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Effects of Low-dose Aspirin and Ticlopidine on β-Thromboglobulin and Platelet Factor 4 Plasma Levels in Lacunar Infarctions

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ABSTRACT

Platelet activation may contribute to the pathogenesis of cerebral thrombosis. Plasma concentrations of β-thromboglobulin (β-TG) and platelet factor 4 (PF4) in 72 patients with symptomatic lacunar infarction were studied before and after treatment with aspirin and ticlopidine. Plasma levels of β-TG and PF4 were significantly reduced by each agent alone or by both agents in combination ($p<0.05$). However, combination therapy with aspirin and ticlopidine did not produce synergistic or additive effects. Furthermore, hypercholesterolemia and hypertension decreased the efficacy of the antiplatelet agents, whereas hyperglycemia, hypertension, and smoking status did not influence treatment efficacy. These results suggest that aspirin and ticlopidine reduce plasma levels of β-TG and PF4 by suppressing platelet aggregation.

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Key words : lacunar infarction, antiplatelet agent, β-thromboglobulin, platelet factor 4, thrombosis

INTRODUCTION

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

β-Thromboglobulin (β-TG) and platelet factor 4 (PF4) are specific markers of platelet alpha-granule release which are related to platelet aggregation and activation and to thrombosis[2]. In addition, PF4 is a potent inhibitor of angiogenesis which has been investigated clinically 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[6-9], Moya-Moya disease[10], coronary artery disease[11-13], inflammatory bowel disease[14], and cancer[15]. Furthermore, antagonists of thromboxane A2 synthesis inhibit platelet aggregation and produce vasodilatation[7,16,17] and are frequently used to treat ischemic cerebral thrombosis.

Aspirin is an antiplatelet agent that completely inhibits platelet aggregation induced by arachidonic acid but inhibits only secondary platelet aggregation i nduced by adenosine diphosphate (ADP) and platelet-activating factor (PAF). Low-dose aspirin also produces vasodilation via activation of prostaglandin I_2[6]. In contrast, ticlopidine inhibits both primary and secondary platelet aggregation induced by ADP and PAF but has little effect on platelet aggregation induced by arachidonic acid[6,18,19].

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The goal of the present study was to investigate the effects of aspirin and ticlopidine on plasma levels of two thrombogenic markers, β-TG and PF4, in patients with lacunar infarction and correlate changes in these levels with the efficacy of antiplatelet treatment.

SUBJECTS AND METHODS

The subjects were 72 consecutive patients with symptomatic lacunar infarction (51 men and 21 women; mean age, 65.9 years; age range, 33 to 92 years). Informed consent was obtained from all patients.

In all patients T_2-weighted and diffusion-weighted magnetic resonance images demonstrated high-intensity lesions in the distribution of the perforators. In addition to imaging, the presence of clinical lacunar syndromes, such as pure motor hemiparesis, pure sensory stroke, ataxic hemiparesis, dysarthria-clumsy hand syndrome, and sensorimotor disturbances, were required to diagnose lacunar infarction.

The cardiac status of each patient was assessed on history. Patients with cardiac valvular diseases or chronic renal failure were excluded. The smoking habits, blood glucose and lipid levels, and blood pressure were documented in each patient. A smoker was defined as a patient 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 the 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.

Blood samples were collected within 3 days after stroke and 3 to 6 months after administration of oral antiplatelet agents. Plasma concentrations of β-TG and PF4 were measured with radioimmunoassay at the Special Reference Laboratories (Tokyo). Blood was obtained 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. Blood sample tubes were placed in ice water for 15 to 30 minutes and then centrifuged at 2,000 g for 30 minutes at 2 to 4°C. Statistical analysis

Table 1. Summary of patients treated with aspirin and ticlopidine

		ticlopidine	aspirin	ticlopidine/aspirin
	n	30	27	15
Sex	male	20	19	12
	female	10	8	3
Age (years)		61.9±9.6	69.6±11.2	66.1±10.0
Serum total cholesterol (mg/dl)		204.4±36.4	200.2±32.2	211.5±33.2
Serum triglyceride (mg/dl)		186.0±183.0	136.0±58.2	164.0±80.0
Hypertension		14(46.7%)	12(43.3%)	5(33.3%)
Hyperglycemia		12(40.0%)	5(18.5%)	6(40.0%)
Smoking		13(43.3%)	11(40.7%)	10(66.7%)

Table 2. Summary of β-TG and PF4 plasma concentrations before and after treatment with aspirin and ticlopidine

	β-TG (ng/ml)		PF4 (ngKml)	
	pretreatment	posttreatment	pretreatment	posttreatment
ticlopidine	123.2±50.0	80.8±43.8*	54.2±29.7	29.2±23.1**
aspirin	93.1±52.7	51.7±24.0*	34.6±28.3	16.2±10.9**
ticlopidine/aspirin	84.5±47.2	47.3±23.7*	39.1±27.7	14.1± 9.4**

*$p < 0.05$ versus pretreatment

**$p < 0.01$ versus pretreatment

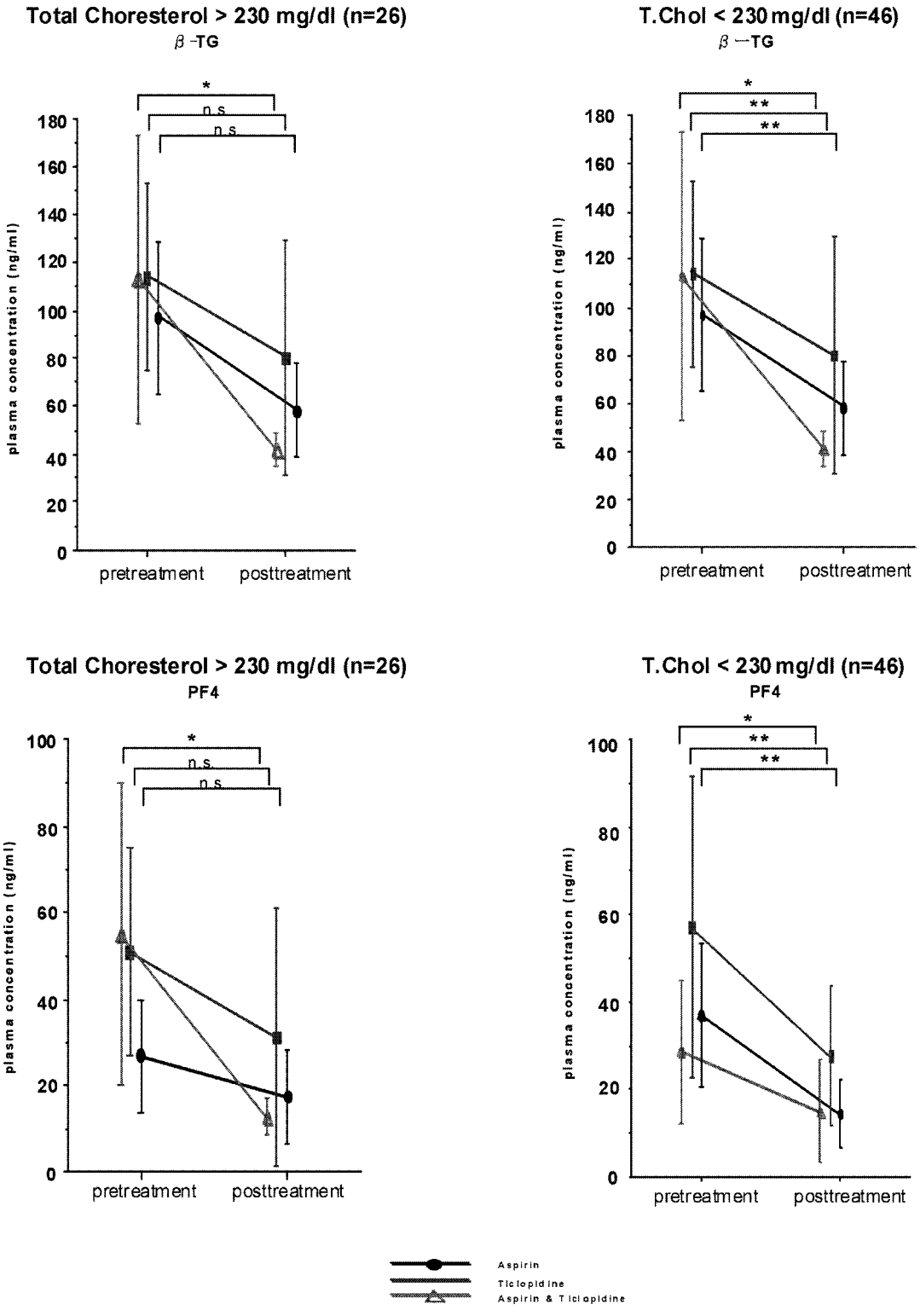

Fig. 1. Plasma concentrations of β-TG and PF4 before and after treatment with aspirin and ticlopidine in patients with or without hypercholesterolemia. Horizontal bars represent standard deviations. (*$p<0.05$, **$p<0.01$, n.s.: not significant)

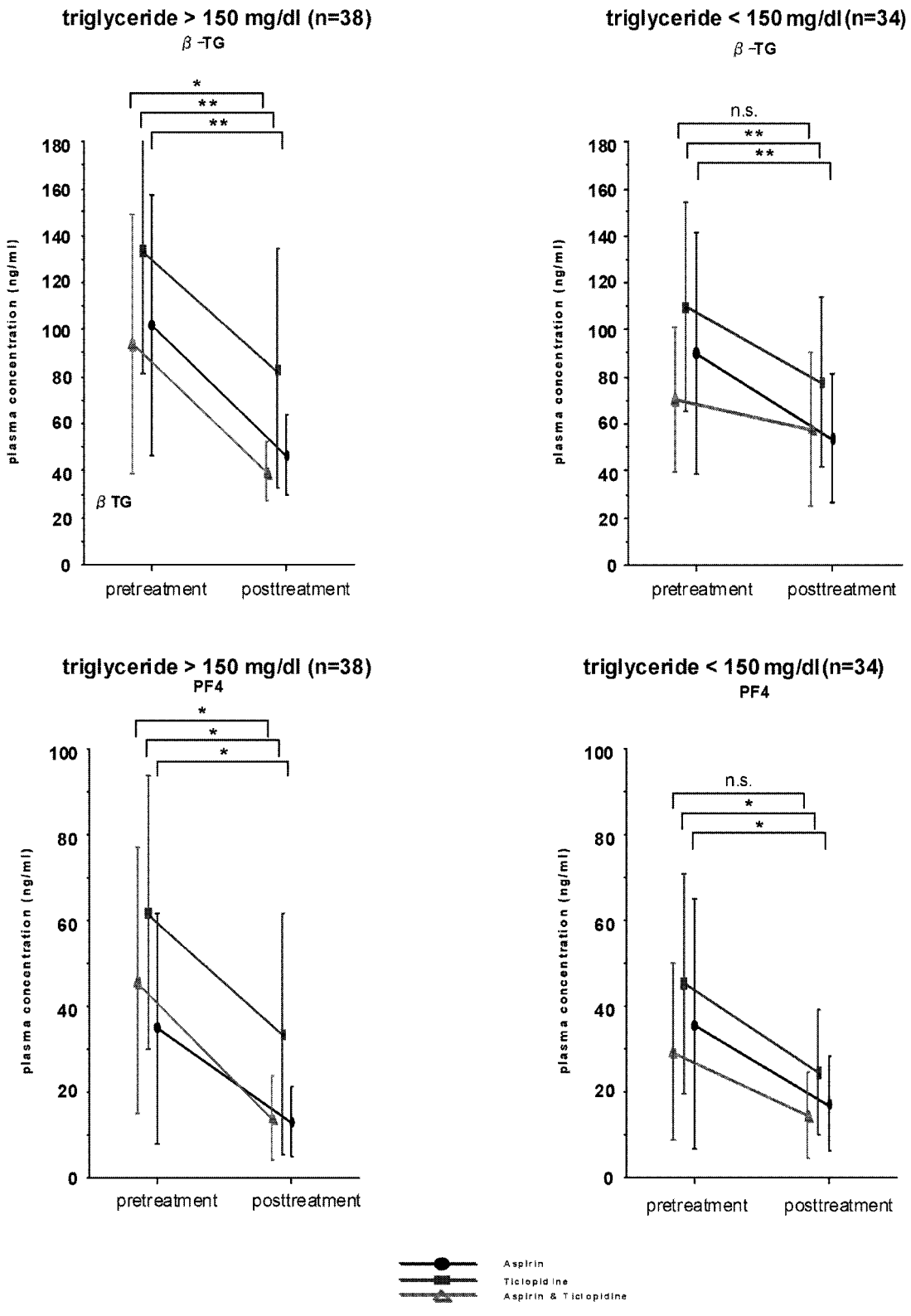

Fig. 2. Plasma concentrations of β-TG and PF4 before and after treatment with aspirin and ticlopidine in patients with or without hypertriglyceridemia. Horizontal bars represent standard deviations. (*$p<0.05$, **$p<0.01$, n.s.: not significant)

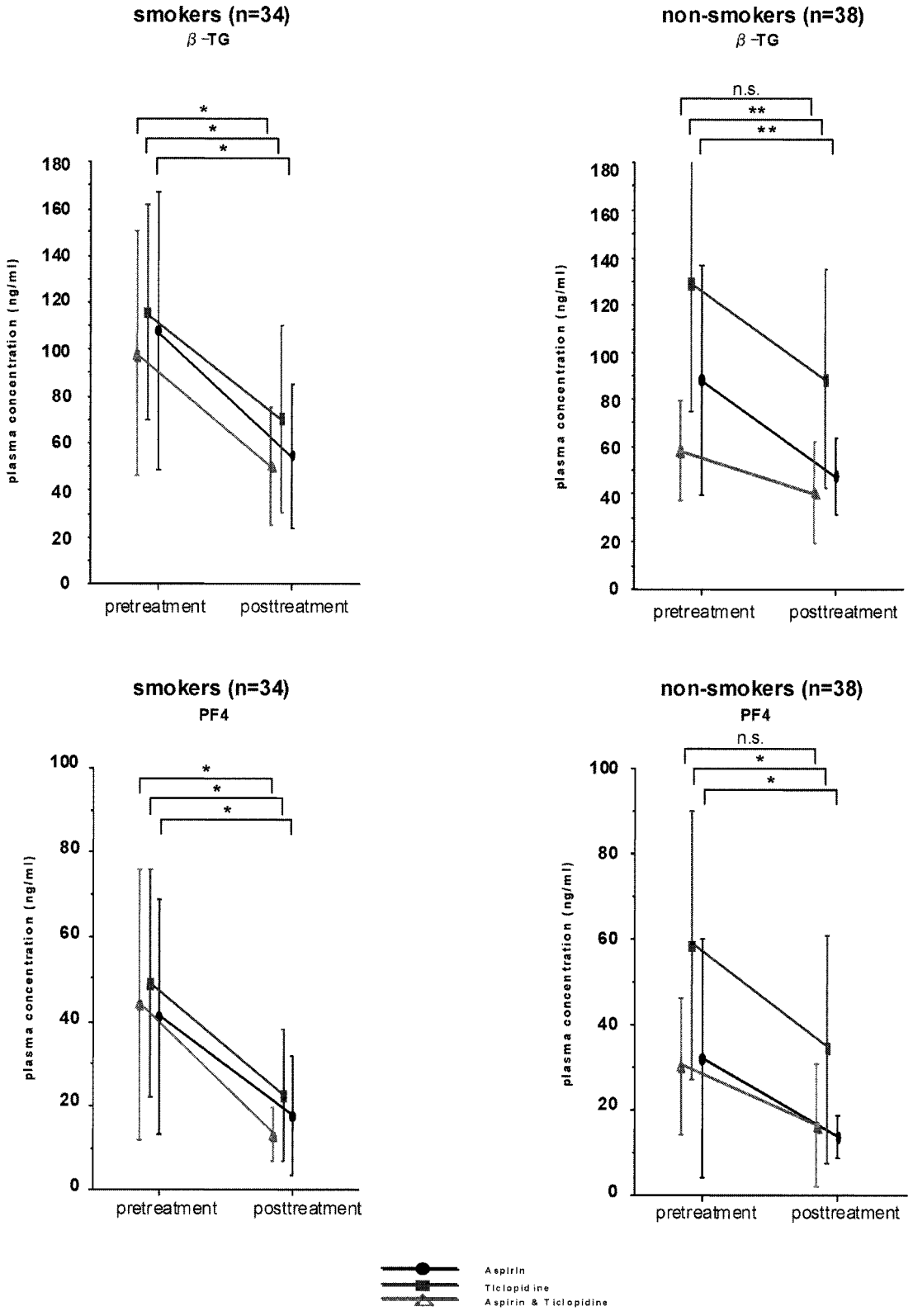

Fig. 3. Plasma concentrations of β-TG and PF4 before and after treatment with aspirin and ticlopidine in smoking and nonsmoking patients. Horizontal bars represent standard deviations.
(*$p<0.05$, **$p<0.01$, n.s.: not significant)

was performed with Student's t-test to compare data obtained before and after treatment with antiplatelet agents.

RESULTS

Patients

The demographic characteristics of each treatment group and their distribution of risk factors are summarized in Table 1. Patients treated with a combination of aspirin and ticlopidine had a higher rate of smoking. There was no evidence of hemorrhagic complications or drug-induced liver damage in any patient.

Plasma levels before and after treatment with aspirin and ticlopidine are summarized in Table 2. Before treatment mean serum levels of β-TG and PF4 were significantly higher in patients treated with ticlopidine alone than in patients treated with aspirin alone or with both aspirin and ticlopidine. After treatment with aspirin and ticlopidine levels of β-TG and PF4 were significantly lower. No significant correlation was noted between the platelet count and levels of β-TG or PF4 (data not shown).

Hypercholesterolemia

In patients with hypercholesterolemia, plasma levels of β-TG and PF4 were significantly reduced only after treatment with both aspirin and ticlopidine ($p<0.05$). In patients without hypercholesterolemia, treatment with aspirin alone, ticlopidine alone, or both aspirin and ticlopidine reduced β-TG and PF4 levels ($p<0.01$; Fig. 1).

Hypertriglyceridemia

In patients with hypertriglyceridemia, plasma concentrations of β-TG and PF4 were significantly reduced by treatment with aspirin or ticlopidine alone ($p<0.01$ and $p<0.05$, respectively). Treatment with both aspirin and ticlopidine also decreased plasma levels of β-TG and PF4, but no synergistic or additive effects were observed (Fig. 2).

Smoking

Plasma concentrations of β-TG and PF4 were significantly reduced by combination therapy with aspirin and ticlopidine ($p<0.05$) in both smokers and nonsmokers. Therefore, smoking had no effect on the reduction of β-TG and PF4 by ticlopidine and aspirin (Fig. 3).

Hypertension

In patients without hypertension, plasma concentrations of β-TG and PF4 were significantly decreased by aspirin or ticlopidine alone ($p<0.01$ and $p<0.001$, respectively). In patients with hypertension, neither β-TG nor PF4 was decreased after treatment with aspirin or ticlopidine. Treatment with both aspirin and ticlopidine did not affect concentrations of β-TG or PF4 (data not shown).

Hyperglycemia

In patients with hyperglycemia, plasma concentrations of β-TG and PF4 were significantly reduced by ticlopidine alone ($p<0.05$) but not by aspirin alone or combination therapy with aspirin and ticlopidine. In contrast, in patients without hyperglycemia β-TG and PF4 concentrations were significantly reduced by aspirin alone ($p<0.05$, data not shown).

DISCUSSION

The pathogenesis of cerebral thrombosis with atherosclerosis is complicated and involves the interplay between platelet aggregation, thrombosis, and atherosclerotic lesions of the vascular wall. In addition, endothelial dysfunction promotes atherosclerosis through vasoconstriction, monocyte and platelet adhesion (via expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1), thrombogenesis, and the stimulation and release of cytokines and growth factors (e.g., tumor necrosis factor-alpha and interleukin-1)[20–23]. Risk-factor modification, particularly lowering concentrations of low-density lipoprotein cholesterol, improves endothelial function[24,25].

Previous studies have demonstrated that aspirin reduces the incidence of subsequent stroke and death in patients with transient ischemic attack and cerebral thrombosis. Furthermore, the Antiplatelet

Trialists' Collaboration study found that the risk reduction in the incidence of stroke, myocardial infarction, or vascular death produced by aspirin was 22% (SD 5%) for patients with ischemic stroke who were treated with low-dose aspirin[6,26].

Aspirin completely blocks platelet aggregation via inhibition of cyclooxygenase, leading to inhibition of thromboxane A2 synthesis[6]. In contrast, ticlopidine inhibits both primary and secondary platelet aggregation in response to ADP and PAF, but its inhibition of platelet aggregation in response to arachidonic acid is not prominent because ticlopidine has no effect on cyclooxygenase[6]. Thus, combination therapy with aspirin and ticlopidine is necessary to inhibit all pathways (e.g., via ADP, arachidonic acid, and PAF) leading to platelet aggregation[6].

Although antiplatelet agents, such as aspirin and ticlopidine, have been widely used in patients with thrombosis, there are no appropriate methods to assess their effects on platelet activation. Both β-TG and PF4, which are stored in the alpha granules of platelets, appear to reflect platelet activation and are often increased in patients with atherogenic diseases[10,11]. For example, β-TG and PF4 are present in increased amounts in patients with lacunar infarction[19] and are decreased after treatment with thromboxane A2 inhibitors[7,27].

In the present study, combination therapy with aspirin and ticlopidine significantly reduced β-TG and PF4 concentrations, whereas aspirin or ticlopidine alone produced reductions but not statistically significant. However, the effect of combination therapy was neither synergistic nor strictly additive, although plasma levels of β-TG and PF4 were within normal limits after treatment, probably because levels of β-TG and PF4 before treatment were lower in patients treated with both aspirin and ticlopidine or with aspirin alone than in patients treated with ticlopidine alone. The therapeutic efficacy of aspirin and ticlopidine might depend on pretreatment levels of β-TG and PF4. As we have previously reported, smokers have significantly higher plasma levels of β-TG and PF4 than do nonsmokers[27]. Other risk factors, such as hypertension, hypercholesterolemia, and hyperglycemia, affect platelet aggregation but do not inhibit antiplatelet activity induced by sodium ozagrel. In the present study, hypercholesterolemia and smoking may have attenuated the efficacy of antiplatelet agents; however, the decreases in β-TG and PF4 levels after antiplatelet therapies were not statistically significant.

Furthermore, we did not find any correlation between β-TG and PF4 levels and cerebral blood flow as measured with single photon emission computed tomography; therefore, the amounts of β-TG and PF4 released from the platelet thrombus occluding a cerebral artery may not be sufficient to cause a significant decrease in cerebral infarction (data not shown).

Recent evidence of interest is the clinical significance of microbleeds in patients with ischemic stroke. Microbleeds, lacunar infarction, and leukoaraiosis can co-exist in patients with hypertension[28]. Cerebral hemorrhage is a major complication of antiplatelet therapy for prevention of ischemic stroke. Microbleeds are not always visible at the site of subsequent hemorrhage, although they may be a risk factor for it. Conventional magnetic resonance often fails to detect microbleeds at the early stage of stroke; however, a novel magnetic resonance technique, T2*-weighted imaging, provides critical information for detecting acute bleeding[29,30]. The cerebral microvasculature can be weakened by aging, sustained hypertension, hyperglycemia, or fibrohyalinostic degeneration of brain blood vessels. Old microbleeds provide further evidence of severe microangiopathy with a subsequent increased vascular vulnerability and activation of platelets.

These findings suggest that monitoring the efficacy of the antiplatelet agents by examining markers of platelet activation, such of the plasma levels of β-TG and PF4, is difficult and cumbersome.

Regression of atherosclerotic plaques and diminution of shear stress against the vascular wall leading to vascular remodeling are required for the treatment of cerebral thrombosis. Risk factor modification, particularly lowering elevated concentrations of low-density lipoprotein cholesterol, improves endothelial function[25,26]. Antiplatelet agents may have such effects; however, methods to monitor them are not

yet available.

To provide information about shear stress on vascular walls, activation of platelet-leukocyte aggregation, and platelet activation, further investigations into methods for monitoring the effects of antiplatelet agents are required.

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