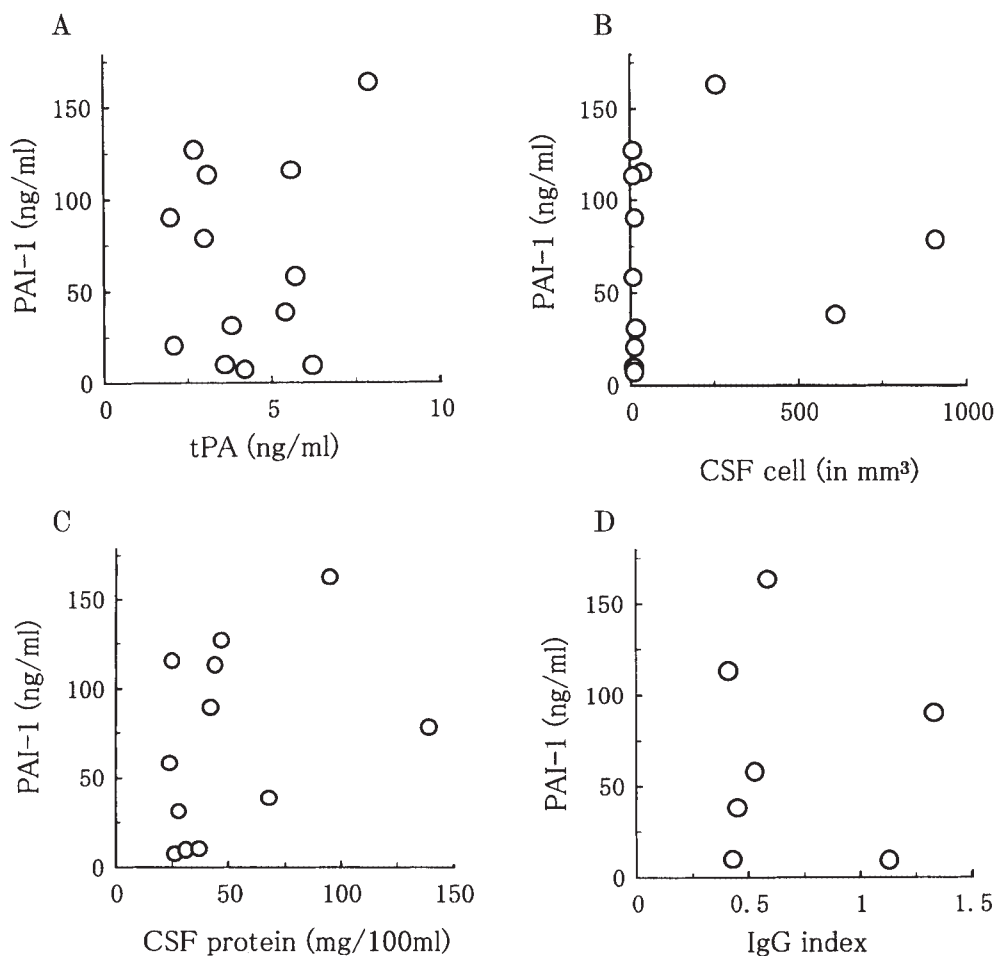
Fig. 2. Correlation between plasma PAI-1 concentration and corresponding, (A)
Plasma values of tissue plasminogen activator (tPA), (B) CSF cell count, (C)
CSF protein concentration, and (D) CSF IgG index in active RRMS patients.

active MS, chronic MS, and the control group were similar and there was no
correlation between the levels of PAI-1 and Lp(a) in active MS ($r = 0.14$).

Pleocytosis (exceeding 10 cells/mm^3) was detected in 3 out of 11 active
RRMS patients but there seemed to be no correlation between CSF cell counts and
PAI-1 values ($r = 0.08$, Fig. 2B). Although 4 out of 11 active RRMS patients
showed an elevated CSF protein concentration, there was no correlation between
CSF protein levels and PAI-1 concentration ($r = 0.32$, Fig. 2C). Moreover, as
shown in Fig. 2D, there was no correlation between IgG index and PAI-1 values.
Lastly, there was no correlation between the EDSS disability value and PAI-1
levels at the active disease stage (data not shown).

DISCUSSION

PAI-1 is a 50-kDa glycoprotein that regulates the plasmin-based pericellular
proteolytic cascade by inhibiting the formation of tPA and urokinase type
plasminogen activator (Stoop et al. 1997). The results obtained in this study
showed a positive relationship between active MS processes and elevated plasma

PAI-1 levels. In 9 of 12 relapses, peak levels for PAI-1 coincided with the onset of clinical symptoms. Thus, an alteration of fibrinolytic variables had occurred in the plasma of patients with MS and PAI-1 could be released as a consequence of inflammation. In a previous report, raised CSF PAI-1 were detected in patients with multiple sclerosis, leukemia and encephalitis (Akenami et al. 1997). Data obtained from studies involving experimental allergic encephalomyelitis and MS also indicates the presence of perivascular inflammation. In certain models of experimental allergic encephalomyelitis, fibrin formation within brain lesions seemed to be a prerequisite for the development of clinical symptoms (Paterson 1976). Fibrin deposition on blood vessels in active MS lesions has been reported (Wakefield et al. 1994; Claudio et al. 1995) although it is rarely seen in chronic lesions. Many reports have also suggested the role of endothelial damage and/or activation in contributing to the disease processes in MS. For instance, MS microvessels express cell adhesion molecules (Dore-Duffy et al. 1993), MHC class II antigens (Wakefield et al. 1994), and the urokinase plasminogen activator receptor (Dore-Duffy et al. 1993).

Plasma concentration of PAI-1 is elevated in vasculitis and arteriosclerotic blood vessels (Stoop et al. 1997). There are several humoral factors that can enhance PAI-1 production. TGF-β is known to be a potent inducer of PAI-1 biosynthesis at the transcriptional level (Riccio et al. 1988) and plays an important role in modifying the disease activity of MS (Navikas and Link 1996). An increased expression of TGF-β in and around blood vessels with acute MS plaque depostition has been reported (Woodroofe and Cuzner 1993). However, in this study, we could not observe significant changes in the plasma TGF-β1 levels in both active and stable MS patients. Although Lp(a) has been reported to upregulate PAI-1 production (Etingin et al. 1991), plasma Lp(a) levels in active MS, chronic MS, and the control group were similar. Thus, we cannot rule out the possibility that increased PAI-1 levels in systemic circulation during an active MS period was not a result of endothelial cell function in active MS lesions but, rather, a consequence of systemic vascular abnormalities that could be closely associated with the relapse. Inflammatory stimuli, such as interleukin-1 and bacterial infections, can enhance PAI-1 production both in vitro and in vivo (Sawdey and Loskutoff 1991; Stoop et al. 1997). Certain humoral factors that could stimulate endothelial cells may selectively activate "preconditioned" endothelial cells in specific brain areas followed by leukocyte infiltration and demyelination (Navikas and Link 1996).

The clinical benefits of interferon β-1b (IFNβ-1b) treatment (IFNB 1993) in MS patients may, in part, be a result of the ability of IFNβ-1 to decrease the gelatinase activities of T-lymphocytes, including the secretion of matrix metalloproteinase-9 (MMP-9), which leads to a reduction of T-lymphocyte infiltration into the matrix (Leppert et al. 1996). Moreover, MMP-9 is known to be responsible for the degradation of adhesive glycoprotein vitronectin (Imai et al.

1995). Because PAI-1 can control cell-matrix interactions by binding to vitronectin (Stefansson and Lawrence 1996), PAI-1 may act as a "molecular switch" within the extracellular matrix that regulates the progression of cellular migration. Further studies are necessary to show whether the differences in PAI-1 levels could influence the relapse rate in patients undergoing IFNβ treatments.

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