Jikeikai Med J 2003 ; 50 : 141-7

Effects of Sodium Ozagrel on β-thromboglobulin and Platelet Factor 4 Plasma Levels in Lacuna Infarctions

Toshihide TANAKA[1,2], Yuzuru HASEGAWA[1], Toshinori KANKI[1], Yasuharu AKASAKI[2], Satoru MOROOKA[2], Kenji YUHKI[2], Toshiaki ABE[3], and Mitsuyoshi URASHIMA[4]

[1]*Department of Neurosurgery, Atsugi Prefectural Hospital*
[2]*Department of Neurosurgery, Fuji City General Hospital*
[3]*Department of Neurosurgery, The Jikei University School of Medicine*
[4]*Division of Clinical and Pharmacokinetics Research, The Jikei University School of Medicine*

ABSTRACT

Plasma concentrations of β-thromboglobulin (β-TG) and platelet factor 4 (PF4) in 50 patients with symptomatic lacuna infarctions were studied before and after treatment with intravenous sodium ozagrel. Smoking habits, levels of blood glucose and lipids, and blood pressure were documented for each patient. The plasma levels of β-TG and PF4 were significantly higher in smoking patients than in nonsmoking patients, whereas neither β-TG nor PF4 levels differed between groups defined by the other measured variables. Plasma levels of β-TG and PF4 were significantly lower after treatment with sodium ozagrel in patients who had higher levels of β-TG (greater than 50 ng/ml) and PF4 (greater than 20 ng/ml) before treatment ($p < 0.05$). Sodium ozagrel decreases plasma levels of β-TG and PF4 by suppressing platelet aggregation ; platelet activation may be related to the pathogenesis of cerebral thrombosis.

(Jikeikai Med J 2003 ; 50 : 141-7)

Key words : lacunar infarction, sodium ozagrel, β-thromboglobulin, platelet factor-4, thrombosis

INTRODUCTION

Recent clinical and experimental evidence suggests that platelet activation and aggregation play important roles in the pathogenesis of thrombotic and atherosclerotic complications of cerebrovascular and cardiovascular diseases[1].

β-Thromboglobulin (β-TG) and platelet factor 4 (PF4) are specific markers of platelet alpha-granule release and are related to the platelet aggregation and activation associated with thrombosis[2]. In addition, PF4 is a potent inhibitor of angiogenesis which has been evaluated as an inhibitor of angiogenesis-dependent tumor growth[3-5]. Plasma levels of β-TG and PF4 are elevated in patients with ischemic cerebrovascular disease[6-8], Moya-Moya disease[9], coronary artery disease[10-12], inflammatory bowel diseases[13], and cancers[14].

Inhibiting synthesis of thromboxane A2 decreases platelet aggregation and induces vasodilation[15,16]. Thus, such antiplatelet agents are frequently used to treat ischemic cerebral thrombosis. Sodium ozagrel, a thromboxane A2 synthetase inhibitor, inhibits platelet aggregation and causes vasodilation, thereby increasing cerebral blood flow in cerebral thrombosis[15,16].

Received for publication, July 31, 2003
田中 俊英, 長谷川 譲, 神吉 利典, 赤崎 安晴, 諸岡 暁, 結城 研司, 阿部 俊昭, 浦島 充佳
Mailing address : Toshihide TANAKA, Department of Neurosurgery, The Jikei University School of Medicine, Kashiwa Hospital, 163-1, Kashiwashita, Kashiwa, Chiba 277-8567, Japan.
E-mail : ttanaka@jikei.ac.jp

We investigated the effects of the antiplatelet agent sodium ozagrel on 2 thrombogenic markers, β-TG and PF4, in patients with lacuna infarction and assessed how these markers reflect the efficacy of and response to sodium ozagrel.

SUBJECTS AND METHODS

The subjects were 50 consecutive patients with symptomatic lacuna infarction (34 men and 16 women; mean age, 65.5 years; age range, 40 to 92 years) and a control group of 24 patients (15 men and 9 women; mean age, 62.2 years; age range, 36 to 78 years) who had no evidence of cerebrovascular disease or history of ischemic diseases. Informed consent was obtained from all subjects.

Lacuna infarction was diagnosed on the basis of clinical lacuna syndromes, such as pure motor hemiparesis, pure sensory stroke, ataxic hemiparesis, dysarthria-clumsy hand syndrome, and sensorimotor strokes. The cardiac status of each patient was assessed on the basis of history.

All patients with lacuna infarction had a high-intensity lesion on T2-weighted and diffusion-weighted magnetic resonance images. All lesions were located in the distribution of the perforating arteries. Patients with cardiac valvular disease or chronic renal failure were excluded.

Smoking habits, levels of blood glucose and lipids, and blood pressure were documented for each subject. A smoker was defined as a subject who smoked at least 10 cigarettes a day.

Hypercholesterolemia was diagnosed when the fasting serum cholesterol level was greater than 230 mg/dl, and hypertriglyceridemia was diagnosed when thge fasting serum triglyceride level was greater than 150 mg/dl. Hyperglycemia was diagnosed when the fasting serum glucose level was greater than 110 mg/dl.

All patients with hypertension received antihypertensive agents, such as angiotensin-converting enzyme inhibitors, calcium-channel blockers, and angiotensin II inhibitors.

Treatment with sodium ozagrel was started within 3 days after stroke. An intravenous infusion of 80 mg of sodium ozagrel was given twice a day for 2 weeks, at which time the diagnostic examinations were repeated. Blood samples were collected before treatment began and after 2 weeks of treatment.

Within 3 days after stroke, plasma concentrations of β-TG and PF4 were measured with radioimmunoassay (Special Reference Laboratories, Tokyo). The blood was drawn with a polystyrene syringe and a 21-gauge needle according to standard procedures. A tourniquet was not used, and blood was allowed to flow freely. The blood drawn was left for 15 to 30 minutes in iced water and then centrifuged at 2,000× g for 30 minutes at a temperature of 2°C to 4°C.

Statistical analysis was performed with Student's *t*-test to compare data from control subjects and data obtained before and after intravenous infusion of sodium ozagrel in patients with lacuna infarction.

RESULTS

Platelet counts were not decreased and bleeding

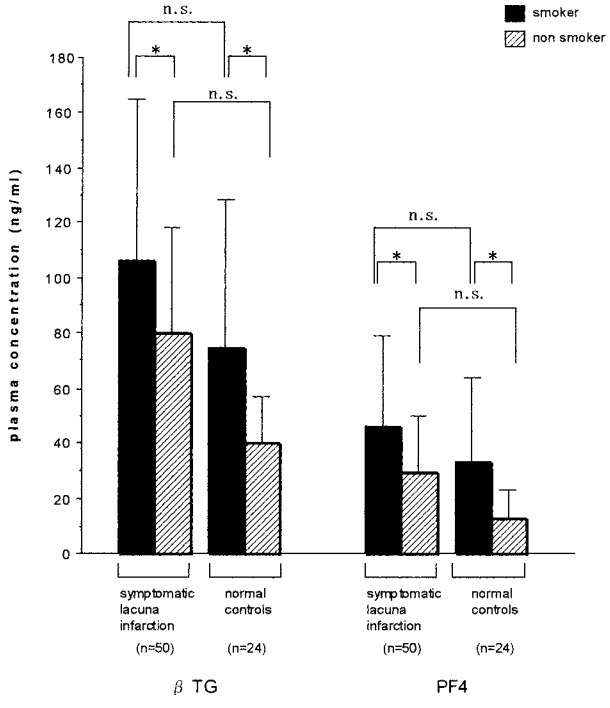

Fig. 1. Plasma concentrations of β-TG and PF4 in 50 patients with lacunar infarctions and 24 control subjects are shown. Horizontal bars indicate standard deviations. (*: $p<0.05$; n.s.: not significant)

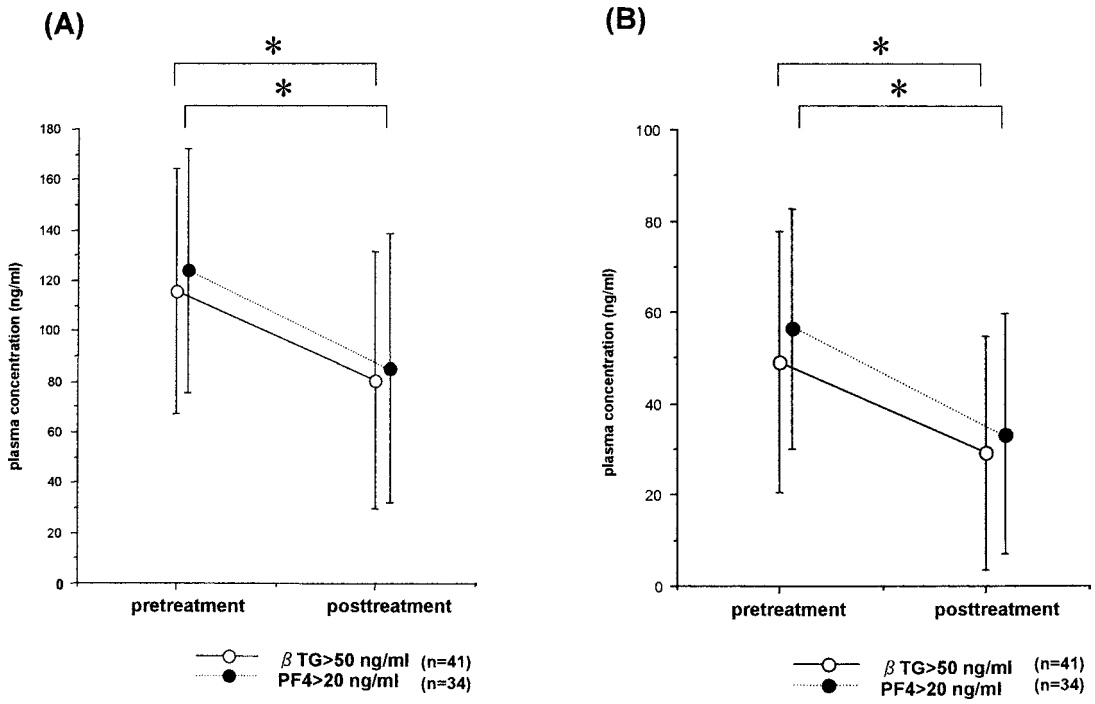

Fig. 2. Plasma concentrations of β-TG (A) and PF4 (B) in patients with initial β-TG levels greater than 50 ng/ml (○: 41 patients) and initial PF4 levels greater than 20 ng/ml (●: 34 patients) before and after treatment with sodium ozagrel. Horizontal bars indicate standard deviations. (*: $p<0.05$)

time was not prolonged in patients treated with sodium ozagrel.

In control subjects mean plasma concentrations of β-thromboglobulin (β-TG) and platelet factor 4 (PF4) were significantly lower in nonsmokers than in smokers (Fig. 1). Patients with hyperlipidemia but without clinical evidence of vascular disease had normal levels of β-TG and PF4 (data not shown).

Mean plasma levels of β-TG and PF4 in patients with symptomatic lacuna infarctions were slightly but not significantly higher than those in control subjects (Fig. 1).

Plasma concentrations of β-TG and PF4 were significantly lower after treatment with sodium ozagrel than before treatment in patients with higher levels of β-TG (more than 50 ng/ml) and PF4 (more than 20 ng/ml) before treatment ($p<0.05$; Fig. 2A and B). In addition, levels of β-TG and PF4 in elderly patients were significantly lower after treatment with sodium ozagrel than before treatment (data not shown).

No significant differences in β-TG and PF4 concentrations were found among patients with hypercholesterolemia, hypertension, and diabetes.

Serum cholesterol levels were not correlated with plasma levels of β-TG or PF4, and levels of β-TG and PF4 did not differ significantly before and after sodium ozagrel treatment in patients with or without hypercholesterolemia (Fig. 3A).

Levels of β-TG and PF4 were lower after treatment with sodium ozagrel treatment in patients with normal triglyceride levels but not in patients with hypertriglyceridemia (Fig. 3B).

In patients with hypertension, levels of β-TG and PF4 showed slight but not significant decreases after treatment with sodium ozagrel (Fig. 3C).

In patients with hyperglycemia, levels of PF4, but not levels of β-TG, were significantly lower after treatment with sodium ozagrel (Fig. 3D).

Plasma levels of β-TG and PF4 were significantly higher in smoking patients than in nonsmoking patients before treatment with sodium ozagrel. Levels of both β-TG and PF4 decreased significantly after treatment with sodium ozagrel in smokers but not in nonsmokers (Fig. 3E).

Plasma levels of β-TG and PF4 increased slightly

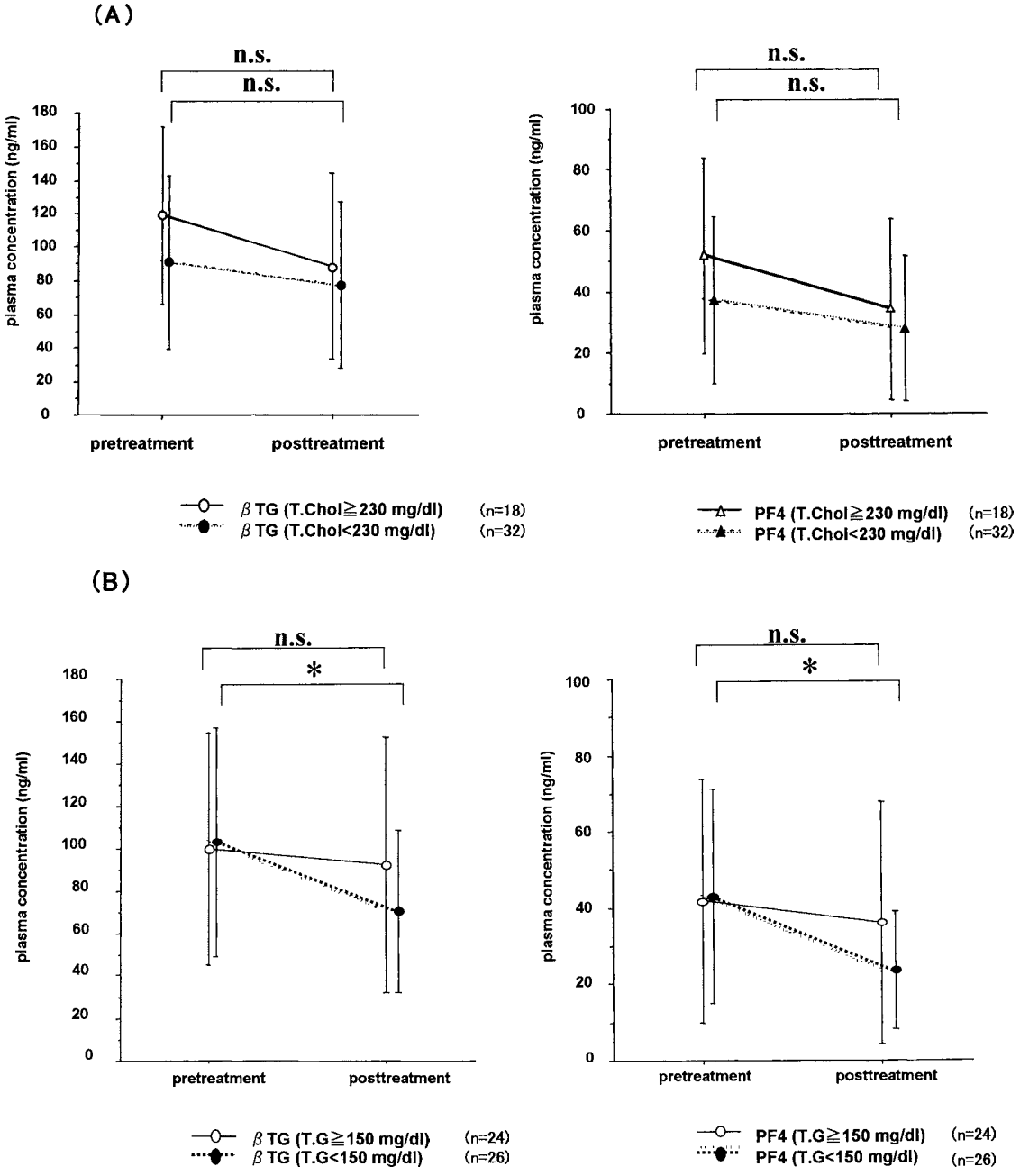

Fig. 3. Plasma concentrations of β-TG and PF4 before and after treatment with sodium ozagrel in patients with and without (A) hypercholesterolemia, (B) hypertriglyceridemia, (C) hypertension, (D) hyperglycemia, and (E) smoking habit. Horizontal bars indicate standard deviations. (*: $p<0.05$; n.s.: not significant; T. Chol: total cholesterol; T.G.: triglyceride; HT: hypertension; FBS: fasting blood sugar level)

in patients with transient ischemic attacks and recurrent lacuna infarctions. In contrast, levels of β-TG and PF4 did not increase markedly in patients who had recurrent lacuna infarction and cardiogenic embolization (data not shown). Plasma levels of β-TG and PF4 were not correlated with the severity of cerebral infarction.

DISCUSSION

β-TG and PF4 are specific proteins released from the alpha granules of platelets. When the platelet is

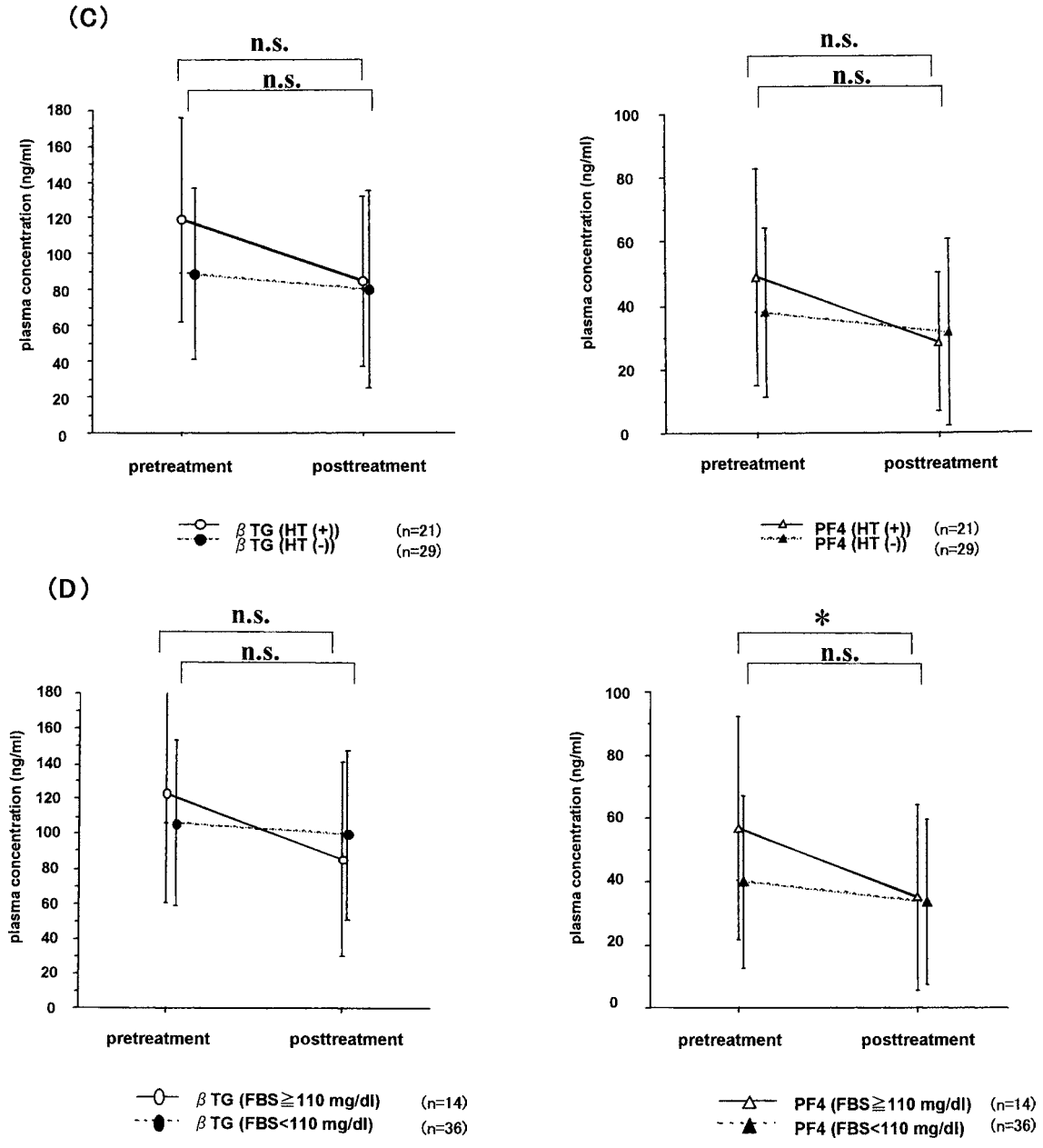

Fig. 3. continued

activated, β-TG and PF4 are released into the blood in similar amounts. In addition, PF4 is a biological marker for thromboischemic disorders and causes local vascular regression[7,9,15].

In the acute phase of cerebral infarction, plasma levels of β-TG and PF4 were lower than in control subjects, suggesting platelet destruction and regression of neovascularization in ischemic lesions.

The aim of our study was to assess whether plasma levels of β-TG and PF4 increase in patients with cerebral lacuna infarction and can be used to predict the pathogenicity and therapeutic efficacy of antiplatelet agents. However, we found no correlation between plasma levels of PF4 and cerebral blood flow as measured with single photon emission computed tomography (data not shown).

We also investigated whether risk factors for ischemic disorders, such as hypertension, diabetes,

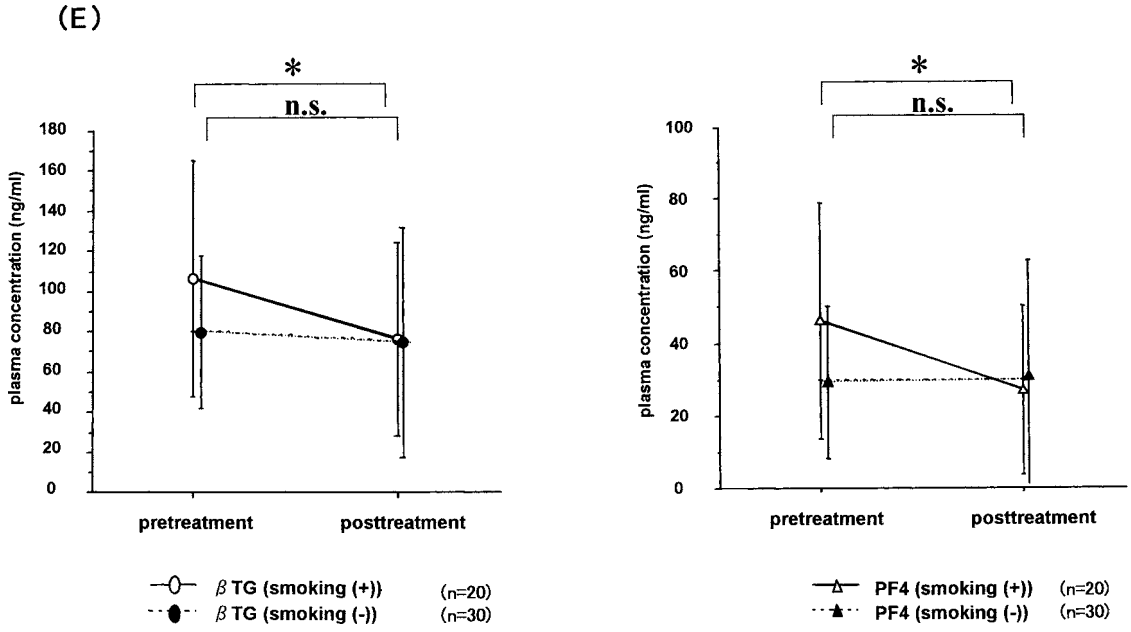

Fig. 3. continued

family history, hyperlipidemia, and smoking, affected platelet aggregation and vasoconstriction. Smokers had significantly higher plasma levels of β-TG and PF4 than did nonsmokers. Of note, levels of β-TG and PF4 were also higher in smokers without thrombosis. Platelet activation occurs more frequently in smokers with lacuna infarction than in nonsmokers.

We investigated the relation between the severity of infarction and the degree of platelet aggregation in patients treated with the antiplatelet agent sodium ozagrel. Sodium ozagrel inhibits synthesis of thromboxane A2, a potent platelet aggregator and vasoconstrictor, and is therefore a useful treatment in the acute stage of cerebral thrombosis, including lacuna infarction[15,16]. Sodium ozagrel decreases platelet aggregation and induces vasodilation. The question of whether normalization of plasma levels of β-TG and PF4 in cerebral thrombosis reflects a complete remission of the disease remains unanswered. If platelet aggregation were to play a major role in the pathophysiology of infarction, then agents that affect platelet activity could be useful therapeutically by normalizing levels of β-TG and PF4.

We found that levels of β-TG and PF4 were significantly reduced after treatment with sodium ozagrel. Previous studies have shown that high levels of β-TG and PF4 in patients with polycythemia decreased after treatment with the antiplatelet agent ticlopidine independently of platelet count variation[6,18]. In addition to affecting the thrombosis induced by platelet aggregation and activation, the therapeutic response to antiplatelet agents may be influenced by the presence of atherosclerosis[1,19,20]. We found that levels of β-TG and PF4 decreased less after antiplatelet therapy with sodium ozagrel in patients with hypercholesterolemia or hypertriglyceridemia than in patients with normal levels of cholesterol and triglyceride. Walls of atherosclerotic contain thrombi or remnants of thrombi[1]. The local production of cytokines and growth-regulatory molecules presumably stimulates changes in smooth muscle phenotypes, with associated increases in proliferation, migration, and collagen synthesis which result in the organization of thrombotic deposits. Studies of atherosclerotic lesion progression have shown that the composition of atherosclerotic lesions is related to the patient's clinical status[1,19,20].

Plasma levels of β-TG and PF4 in patients with recurrent lacuna infarction were not as high as those in patients in the acute phase of lacuna infarction, suggesting that platelet aggregation and activation are not the only factors that can predict and prevent

recurrent ischemic attacks[17].

We were surprised by our failure to demonstrate elevations of plasma β-TG and PF4 levels after recurrent infarction. If infarction is associated with considerable β-TG and PF4 release, we should have observed a subsequent decline in plasma levels of β-TG and PF4 from an initial high level. Our negative findings suggest that β-TG and PF4, with their short half-lives, were cleared from the blood before samples were taken[19] and that plasma levels of β-TG and PF4 may be affected by hypertensive agents and smoking cessation.

Furthermore, our finding of no correlation between PF4 levels and cerebral blood flow, as measured with single photon emission computed tomography, suggests that the amounts of β-TG and PF4 released from a platelet thrombus occluding a cerebral artery may not be sufficient to cause a measurable decrease in cerebral circulation.

In conclusion, our results suggest that platelet activation is related to the pathogenesis of cerebral thrombosis and that sodium ozagrel decreases plasma levels of β-TG and PF4 by suppressing platelet aggregation. Although plasma concentrations of β-TG and PF4 are useful markers of platelet activity, other biological variable that more fully reflect the status of thrombosis and atherosclerosis may be required.

References

1. Nomura T, Kohriyama T, Yamaguchi S, Kajiwara H, Nakamura S. Association between carotid atherosclerosis and hemostatic markers in patients with cerebral small artery disease. Blood Coagul Fibrinolysis 1998; 9: 55-62.
2. Gurney D, Lip GYH, Blann AD. A reliable plasma marker of platelet activation: Does it exist? Am J Hematol 2002; 70: 139-44.
3. Tanaka T, Manome Y, Wen PY, Kufe DW, Fine HA. Viral vector-mediated transduction of a modified platelet factor 4 cDNA inhibits angiogenesis and tumor growth. Nat Med 1997; 3: 437-42.
4. Staddon A, Henry D, Bonnen E. A randomized dose finding study of recombinant platelet factor 4 (rPF4) in cutaneous AIDS-related Kaposi's sarcoma. Proc ASCO 1994; 13: 50.
5. Maione TE, Gray GS, Petro J, Hunt AJ, Donner AL, Bauer SI, Carson HF, Sharpe RJ. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science 1990; 247: 77-9.
6. Uchiyama S, Sone R, Nagayama T, Shibagaki Y, Kobayashi T, Maruyama S, Kusakabe K. Combination therapy with low-dose aspirin and ticlopidine in cerebral ischemia. Stroke 1989; 20: 1643-7.
7. Oishi M, Mochizuki Y, Shikata E. Single lacuna brain infarction with transient signs versus those with long-lasting signs. Int Angiol 1999; 18: 206-9.
8. Oishi M, Mochizuki Y. Cerebrovascular acetazolamide reactivity and platelet function in asymptomatic cerebral thrombosis. J Neurol Sci 1999; 166: 81-4.
9. Albala MM, Levine PH. Platelet factor 4 and s-thromboglobulin in Moya-Moya disease. Am J Pediat Hematol/Oncol 1984; 96-9.
10. Pumphrey CW, Dawes J. Plasma beta-thromboglobulin as a measure of platelet activity. Effect of risk factors and findings in ischemic heart disease and after acute myocardial infarction. Am J Cardiol 1982; 50: 1258-61.
11. Nichols AB, Dwen J, Kaplan KL, Sciacca RR, Cannon PJ, Nossel HL. Fibrinopeptide A, platelet factor 4 and s-thromboglobulin levels in coronary heart disease. Blood 1982; 60: 650-4.
12. Ffrench P, McGregor JL, Berruya M, Belleville J, Touboul P, Dawes J, Dechavanne M. Comparative evaluation of plasma thrombospondin, β-thromboglobulin and platelet factor 4 in acute myocardial infarction. Thrombosis Res 1985; 39: 619-24.
13. Vrij AA, Rijken J, van Wersch JWJ, Stockbrugger RW. Platelet factor 4 and β-thromboglobulin in inflammatory bowel disease and giant cell arteritis. Eur J Clin Invest 2000; 30: 188-94.
14. Al-Mondhiry H. β-thromboglobulin and platelet factor 4 in patients with cancer: correlation with the stage of disease and the effect of chemotherapy. Am J Hematol 1983; 14: 105-11.
15. Oishi M, Mochizuki Y, Hara M, Yoshihashi H, Takasu T. Effects of sodium ozagrel on hemostatic markers and cerebral blood flow in lacuna infarction. Clin Neuropharmacol 1996; 19: 526-31.
16. Nihei C, Metoki H, Shimanaka Y. Clinical study on effect of thromboxane A2 synthetase inhibitor (OKY-046) to clarify the regulation of cerebral blood flow in cerebral thrombosis. Adv Prostaglandin Thromboxane Leukotriene Res 1987; 17: 953-7.
17. Woo E, Huang CY, Chan V, Chan YW, Yu YL, Chan TK. Beta-thromboglobulin in cerebral infarction. J Neurol Neurosurg Psychiatry 1988; 51: 557-62.
18. Najean Y, Poirier O. Beta-thromboglobulin and platelet factor 4 in polycythemia patients treated by ticlopidine. Acta Haemat 1984; 72: 83-9.
19. Kurabayashi H, Tamura J, Naruse T, Kubota K. Possible existence of platelet activation before the onset of cerebral infarction. Atherosclerosis 2000; 153: 203-7.
20. Oishi M, Mochizuki Y. Intima-media thickness of carotid artery and platelet function in asymptomatic cerebral thrombosis. Nihon Univ J Med 1999; 41: 351-6.