Detection of von Willebrand Factor and Tissue Factor in Platelets-Fibrin Rich Coronary Thrombi in Acute Myocardial Infarction

Atsushi Yamashita, MD[a], Takahiro Sumi, MD[b], Shinya Goto, MD[c], Yasunari Hoshiba, MD[c], Kensaku Nishihira, MD[b], Riichirou Kawamoto, MD[b], Kinta Hatakeyama, MD[a], Haruhiko Date, MD[b], Takuroh Imamura, MD[b], Hisao Ogawa, MD[d], and Yujiro Asada, MD[a,*]

The rapid closure of coronary arteries due to occlusive thrombi is the major cause of acute myocardial infarction. However, the mechanisms of coronary thrombus formation have not been elucidated. We immunohistochemically assessed the localizations and their changes over time of glycoprotein IIb/IIIa, fibrin, von Willebrand factor (vWF), and tissue factor (TF), after the onset of chest pain (<4, 4 to 6, or 6 to 12 hours), in fresh coronary thrombi causing acute myocardial infarction. The occlusive thrombi were consistently composed of platelets, fibrin, vWF, and TF from the early phase of onset, and glycoprotein IIb/IIIa and fibrin were closely associated with vWF and TF, respectively. vWF and/or TF may contribute to occlusive thrombus formation and be novel therapeutic candidates for treating patients with coronary thrombosis. (Am J Cardiol 2006;97:26–28)

Many thrombotic factors are involved in acute thrombus formation. In particular, von Willebrand factor (vWF) binding to glycoprotein (GP) Ibα and GP IIb/IIIa plays an important role in initial platelet aggregation under rapid flow conditions.[1] Tissue factor (TF) expressed in disrupted atheromatous plaques potently activates the coagulation cascade, leading to thrombin generation. Thrombin is a powerful platelet agonist, in addition to fibrin formation, and may amplify thrombus growth.[2] Although vWF and TF play significant roles in thrombus formation, their localization and how they change over time have not been examined in fresh thrombi that cause acute myocardial infarction (AMI). We therefore examined platelet, vWF, TF, and fibrin localization immunohistochemically in fresh occlusive thrombi from patients with AMI. We also examined the proportion of these constituents <4, 4 to 6, and 6 to 12 hours after the onset of chest pain. Thrombi were obtained with a novel ultra-thin aspiration catheter, which allows the pathologic evaluation of occlusive thrombi in vivo without postmortem changes.[3,4]

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We examined 21 patients with AMI who had undergone thrombus removal using an aspiration catheter (Thrombuster, Kaneka, Japan). The diagnosis of AMI was based on published clinical guidelines.[5] All patients provided written informed consent to participate in the study, and the institutional ethics committees approved the study protocol. We examined thrombi obtained from 17 patients within 12 hours of chest pain onset. Thrombi that appeared organized (2 patients) were excluded from this study. Thus, 15 patients were separated into 3 groups according to the time after onset (<4, 4 to 6, and 6 to 12 hours). The risk factors for coronary artery disease consisted of hypertension (systolic pressure >140 mm Hg and/or diastolic pressure >90 mm Hg), hypercholesterolemia (total cholesterol >220 mg/dl), diabetes mellitus, smoking, and obesity (body mass index >30 kg/m^2). Aspirated thrombi were immediately fixed in 4% paraformaldehyde and stained with hematoxylin and eosin. Sections were stained immunohistochemically using antibodies against platelet GP IIb/IIIa (Affinity Biologicals, Hamilton, California), vWF (Santa Cruz Biotechnology, Santa Cruz, California), TF (Kaketsuken, Kumamoto, Japan)[6] and fibrin (CHEMICON International, Temecula, California) with the peroxidase-labeled secondary antibody (Jackson ImmunoResearch, West Grove, Pennsylvania), or were double-stained with fluorescein isothiocyanate- or Cy3-labeled secondary antibodies (Jackson ImmunoResearch). Immunofluorescent and immunohistochemical positive areas were analyzed using a spectral confocal scanning system (TCS SP2, Leica, Tokyo, Japan) and a color imaging morphometric analysis system (Mac SCOPE, Mitani, Fukui, Japan), respectively.[6] One-way analysis of variance was used to

[a]Department of Pathology and [b]First Department of Internal Medicine, University of Miyazaki Faculty of Medicine, Miyazaki; [c]Department of Medicine, Tokai University School of Medicine, Ishehara; and [d]Department of Cardiovascular Medicine, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan. Manuscript received May 22, 2005; revised manuscript received and accepted July 25, 2005.

Dr. Asada was supported by Grants-in-Aid for Scientific Research (C-2) (No. 16590284) and the 21st Century COE program (Life Science) from The Ministry of Education, Science, Sports, and Culture, Tokyo, Japan; Drs. Asada and Ogawa were supported by a Research Grant for Cardiovascular Disease (14C-4) from the Ministry of Health, Labor and Welfare, Tokyo, Japan; and Dr. Goto was supported by Tokai University School of Medicine, Project Research 2004 and 2005, Isehara, a grant from the Vehicle Racing Commemorative Foundation, and a Grant for Regulatory Science supported by the Ministry of Health, Labor and Welfare, Tokyo, Japan.

* Corresponding author: Tel: 81-985-85-0872; fax: 81-985-85-6596.
E-mail address: yasada@fc.miyazaki-u.ac.jp (Y. Asada).

0002-9149/06/$ – see front matter
doi:10.1016/j.amjcard.2005.07.105

Table 1
Clinical characteristics of study patients

	Hours After Onset		
	0–4 (n = 5)	4–6 (n = 5)	6–12 (n = 5)
Age (yrs)	65.2 ± 7.6	60.6 ± 2.3	67.4 ± 3.7
Men	5	4	5
Hypercholesterolemia*	3	3	3
Hypertension†	3	3	3
Diabetes mellitus	2	1	1
Obesity	2	3	4
Smoker	3	4	4

* Hypercholesterolemia (total cholesterol >220 mg/dl).
† Hypertension (systolic pressure >140 mm Hg and/or diastolic pressure >90 mm Hg).

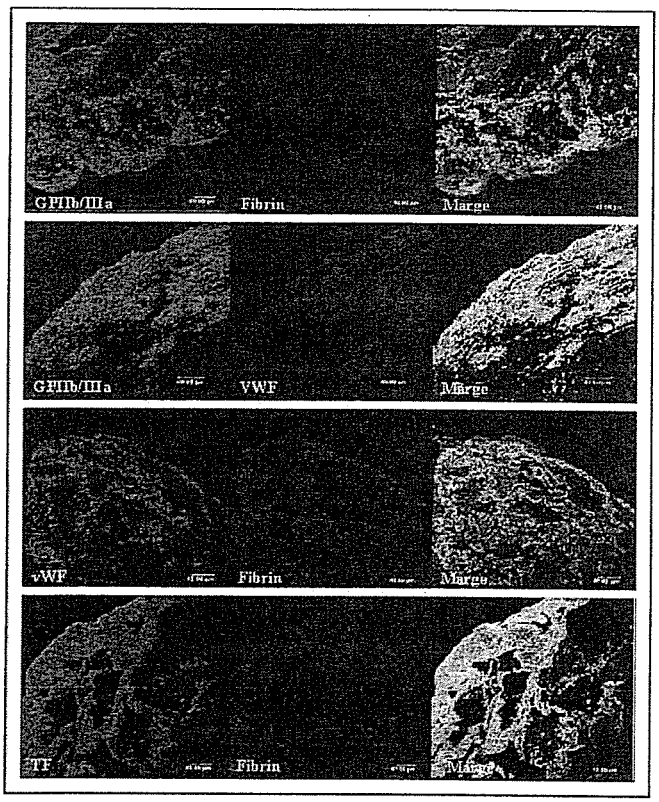

Figure 1. Representative immunofluorescent micrographs of fresh coronary thrombi from patients with AMI. *Left*, staining with fluorescein isothiocyanate-labeled GP IIb/IIIa, vWF, and TF *(green). Center*, staining with Cy3-labeled with fibrin and vWF *(red). Right*, merged immunofluorescent images. Co-localized areas of each factor are stained *yellow.*

compare values among the groups. The data are expressed as means ± SE. A p value <0.05 was considered statistically significant.

Table 1 shows the clinical characteristics of the patients. Risk factors for coronary artery disease among the 3 groups did not differ significantly. All coronary thrombi specimens were composed mostly of aggregated platelets and densely packed fibrin (Figure 1), along with inflammatory-related polymorphonuclear and mononuclear cells (data not shown). Immunohistochemical staining revealed the constitutive presence

Table 2
Immunopositive area for antibodies in thrombi

	Hours After Onset		
	0–4 (n = 5)	4–6 (n = 5)	6–12 (n = 5)
GP IIb/IIIa (%)	24.1 ± 3.1	30.7 ± 5.4	34.9 ± 8.0
Fibrin (%)	28.4 ± 4.3	29.3 ± 7.0	30.5 ± 5.9
vWF (%)	28.3 ± 4.2	30.5 ± 8.9	34.3 ± 6.2
TF (%)	19.9 ± 4.6	26.2 ± 4.8	18.9 ± 3.5

of GP IIb/IIIa, fibrin, vWF, and TF in all the thrombi. The immunopositive area (percentage) of GP IIb/IIIa and vWF tended to increase in older thrombi, but did not significantly differ with time after onset (Table 2). Immunofluorescent staining showed that GP IIb/IIIa intermingled with fibrin, and that the surface of the thrombi was mainly covered with GP IIb/IIIa and vWF. GP IIb/IIIa co-localized with vWF, TF was closely associated with fibrin, and vWF somewhat co-localized with fibrin (Figure 1).

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The occlusive thrombi causing AMI were rich in fibrin, as well as platelets, from the early phase of onset. In contrast to our findings, Nagata et al[7] recently reported a higher platelet component of many aspirated thrombi using thrombectomy catheters during the early phase of AMI. This discrepancy may have been because they stained samples with hematoxylin and eosin or azan, and we used immunohistochemical means with a specific antibody to detect fibrin. Therefore, the results of the present study seem to be more objective and specific. We found that the proportion of platelets and fibrin in thrombi did not significantly differ within 12 hours after onset. This suggests that the coagulation system, as well as platelets, plays a crucial role in thrombus formation in AMI. In contrast, thrombin generation on activated platelets involves complex interactions between platelets and coagulation proteins. Activated platelets provide a procoagulant lipid surface to assemble coagulation factors and promote thrombin generation, resulting in additional platelet activation and fibrin formation. Immunofluorescent staining showed that GP IIb/IIIa and vWF intermingled with fibrin, which closely co-localized TF. The findings suggest that accumulation of these molecules on activated platelets amplifies thrombus growth. Because fibrin is essential for stabilizing initial and loosely packed platelets aggregates during high blood flow, our results support the relevance of fibrinolysis therapy for AMI.[8]

Many in vitro studies have revealed that vWF plays a pivotal role during the initial step of platelet adhesion and aggregation under rapid flow conditions.[9,10] We found that vWF was closely localized with platelets and partly with fibrin, which suggests that vWF plays an important role in platelet–platelet[9,10] and platelet–fibrin interactions[11] during thrombus growth in AMI. Because vWF is present in plasma and in platelet α-granules, a positive immunoreaction for vWF in thrombi does not necessarily reflect functional vWF. How-

ever, plasma vWF levels have been correlated positively with the incidence of coronary thrombosis.[12,13] In addition, fibrin-dependent platelet procoagulant activity requires vWF and its GP Ib receptor,[14] and our animal experimental study showed that a monoclonal antibody against vWF inhibits occlusive thrombus formation in arteries.[15] Such evidence suggests that vWF in thrombi actually contributes to coronary occlusive thrombus formation.

Occlusive thrombi were also consistently rich in TF, which was closely localized with fibrin. Himber et al[16] found that active TF in human thrombi is associated with fibrin, leukocytes, and platelets. Our results support this observation. Whether intravascular TF originates from leukocytes, platelets, or the vascular wall remains controversial. Chou et al[17] reported that hematopoietic cell-derived microparticle TF contributes to thrombus propagation. Conversely, Day et al[18] demonstrated that vascular TF is more important for thrombus formation than TF derived from blood cells. We showed that fibrin-rich thrombus formation in arteries depends largely on increased vascular wall thrombogenicity.[15] Although our studies did not address this issue, the obvious co-localization of TF and fibrin suggests that intravascular TF would be active and thus play an important role in activating the intravascular coagulation cascade.

One limitation of this study was a possible discrepancy between thrombus age and the time after the onset of clinical symptoms.[3,4] Although we could not define the exact onset of thrombus formation, we excluded organized thrombi to minimize this limitation. The possibility that the spatial distribution of the constituents of thrombi is broken by catheter aspiration could not be excluded. Also, the study cohort was small, so the inherent bias in patient selection could not be evaluated.

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