ENHANCED PLATELET AGGREGABILITY UNDER HIGH SHEAR STRESS IN CORONARY CIRCULATION OF PATIENTS WITH UNSTABLE ANGINA

NAOFUMI DOI
First Department of internal Medicine, , Nara Medical University
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Abstract: Mechanical forces, including high shear stress, have been found to cause platelet aggregation. Although increased platelet aggregation is also associated with the pathophysiology of unstable angina, it is not known whether platelet aggregation induced by high shear stress occurs in the coronary circulation of patients with unstable angina. We assayed high shear stress induced platelet aggregation (h-SIPA) in each of 25 patients with unstable angina and a severe stenotic lesion of the left coronary artery, and in 5 patients with chest pain syndrome as controls.

We obtained blood from the coronary ostium and coronary sinus of each subject during angiography and measured h-SIPA of each sample with a modified cone-and-plate type viscometer set at 108 dyne/cm^2. In the patients with unstable angina, h-SIPA of the samples from the coronary sinus was significantly higher than in those from the coronary ostium ($p<0.01$). However, we detected no differences between blood from the coronary ostium and coronary sinus in samples from the control subjects. Although h-SIPA has been known to be mediated by the interaction between platelets and von Willebrand factor (vWF), we found no differences in the concentrations of vWF antigen or ristocetin cofactor activity between plasma from coronary ostium and coronary sinus of the patients with unstable angina.

Platelet aggregation under conditions of high shear stress is enhanced in the coronary circulation of patients with unstable angina. These results suggest that treatment of these patients with an agent that prevents h-SIPA may be beneficial.
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Key words: coronary circulation, platelet, shear stress, unstable angina, von Willebrand factor

INTRODUCTION

The intracoronary formation of thrombus is a major cause of acute myocardial infarction in patients with unstable angina. This latter condition in associated with the adhesion of platelets onto the thrombogenic surfaces of vessel walls[1,2], the activation and aggregation of platelets, an increase of blood coagulation activity, a decrease of anticoagulation activity and a decrease of blood fibrinolytic activity. Indeed, patients with unstable angina have been treated with antiplatelet, anticoagulant, and fibrinolytic agents[3]. In these mechanisms the adhesion and the aggregation of platelets are the primary steps in the evolution of unstable angina to myocardial infaction. The mortality of the unstable angina is decreased by blockade of platelet aggregation with aspirin. The process of platelet activation and aggregation also plays a central role

in thrombus fromation in atherosclerotic coronary arteries[2]. It has been reported that the aggregation of platelets by collagen or ristocetin is enhanced. P-selectin is an adhesional molecule that is present on the surface of activated platelets[4,5]. It was recently reported that the expression of P-selectin on platelets is increased at the time of anginal episodes in patients with unstable angina[6], suggesting that the activation of circulating platelets occurs during these attaacks. This platelet activation may be stimulated by injury to the endothelium, by the fissuring or rupture of an atherosclerotic plaque[7], by the presence of local humoral factors, such as thromboxane A_2, serotonin and/or by high shear stress, a flow dynamic force that is present in arterial stenotic lesions. In addition, high shear stress has been shown to induce platelet aggregation, a process that is also stimulated by such agonists as ristocetin, collagen, thrombin or epinephrine.

In arterial stenotic lesions, the aggregation of platelets has been found to be caused by mechanical forces such as high shear stress, and not by exogenous agosists[8,9]. The platelet aggregation induced by high shear stress (h-SIPA) is mediated by the interaction between von Willebrand factor and the platelet glycoprotein (GP) adhesion receptors, GPIb/IX complex and GP IIb/IIIa. In contrast, the platelet aggregation induced by low shear stress is mediated by the binding of fibrinogen to GP IIb/IIIa[10-12]. Although h-SIPA is thought to play an important role in the platelet aggregation that occurs during arterial thrombosis, it is not known whether this type of platelet aggregation is also enhanced in coronary sinus blood that has been subjected to the shear stress occurring in the coronary circulation. We therefore investigated h-SIPA in blood from the coronary sinus and the coronary ostium in patients with unstable angina.

METHODS

Study population

Each subject who participated in this study was aware of the experimental nature of the research and gave informed consent, in accordance with the Declaration of Helsinki. Included in this study were 25 Japanese patients (18 men and 7 women, mean age 62 years, range 52-76), with unstable angina, each of whom had undergone angiography that revealed a severe stenotic lesion of the left coronary artery. Excluded from this study were patients who had received any antiplatelet agent or anticoagulant within 7 days of angiography. To inhibit blood coagulation, each subject was administered 100 IU/kg heparin prior to angiography. As controls, we also utilized five patients (4 men and 1 woman, mean age 60 years, range 48-70), with the chest pain syndrome, each of whom had a normal coronary angiogram and no spastic reaction to an acetylcholine loading test.

Measurement of h-SIPA

Blood samples were obtained from the coronary ostium and coronary sinus of each patients during angiography. The blood was mixed with 1/10 volume of 3.8 % sodium citrate, and immediately centrifuged at 800 rpm for 10 min at 25℃ to obtain platelet-rich plasma. The h-SIPA was measured with a modified cone-and-plate type viscometer apparatus (Toray Medical Co, Ltd. Tokyo, Japan)[13,14], which consisted of a helium-neon laser light source at 633 nm, a thermostated cone-plate streaming chamber, and a photocounting unit whose output was analyzed in a computer (Fig. 1A). Platelet-rich plasma samples were exposed to a constant high-shear stress of 108 dynes/cm^2, while the cone was rotated at 1,800 rpm. Platelet aggrega-

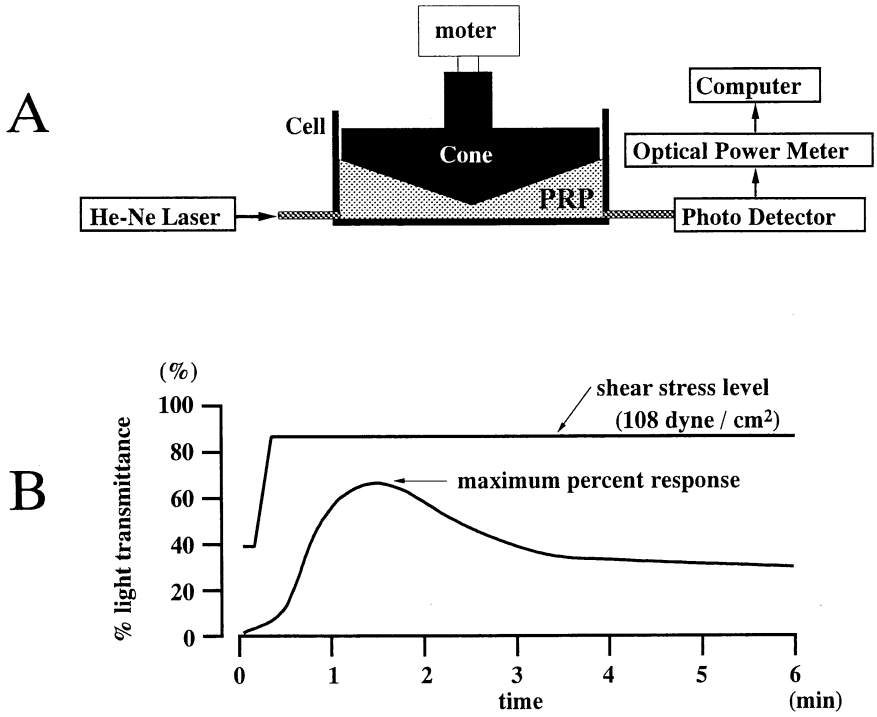

Fig. 1. (A) Schema of the device used for continuous recording of shear -induced platelet aggregation. The measuring device consists of three components: a light source, a thermostated cone-plate streaming chamber and an optical detection unit. The distance from the cone apex to the base plate is adjusted to 0.04 mm. Platelet rich plasma samples were exposed to constant high-shear stress of 108 dynes/cm², while rotating the cone et 1,800 rpm. Platelet aggregation was detected as a rapid increase in transmitted light intensity, which was analyzed by a computer. (B) Sample curve of high shear stress induced platelet aggregation. The extent of aggregation was determined from the peak light transmittance. PRP=platelet rich plasma.

tion was detected as a rapid increase in the intensity of the transmitted light (Fig. 1B).

Assays for von Willebrand factor (vWF)

Citrated whole blood samples drawn from the coronary sinus and coronary ostium were immediately centrifuged at 2,300 rpm for 10 min at 25℃ and stored at −80℃. The concentration of von Willebrand factor antigen (vWF : Ag) was measured by an enzyme immunoassay. The activity of von Willebrand factor was determined by platelet aggregation in the presence of ristocetin, referred to as the von Willebrand ristocetin cofactor (RCof), using the von Willebrand Reagent™ kit (Behring Diagnostics GmbH, Marburrng, Germany), according to the manufacturer's instructions.

Statistical analysis

Continuous variables are expressed as mean±standard deviation and compared by the

Wilcoxon test. Difference were considered statistically significant when $p<0.05$.

RESULTS

The platelet aggregation curves of the representative case are shown in Fig. 2. The peak and the maximum solpe of the curve from coronary sinus was higher than that from coronary ostium.

The maximum percent response of h-SIPA in the samples from the coronary sinus of patients with unstable angina were significantly higher (53.6±15.6 %) than those from the coronary ostium (41.7±15.6 %, $p<0.01$) (Fig. 3). Control subjects exhibited no difference between the coronary sinus 51.4±11.5 %) and the coronary ostium (46.6±12.4 %) (Fig. 3).

The maximum aggregation slope of h-SIPA in the samples from the coronary sinus of patients with unstable angina were significantly higher (5.8±1.4 nW/min) than those from the coronary ostium (4.3±1.6 nW/min, $p<0.01$) (Fig. 4). Control subjects exhibited no difference between the coronary sinus (5.2±1.9 nW/min) and the coronary ostium (5.4±1.8 nW/min) (Fig. 4).

When we assayed the concentrations of vWF antigen and ristocetin cofactor activity in these plasma samples, we detected no differences between coronary sinus and coronary ostium in these patients with unstable angina (Fig. 5).

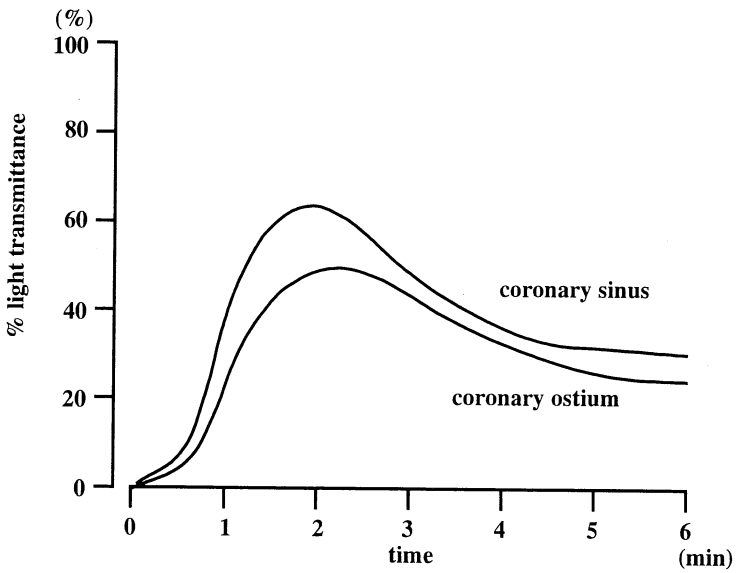

Fig. 2. The curves shows the time course of high-shear induced platelet aggregation (h-SIPA) from the sample of coronary sinus and that of coronary ostium in patients with unstable angina.

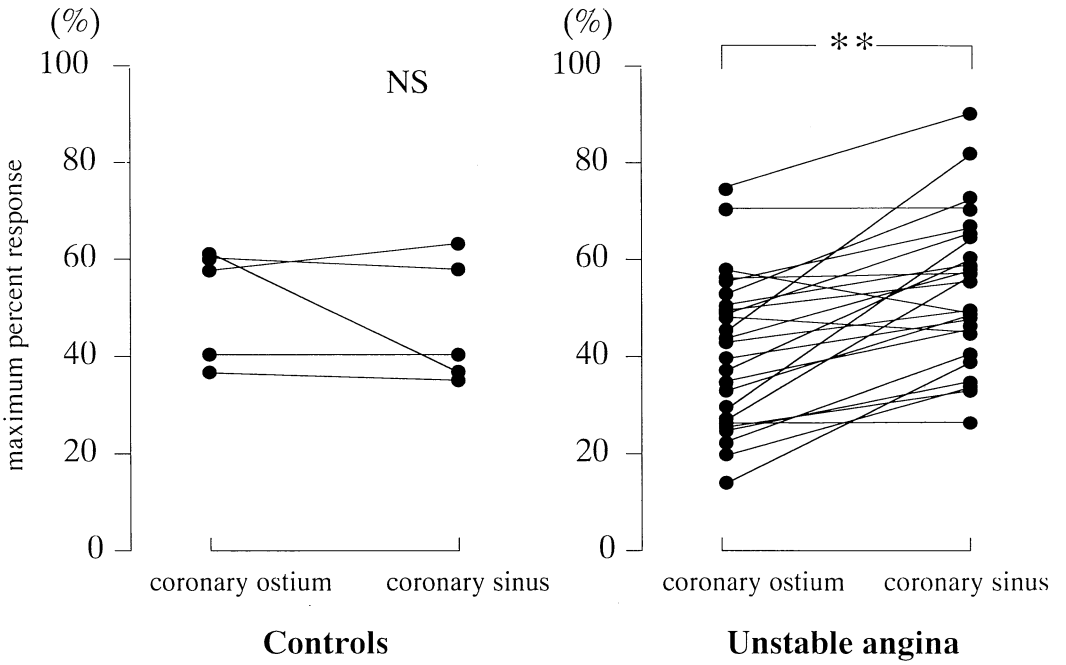

Fig. 3. Maximum percent response of high-shear induced platelet aggregation (h-SIPA) in control subjects (left) and unstable angina patients (right). ** ; $p < 0.01$.

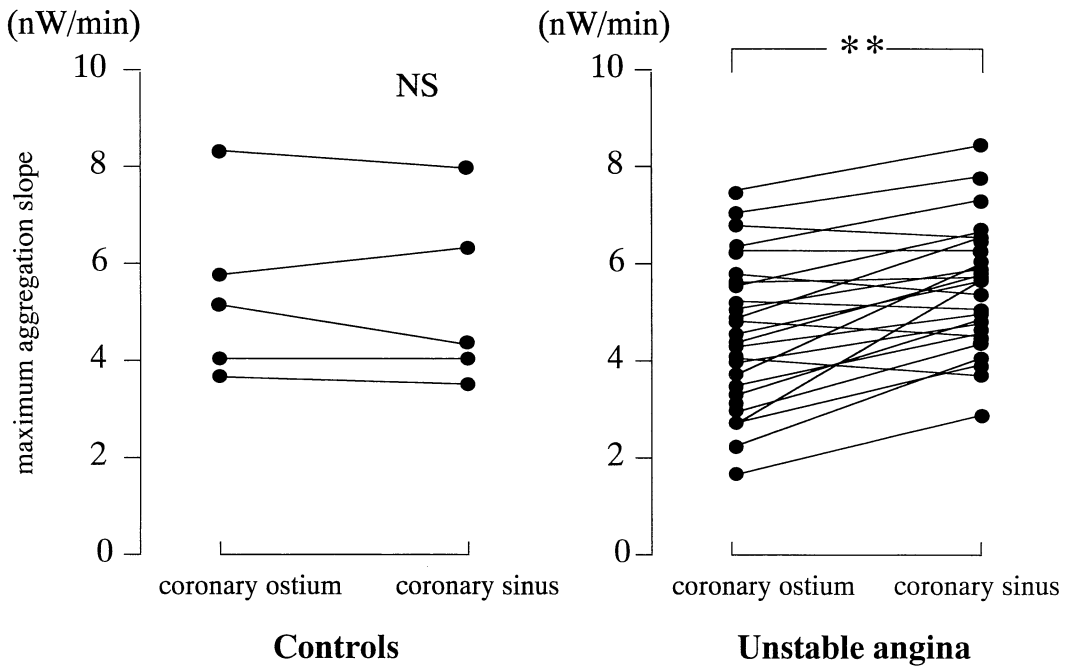

Fig. 4. Maximum aggregation slope of high-shear induced platelet aggregation (h-SIPA) in control subjects (left) and unstable angina patients (right). ** ; $p < 0.01$.

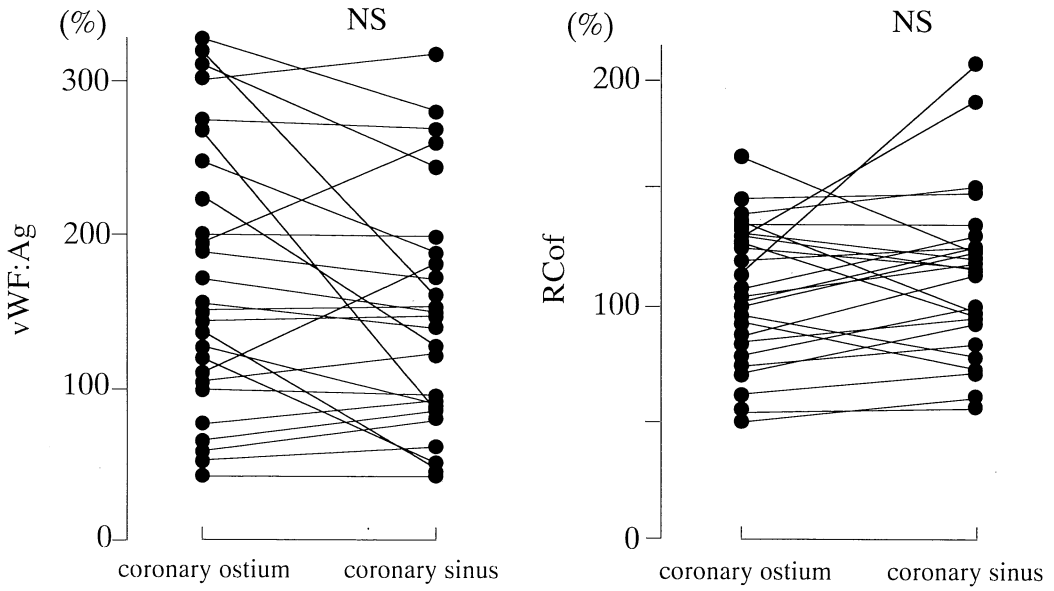

Fig. 5. Relative concentration of von Willebrand factor antigen (left), and ristcetin cofactor (right) in plasma samples from the coronary ostium and coronary sinus of patients with unstable angina. vWF:Ag ; von Willebrand factor antigen ; RCof=ristocetin cofactor.

DISCUSSION

We have shown that, in patients with unstable angina, h-SIPA is significantly higher in blood from the coronary sinus than in that from the coronary ostium. In control subjects, no difference was observed. Platelet aggregation under high shear force is an important component of atherosclerotic coronary thrombogenesis. In a model of coronary artery stenosis under pulsatile, viscous blood flow, shear stress was found to exceed 100 dynes/cm^2 [15,16]. In assaying platelet aggregation, we therefore subjected our samples to a shear force of 108 dynes/cm^2, a stress which may occur at coronary stenotic lesions in vivo.

Aspirin inhibits platelet activation by acetylating the enzyme, cyclo-oxygenase[17,18]. This agent is now part of the standard therapy for patients with unstable angina[19]. This therapy is supported by the previous studies showing that aspirin markedly reduces the risks of myocardial infaction and cardiac death in patients with unstable angina[20-23]. Aspirin has no effect, however, on h-SIPA, which occurs through a dependent mechanism on platelet glycoproteins, vWF and ADP[24].

Agents that inhibit platelet aggregation have recently been described. For example, prospective randomized double-blind clinical trials have shown that either chimeric Fab fragment of a monoclonal antibody (c7E3) directed against the platelet GPIIb/IIIa receptor, or integrelin, a specific GPIIb/IIIa antagonist, reduced the ischemic complications of coronary angioplasty[25-29]. More directly, intravenous administration of c7E3 to patients undergoing coronary angioplasty has been shown to inhibit h-SIPA[30].

In addition, ticlopidine, which is widely administered to patients with unstable angina, or those who have received a coronary intervention (e.g. balloon angioplasty and/or stent placement), has been reported to inhibit h-SIPA[31]. It is reported that ticlopidine inhibits platelet aggregation because it blocks ADP receptors of platelets.

It has been established that h-SIPA is mediated by the binding of von Willebrand factor to both GPIb/IX complex and GPIIb/IIIa[32,33]. A monoclonal antibody against vWF, which recognized the domain on vWF that binds to GPIb/IX complex, has been shown to inhibit platelet adhesion and thrombus growth completely, but to have no effect on platelet adhesion and thrombus growth under low shear force[34]. In preliminary experiments, it has been found that an antibody blocks the increased platelet aggregation observed in the coronary sinus of patients with unstable angina, indicating that enhanced aggregation is caused by high shear stress (unpublished observations).

It the present study, the reason why h-SIPA was enhanced in blood from coronary sinus compared with that from coronary ostium in patients with unstable angina is unknown. However, a possible explanation is as follows : (1) plasma factors, such as vWF, might enhance platelet aggregability ; or (2) platelet factors, such as platelet membrane receptors GPIb/IX complex and/or GPIIb/IIIa, be activated in coronary circulation, causing enhancement of platelet aggregability.

The present study showed that the concentrations of plasma vWF antigen and RCof activity were not increased in the coronary sinus of patients with unstable angina, where the h-SIPA increased. It is reported that vWF binding affinity to GPIb/IX complex and GPIIb/IIIa is related to multimeric size[35]. The multimeric patterns of vWF were analyzed using samples from two patients whose h-SIPA was markedly enhanced. The patterns of vWF multimer, however, did not show any differences between samples from coronary sinus and those from coronary ostium (not shown). Since h-SIPA is mediated by the binding of vWF to platelet GPIb/IX complex and GPIIb/IIIa, this finding suggests that the increased platelet aggregability observed in blood from coronary sinus of patients with unstable angina may be due to alterations in platelet membrane glycoproteins or in activation mechanisms. So, the membranous expression levels of GPIb/IX complex and GPIIb/IIIa receptors were measured with flowcytometric analysis using samples from patients whose h-SIPA was enhanced in coronary sinus. These were no differences in binding levels of anti-activated GPIIb/IIIa receptor monoclonal antibody between samples from coronary sinus and those from coronary ostium. On the other hand, the binding levels of anti-GPIb/IX complex receptor antagonist, which recognize the binding site of vWF on GPIb/IX complex receptors and bind the site competitively, were increased in blood from coronary sinus compared with coronary sinus (unpublished observations). This finding indicates that platelets obtained from coronary sinus have lower membranous expression of GPIb/IX complex and/or have higher level of vWF bound to GPIb/IX complex than those from coronary ossium in patients with unstable angina. Recently it is reported that membranous expression of GPIb/IX complex is decreased by thrombin stimulation[36,37]. This finding may show a possible cause for the enhancement of platelet aggregability in coronary circulation.

Present results show that some relation exists between enhanced h-SIPA in coronary circulation and the pathophysiology of unstable angina. Although these results do not detect

directly the causal mechanism of the enhancement of platelet aggregability, this finding that the platelet aggregability enhance in coronary circulation indicates an association with the pathophysiology of this condition. From this point of view, these results suggest that treatment with agents preventing h-SIPA may be beneficial in patients with unstable angina.

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