

Plasma Level of IL-6 and Its Relationship to Procoagulant and Fibrinolytic Markers in Acute Ischemic Stroke

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Procoagulant or impaired fibrinolytic states as well as inflammatory reactions mediated by cytokines are likely involved in the pathogenesis of acute ischemic stroke. We examined the potential relationship between interleukin 6 (IL-6) and hemostatic markers. The procoagulant and fibrinolytic states were assessed in 46 patients with acute stroke by measuring plasma levels of plasminogen activator inhibitor-1 (PAI-1), thrombin-antithrombin complex (TAT), and plasminogen-antiplasmin complex (PAP). Circulating IL-6 levels were measured using ELISA (Quantikine, R & D systems, MN, USA). Circulating IL-6 (mean, 26.5 pg/mL) and PAI-1 (mean, 19.9 ng/mL) levels were higher in patients with acute stroke than in healthy subjects (mean, 3.0 pg/mL, 10.4 ng/mL, respectively). TAT levels were statistically different according to the etiologic subtypes of stroke (atherogenic, 2.5 ng/mL; lacunar 3.2 ng/mL; cardiogenic 9.9 ng/mL, $p = 0.021$). Neither procoagulant levels nor fibrinolytic markers significantly correlated with circulating IL-6 levels. Our findings suggest that elevated proinflammatory cytokines during the initial hours of ischemic stroke may be an independent pathogenic factor or a consequence of the thrombotic event with no relationship to the procoagulant or fibrinolytic states.

Key Words: Stroke, cytokine, procoagulant, fibrinolysis

INTRODUCTION

Atherosclerosis is the main cause of cerebral infarction.¹ The revised response to injury hypothesis highlights the importance of inflammatory reactions involved in the pathogenesis of athero-

sclerosis.² Injury of endothelial cells may alter the balance of procoagulant and antithrombotic factors on the surface of endothelium to favor thrombosis.³ Consonant with the role of monocytes/macrophages, vascular smooth muscle cells and platelets, there are several important interactions between the blood components of both the coagulation and fibrinolytic systems.⁴ Thus, a growing body of evidence supports a relationship between the inflammatory response, thrombosis, and the development of atherosclerotic lesions.⁵ In addition, monocyte-derived cytokines, such as IL-6, are in part responsible for the elevations of plasma fibrinogen that accompany inflammation. To better understand the relationship between the inflammatory response and thrombosis in ischemic stroke, we assessed procoagulant and fibrinolytic states in patients with acute ischemic stroke by measuring plasma levels of plasminogen activator inhibitor-1 (PAI-1), thrombin-antithrombin complex (TAT) and plasminogen-antiplasmin complex (PAP), and examined the potential relationship between these hemostatic markers and interleukin 6 (IL-6).

MATERIALS AND METHODS

Forty-six patients (34 men and 12 women, mean age, 64 yrs) presenting with acute ischemic stroke were studied according to a standard protocol entailing clinical and imaging studies (CT, MRI and/or angiography) of the brain. Patients were admitted with atherosclerotic ($n = 28$), lacunar ($n = 14$), and cardioembolic ($n = 4$) infarcts within the first 24 hours, and 28 age- and sex- matched healthy subjects were included for comparison

Received September 28, 2005

Accepted October 27, 2005

This study was supported by the Brain Korea 21 Project for Medical Science, Yonsei University, 2001.

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Table 1. Characteristics of Study Groups

Characteristics	Stroke (n = 46)	Controls (n = 28)
Age (yrs)		
Mean \pm SD	63.7 \pm 7.3	59.6 \pm 8.0
Range	36 - 89	43 - 76
Men/women (n)	34/12	20/8
Smokers (n)	22	9
Hypertension (n)	30	11
Diabetes mellitus (n)	7*	0
Ischemic heart disease (n)	4	0
Medications used		
Antihypertensive drugs	27	10
Anticoagulants	4	0
Aspirin	11*	0
Infections	3	0
Other illness [†]	8*	0

* $p < 0.05$ versus control group.

[†]Other illness: chronic bronchitis, hyperthyroidism, multiple osteoarthritis, fatty liver, proteinuria, glaucoma, oral ulcer/gingivitis, degenerative spondylosis.

(Table 1). Citrated blood was drawn early in the morning on admission to determine plasma levels of TAT (Dade-Behring, Germany), PAP (Dade-Behring, Germany), PAI-1 (Diagnostica Stago, France), IL-6 (Quantikine, R&D systems, MN, USA), and anti-phospholipid antibody (IgG/IgM Diagnostica Stago, France) by ELISA methods.

RESULTS

Circulating IL-6 levels (mean, 26.5 pg/mL, range, 6.4-161.3 pg/mL) were significantly higher in all patients with acute stroke than in healthy subjects (median, 3.0 pg/mL, range, 2.3-5.9 pg/mL) ($p < 0.001$, Table 2).

PAI-1 and PAP levels were also significantly different between patients with acute stroke (mean, 19.9 ng/mL, 522.9 ng/mL, respectively) and healthy subjects (mean 10.4 ng/mL, 240.4 ng/mL, respectively) ($p < 0.001$, Table 2).

Mean levels of TAT (3.4 ng/mL) in the stroke patient group were not significantly increased

compared with normal subjects (2.8 ng/mL) ($p < 0.001$, Table 2), however, TAT levels were statistically different according to the etiologic subtypes of stroke (atherogenic, 2.5 ng/mL; lacunar 3.2 ng/mL; cardiogenic, 9.9 mg/mL; $p = 0.021$).

PAI-1 and PAP levels were higher in the lacunar subtype and in the cardiogenic subtype, respectively, than in other subtypes, with weak statistical significance ($p = 0.070$ and $p = 0.058$, respectively). However, there were no significant differences in levels of IL-6 and other hemostatic markers among the different types of stroke (Table 3).

Levels of IL-6 and hemostatic parameters were not different between positive and negative groups for anti-phospholipid antibodies (Table 4).

There were significant correlations between TAT and PAP markers ($r = 0.455$, $p = 0.0015$), and TAT correlated weakly with PAI-1 ($r = 0.264$, $p = 0.0787$). However, neither procoagulant nor fibrinolytic markers were significantly correlated with circulating IL-6 levels (Table 5).

Table 2. Plasma Concentrations of IL-6 and PAI-1 in Patients with Acute Ischemic Stroke and Healthy Subjects

Variables	Stroke (n = 46)	Healthy subjects (n = 28)	p value*
IL-6 (pg/mL)	26.5 (6.4 - 161.3)	3.0 (2.3 - 5.9)	< 0.001
PAI-1 [†] (ng/mL)	19.9 (3.3 - 56.7)	10.4 (1.3 - 23.1)	< 0.001
TAT (ng/mL)	3.4 (1.1 - 24.5)	2.8 (1.6 - 4.0)	0.071
PAP (ng/mL)	522.9 (163 - 2182)	240.4 (120 - 700)	< 0.001

Values are expressed as mean (range).

*Mann-Whitney test was used.

[†]Data on PAI-1 were missing in one stroke patient.

Abbreviations: IL-6, interleukin-6; PAI, plasminogen activator inhibitor; TAT, thrombin-antithrombin; PAP, plasmin-antiplasmin.

Table 3. Circulating Levels of Hemostatic Parameters According to the Etiologic Subtypes of Acute Ischemic Stroke

Parameters	All patients	Atherogenic (n = 28)	Lacunar (n = 14)	Cardiogenic (n = 4)	p value*
PAI-1 [†] (ng/mL)	19.9 (3.3 - 56.7)	17.1 (3.3 - 33.5)	24.6 (7.7 - 56.7)	22.1 (19.8 - 23.8)	0.070
PAP (ng/mL)	522.9 (163 - 2182)	476.2 (163 - 1028)	403.5 (209 - 698)	1268.0 (366 - 2182)	0.058
TAT (ng/mL)	3.4 (1.1 - 24.5)	2.5 (1.1 - 8.6)	3.2 (1.4 - 13.2)	9.9 (3.9 - 24.5)	0.021
APA					0.510
Positive	18	12	6	0	
Negative	28	16	8	4	

Values are given as number of subjects or mean (range).

*Chi-square test for categorical and ANOVA test for continuous variables were used.

[†]Data on PAI-1 were missing in one cardiogenic subtypes.

Abbreviations: IL-6, interleukin-6; PAP, plasmin inhibitor complex; TAT, thrombin-antithrombin complex; PAI, plasminogen activator inhibitor; APA anti-phospholipid antibody (IgG or IgM).

Table 4. Circulating Levels of Cytokines and Hemostatic Parameters According to the Positivity (IgG or IgM) for Anti-phospholipid Antibody

Parameters	Anti-phospholipid antibody		p value*
	Positive (n = 18)	Negative (n = 28)	
Sex (M/F)	14/4	20/8	0.739
IL-6 (pg/mL)	31.8 (36.7)	39.8 (43.8)	0.510
PAI-1 [†] (ng/mL)	18.8 (10.2)	20.5 (10.1)	0.597
PAP (ng/mL)	446.8 (197.3)	571.8 (430.2)	0.189
TAT (ng/mL)	2.6 (1.7)	3.9 (4.7)	0.193

Values are given as number of subjects or mean values (SD).

*Chi-square test for categorical and Mann-Whitney test for continuous variables were used.

[†]Data on PAI-1 were missing in 1 positive APA.

Abbreviations: see Table 2.

Table 5. Correlation Coefficients (r) Among Various Parameters in the Patients with Acute Ischemic Stroke

	IL-6	PAP	TAT	PAI-1
Age	0.284 (n = 46)	0.167 (n = 46)	0.188 (n = 46)	0.073 (n = 45)
IL-6	1	0.223 (n = 46)	0.131 (n = 46)	-0.083 (n = 45)
PAP		1	0.455* (n = 46)	0.061 (n = 45)
TAT			1	0.264 [†] (n = 45)
PAI-1				1

* $p = 0.0015$, [†] $p = 0.0787$ (Spearman correlation coefficients).

Abbreviations: see Table 2.

DISCUSSION

Elevated plasma fibrinogen is a recognized risk factor for stroke⁶ and elevations in fibrinogen may in fact be a surrogate for elevated IL-6, a cytokine which plays a critical role in regulating fibrinogen production.⁷ IL-6 levels have also been reported to be elevated in stroke patients,⁸⁻¹⁰ and the increase of IL-6 significantly correlated with increased brain lesion volumes and was associated with poor functional and neurological outcome.⁹ In addition to elevated IL-6 levels in patients, gene expression of interleukin-1 β (IL-1 β) and TNF- α in rats are elevated after cerebral infarction.¹¹ IL-6 also regulates the production of tumor necrosis factor (TNF- α),¹² which in turn is capable of inducing tissue factor-like procoagulant activity on the surface of endothelial cells.¹³ In addition, these mediators activate the hepatic synthesis of acute-phase reactants such as C-reactive protein, serum amyloid A protein, fibrinogen, haptoglobin, ceruloplasmin, and C4b-binding protein (C4b-BP).^{11,14} C4b-BP possesses a binding site for protein S, a cofactor for protein C, and another natural anticoagulant which inactivates factors Va and VIIIa.¹⁵ In fact, during stroke, C4b-BP causes a decrease of free protein S,¹⁶ which may promote a procoagulant state. As a result, IL-6 may influence the disease course in the stroke by changing coagulation and fibrinolysis, in addition to causing hemorheologic changes by increasing fibrinogen levels and blood viscosity.¹⁷ Therefore, the aim of this study was to assess the relationship between circulating levels of IL-6 and hemostatic status in acute ischemic stroke.

In our study, IL-6 levels were significantly ele-

vated early after stroke. This observation is consistent with previous studies⁸⁻¹⁰ which suggest that IL-6 may play an important role in the inflammatory changes that occur following brain ischemia and the subsequent tissue damage.

Despite a significant increase in IL-6 levels, circulating levels of TAT (a reflection of systemic procoagulant activity¹⁸) did not correlate with IL-6. This observation supports the hypothesis that IL-6 production is a localized inflammatory response to acute hypoxic-ischemic injury and is not related to enhanced thrombin activity or intravascular hypercoagulation status.

In this study we found that mean TAT levels shortly after stroke were mildly elevated (3.4 ng/mL), an observation similar to a previous report by Geiser et al. (2.9 ng/mL).¹⁹ These findings suggest that thrombin formation is only partially suppressed, despite adequate anticoagulation, in patients with cerebrovascular events with prosthetic heart valves. This is present in atherogenic or lacunar subtypes, suggesting increased procoagulant activity in patients with cardioembolism and different pathophysiology of each subtype. Even though there was no statistical difference in IL-6 levels among the three different subtypes, the mean value of IL-6 was highest in the cardiogenic type, an observation that needs to be studied further using a large number of patients of different subtypes.

Although the importance of the thrombotic process and fibrin metabolism in stroke has been recognized for years, the role of fibrinolytic abnormalities have not been clearly delineated, probably because of the heterogeneous nature of stroke syndrome and because of the variety of *in*

in vitro methods for assessing fibrinolysis.

The recent development of specific immunoassays including t-PA,²⁰ PAI-1,²¹ and PAP²² allow more advanced assessment of the fibrinolytic system. In our study, IL-6 levels did not correlate with fibrinolytic markers, whereas there were significant correlations between procoagulant TAT and fibrinolytic markers (PAP and PAI-1). These findings reflect systemic derangements of both the clotting and fibrinolytic processes in stroke that do not involve IL-6.

The proinflammatory cytokines are produced not only by endothelial cells, monocytes, fibroblasts and T-lymphocytes,^{23,24} but also by microglia²⁵ and astrocytes.²⁶ Localized inflammatory response to acute brain lesion may have multiple local and systemic effects that modulate the disease course, including cytotoxicity,²⁷ astrocyte proliferation,²⁸ and leukocyte activation.²⁹ Recently, Johansson et al.³⁰ reported that neuroendocrine disturbances are often profound shortly after stroke, and cytokines including IL-6 seem to be important modulators of these disturbances. Vila et al.³¹ have also assessed the implication of IL-6 in early neurological worsening in ischemic stroke. More directly, IL-6 infusion has been shown to stimulate coagulation³² and to significantly correlate with coagulation markers.³³ On the other hand, IL-6 levels during the acute phase should be interpreted with caution because the pattern of the related changes depends on the disease severity. Other contributing factors can also affect the coagulation system.^{34,35} Taken together, these results suggest that elevated proinflammatory cytokines during the initial hours of ischemic stroke may either be an independent pathogenetic factor or the consequence of the thrombotic event, with no relationship to the procoagulant or fibrinolytic states.

Further investigation into the role of IL-6 in stroke may provide a basis for new therapies of stroke prevention or treatment.

ACKNOWLEDGMENTS

The authors wish to thank Hae Sun Chun for her technical assistance in this study.

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