ORIGINAL ARTICLES

Evaluation of platelet function under high shear condition in the small-sized collagen bead column

MAKOTO KANEKO, TOSHIRO TAKAFUTA, OLGA CUYUN-LIRA, KANEO SATOH, MORIO ARAI, YUTAKA YATOMI, and YUKIO OZAKI

YAMANASHI, KOBE, and TOKYO, JAPAN

We previously reported that platelet retention rates as measured with collagen-coated bead columns (the conventional column) reflect the processes of platelet adhesion and aggregation under low shear stress, and that this system could serve as an easy-to-use platelet aggregometry. With this column, platelet glycoprotein (GP) VI and GPIIb/IIIa, but not the GPIb–von Willebrand factor (VWF) interaction, play major roles in platelet activation. To develop a system that can better reflect the GPIb-VWF interaction under high shear stress, we designed a column containing small-sized beads (125–212 μm) coated with porcine collagen type I. As expected, the GPIb-VWF interaction played a crucial role in platelet retention rates at higher flow rates. Adenosine 5′-diphosphate, but not thromboxane A_2, appears to support platelet activation in this system. The platelet retention rates among healthy individuals with the new columns are in the range wider than the conventional columns, and this diversity could be attributed to the broad range of the VWF antigen and/or its activity. It is suggested that this new column can serve as an easy-to-use method for evaluating the VWF antigen levels and its activity and for monitoring patients with thrombotic or bleeding disorders related to the VWF-GPIb interaction. (J Lab Clin Med 2005;146:64–75)

Abbreviations: A3P5P = adenosine 3′ 5′-diphosphate; ADP = adenosine 5′-diphosphate; AR-C69931MX = *N*6-(2-methylthioethyl)-2-(33,3-trifluoropropylthio)-β,γ-dichloromethylene adenosine triphosphate; ASA = acetylsalicylic acid; CP = creatine phosphate; CPK = creatine phosphokinase; EDTA = ethylenediamine tetraacetic acid; GP = glycoprotein; MoAb = monoclonal antibody; PPP = platelet-poor plasma; PRP = platelet-rich plasma; TTP = thrombotic thrombocytopenic purpura; TXA_2 = thromboxane A_2; VWD = von Willebrand disease; VWF = von Willebrand factor; VWF:Ag = VWF:Antigen; VWF:RCo = VWF:Ristocetin Cofactor activity

Blood flow through arteries often results in high shear rates. Under such hemodynamic conditions, platelets initially adhere to the subendothelium at sites of vascular injury through the platelet membrane receptor glycoprotein Ib (GPIb)-von Willebrand factor (VWF) interaction. This reversible interaction leads to the conformational activation of integrin αIIbβ3 (GPIIb/IIIa) followed by platelet activation, which finally culminates in aggregate formation. Thus, the interaction between VWF and platelets is an impor-

From the Department of Clinical and Laboratory Medicine, Faculty of Medicine, University of Yamanashi, Yamanashi, Japan; Department of Hematology and Clinical Immunology, Nishi-Kobe Medical Center, Kobe, Japan; Department of Laboratory Medicine, Tokyo Medical University, Tokyo, Japan; and Department of Clinical Laboratory Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

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Reprint requests: Yukio Ozaki MD, PhD, Department of Clinical and Laboratory Medicine, Yamanashi Medical University, 1110 Shimokato, Tamaho, Nakakoma, Yamanashi 409-3898, Japan; e-mail: yozaki@yamanashi.ac.jp

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tant step in primary hemostasis, and moreover, it can also contribute to the generation of pathologic thrombi at sites of atherosclerotic plaque rupture under high shear stress.

The importance of VWF-platelet interaction in thrombus formation implies that the level of plasma VWF or VWF activity enhances platelet aggregate formation. A number of epidemiologic studies have shown a positive correlation between plasma VWF levels and the incidence of acute coronary syndromes.[1–5] High levels of VWF are also related with cerebral vascular accidents.[6–8] The pathogenesis of thrombotic thrombocytopenic purpura (TTP), which remained obscure until recently, is now suggested to be a dysfunction of ADAMTS13,[9,10] a VWF cleaving metalloprotease with resultant increase in large VWF multimers. It is suggested that excessive adhesive activity of large VWF multimers results in platelet aggregate formation and thrombus formation in this disorder. The detection of large VWF multimer or enhanced platelet-VWF interaction at the early stage will provide useful information for the prevention of overt and/or full-blown symptoms. Thus, for the purpose of diagnosing TTP at the early stage or for monitoring patients with thrombotic disorders, it is important to develop appropriate methods for evaluating platelet functions related to VWF under various shear conditions.

Among many methods for the evaluation of platelet functions, platelet adhesion and aggregation tests have been most frequently used.[11,12] They have met with limited approval in clinical settings, because they have several shortcomings including relative insensitivity to detect weak platelet activation, lack of reproducibility or clinical relevance, and tedious procedures for measurements. To overcome these hurdles, a number of platelet function tests with improved technology and novel approaches to analysis have been developed recently. Some can only measure platelet function in the absence of red blood cells or white blood cells, which may also contribute to thrombus formation in vivo. Others only measure platelet activation under shear stress of a low degree. More recently, new instruments have been developed that can evaluate platelet function using whole-blood samples under high shear stress.[13–18] However, specialized and expensive instruments are required for these methods, and thus these methods may not be suitable for screening tests in clinical settings in ordinary facilities.

The glass bead column system was developed by Salzman, Hellem, and others, and has been modified to assess platelet adherent function.[19–21] However, because the interaction between platelets and the glass surface may not accurately represent physiologic platelet function in vivo and not only platelet adhesion but also platelet aggregation occurs in the glass bead column, this method has not seen wide clinical application. Recently, copolymer plastic sphere beads coated with porcine type I collagen have been developed to replace glass beads, and this collagen-coated bead column (conventional column) is currently used for the measurement of platelet retention.[22] We investigated the factors responsible for platelet retention and characterized this as an easy-to-use platelet function test. Our findings in conventional columns suggest that GPVI and GPIIb/IIIa are mainly involved in platelet retention, and that the results mainly reflect platelet aggregate formation at relatively lower shear rates. We have suggested that this can be a simple and reliable method for evaluating platelet aggregation using whole blood as samples.

To develop a new aggregometer that can evaluate platelet activation related to VWF under high shear stress, we designed a new column that contains small-sized collagen beads. In the present study, we investigated the factors responsible for platelet retention in a small-sized collagen bead column. Our findings suggest that the level of platelet retention in this new type of column reflects the GPIb-VWF interaction and that it positively correlates with the plasma VWF activity. We suggest that this column, using whole blood as samples, can be a simple and reliable method for the evaluation of platelet function under high shear conditions.

METHODS

Materials. AJvW-2,[2] an anti-VWF monoclonal antibody (MoAb), was a gift from Pharmaceutical Research Laboratories (Ajinomoto Co, Kawasaki, Japan). Dr. Jan J. Sixma (Thrombosis and Hemostasis Laboratory, Department of Hematology, University Medical Centre Utrecht, Netherlands) provided us with RU5,[23] an anti-VWF MoAb that blocks the interaction between VWF and collagen. Confact F, a human factor VIII/VWF concentrate, was provided by Kaketsuken (Kumamoto, Japan). The following materials were obtained from the indicated suppliers: abciximab (ReoPro™) (Eli Lilly & Co. Ltd., Indianapolis, Ind); Asserachrom VWF (commercial kit for assay of factor VIII/VWF antigen) (Roche, Tokyo, Japan); peroxidase-conjugated rabbit polyclonal anti-human VWF Ab (Dako Cytomation Co. Ltd., Glostrup, Denmark); ristocetin (Sigma, St. Louis, Mo); and SeaKem HGT (P) Agarose (Takara Bio Inc., Otsu, Japan).

Blood collection. This study was carried out according to the principles of the Declaration of Helsinki, and informed consent was obtained from all blood donors. Furthermore, the study was approved by the review board of the University of Yamanashi. Healthy drug-free volunteers and a patient with type 3 von Willebrand disease (VWD) served as donors, and blood withdrawn by venipuncture was mixed with 3.13% citrate (9:1, v/v) to make whole-blood samples as described previously.[22]

Platelet retention tests. Small-sized collagen bead column (PLA BEADS COLUMN-PAG, diameter 0.1–0.2 mm) and conventional column (PLA BEADS COLUMN, diameter