Thrombosis Research 123 (2009) 856–861

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Measurement of the platelet retention rate in a column of collagen-coated beads is useful for the assessment of efficacy of antiplatelet therapy

Akira Mende [a], Jyun-ei Obata [a], Keita Sano [a], Mitsumasa Hirano [a], Yoshinobu Kitta [a], Yasushi Kodama [a], Takamitsu Nakamura [a], Ken-ichi Kawabata [a], Yukio Saitoh [a], Daisuke Fujioka [a], Tsuyoshi Kobayashi [a], Kaneo Satoh [b], Yukio Ozaki [b], Toshiaki Yano [a], Kiyotaka Kugiyama [a,*]

[a] Department of Internal Medicine II, University of Yamanashi, Chuo city, Yamanashi prefecture, Japan
[b] Department of Clinical and Laboratory Medicine, University of Yamanashi, Chuo city, Yamanashi prefecture, Japan

ARTICLE INFO

Article history:
Received 4 February 2008
Received in revised form 3 July 2008
Accepted 15 September 2008
Available online 12 November 2008

Keywords:
Platelet function
Antiplatelet drugs
Coronary artery disease

ABSTRACT

Introduction: A simple, validated method to measure platelet function is unavailable for bedside use. Measurement of platelet retention rate using a column of collagen-coated beads and whole blood is a new, simple assay that reflects platelet aggregation. This study was aimed to examine the utility of this assay to assess efficacy of antiplatelet drug therapy.

Methods: Citrated whole blood (1.5 ml) in a syringe was passed through a polyvinyl tube packed with collagen-coated beads for 40 seconds using a syringe pump. Platelet retention rate in the column was calculated from platelet counts in blood before and after passage. An increase in the retention rate reflects an increase in platelet activity. This new platelet retention assay and the traditional optical aggregometry assay were performed in 331 patients with stable coronary artery disease (CAD).

Results: The retention rate was significantly reduced in patients taking dual antiplatelet therapy (aspirin plus clopidogrel or ticlopidine) compared with aspirin alone. There was a significant linear correlation between the platelet retention rate and platelet aggregability measured by the traditional method ($r = 0.44$, $p < 0.001$). In multivariate Cox proportional hazards analysis, higher platelet retention rate was an independent predictor of future cardiovascular events in patients on dual antiplatelet therapy (hazard ratio 3.9, 95% CI 1.6 to 9.5, $p = 0.003$).

Conclusions: Measurement of the platelet retention rate in a column of collagen-coated beads may be useful for monitoring the efficacy of antiplatelet drug therapy in patients with CAD.

Introduction

Platelet-thrombus formation is the first critical step in the pathogenesis of athero-thrombotic occlusive disease [1,2]. Antiplatelet drugs have been proven effective in preventing ischemic events in patients with athero-thrombotic cardiovascular disease [3–5]. It has been shown that there is a considerable variation in response to antiplatelet treatment and that "resistance" to antiplatelet treatment leads to adverse outcomes in patients with coronary artery disease (CAD) [6,7]. Therefore, it is important to monitor the efficacy of antiplatelet treatment in patients with CAD. However, a simple, practical method to measure platelet function is unavailable for bedside use. Light-transmission aggregometry using platelet rich plasma (PRP) with agonists added is the standard method to measure platelet aggregation, and this process is mediated by glycoprotein (GP) IIb/IIIa. However, light transmission aggregometry requires expensive, specialized equipment that cannot be easily used at the bedside and advanced technical skills to ensure accurate results [8–10]. Currently, several point-of-care assays of platelet function, including platelet function analyzer (PFA)-100, have been developed [8–11].

It is known that red and white blood cells affect platelet function in the circulation, and that the shear stress produced in a stenotic artery causes platelet activation leading to thrombus formation [12–14]. Moreover, at the injured artery where high shear stress is generated, an interaction between plasma von Willebrand factor (vWF) and subendothelial collagen with subsequent binding of platelet GPIb to vWF also plays an essential role in platelet thrombi formation [14]. Thus, a system that can measure platelet activation in vitro induced by high shear stress in whole blood exposed to collagen may be ideal for evaluating platelet activation in a pathophysiologic milieu. Recently, Ozaki and his group [10,15] developed a new, simple assay of platelet function using a column of collagen-coated beads and whole blood. In

Abbreviations: CAD, coronary artery disease; PRP, platelet rich plasma; GP, glycoprotein; PFA-100, platelet function analyzer-100; vWF, von Willebrand factor; PPP, platelet-poor plasma; ADP, adenosine diphosphate; ROC, receiver-operating characteristic.

* Corresponding author. Department of Internal Medicine II, University of Yamanashi, Faculty of medicine, 1110 Shimokato, Chuo City, 409-3898 Japan. Tel.: +81 55 273 9590; fax: +81 55 273 6749.
E-mail address: kugiyama@yamanashi.ac.jp (K. Kugiyama).

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doi:10.1016/j.thromres.2008.09.010

this assay system, the small fluid-flow space in a column packed with small collagen-coated beads produces shear stress high enough to activate platelets via GPIb-vWF interaction [10,15]. Thus, this system may mimic platelet aggregation leading to thrombus formation in a stenotic artery in vivo. The platelet retention rate in the column principally represents platelet aggregation and adhesion at the injured artery and serves as a new form of platelet aggregometry with high reproducibility [10,15]. Thus, this study examined whether platelet retention rate differs significantly in stable CAD patients with dual antiplatelet therapy compared with those treated with aspirin alone. And, this study evaluated the agreement between platelet retention rate and platelet activity measured with other tests and the prognostic significance of platelet retention rate in stable CAD patients.

Methods

Study patients

Three hundred thirty-one patients with angiographically-proven stable CAD admitted to Yamanashi University Hospital were enrolled in this study from September 2003 to June 2008. Among them, 257 patients were on antiplatelet therapy with aspirin (100 mg/day) alone or in combination with ticlopidine (200 mg/day) or clopidogrel (75 mg/day) for >1 month prior to the time of enrollment. In the remaining 74 patients, there was no antiplatelet therapy for >1 month prior to enrollment. The platelet retention rate in these 74 patients with CAD was measured before and at a steady state phase (>1 month) of antiplatelet treatment with aspirin alone or in combination with ticlopidine or clopidogrel at the same doses as described above. This study also included a consecutive series of 59 control subjects without obvious heart disease who underwent diagnostic coronary angiography for atypical chest pain at Yamanashi University Hospital during the same time period as the patients with CAD. These control subjects also fulfilled all of the following inclusion criteria: 1) normal coronary angiography and left ventriculogram and no coronary artery spasm; 2) no significant S-T segment changes during the chest pain on 12-lead ECG and ambulatory ECG; 3) neither chest pain nor S-T segment changes during the treadmill test; 4) taking no antiplatelet drug. The platelet retention rate in these control subjects was compared to the retention rate in the absence of antiplatelet drugs in the 74 CAD patients taking no antiplatelet drug at the enrollment. None of the study patients and the control subjects had any of the following exclusion criteria: (1) personal or family history of bleeding or thrombotic disorder, platelet counts <100,000 or >450,000/μL, hemoglobin <9 g/dL, or hematocrit <35%, (2) antiplatelet drug use other than aspirin, ticlopidine, or clopidogrel and the use of nonsteroidal antiinflammatory drugs and GP IIb/IIIa inhibitors within 2 weeks prior to enrollment, (3) acute coronary syndrome, stroke, cardiogenic shock, pulmonary edema, major surgery, trauma, or serious infectious disease within 4 weeks prior to enrollment, (4) neoplasm, significant hepatic or inflammatory disease, (5) serum creatinine levels >3.0 mg/dL, or (6) untreated endocrine disorder. The clinical characteristics of the study patients and subjects are shown in Table 1. Written informed consent was obtained from all patients and subjects prior to enrollment. The study was approved by the ethics committee at our institution.

Blood sampling

Blood was obtained from all patients and subjects at 7 a.m. in the fasting state. In the 74 patients with CAD not taking antiplatelet drugs at enrollment, blood sampling was performed twice before and at least 1 month after starting antiplatelet treatment. Blood samples were drawn from the antecubital vein under minimal tourniquet pressure using a sterile 21-gauge needle syringe. The initial 3 mL of blood was used for clinical chemistry analysis. Then, 2.7 or 7.0 mL of blood was collected into a vacutainer tube containing 3.8% sodium citrate for measuring platelet function, and 1.5 mL of additional blood was collected in another tube containing EDTA for the assay of plasma vWF levels. The citrated blood was used for the platelet retention test (2.7 mL) and PFA-100 (2.0 mL). When light-transmission aggregometry was performed, part of the citrated sample was centrifuged to obtain PRP and platelet-poor plasma (PPP) [10]. The EDTA samples for the vWF assays were centrifuged immediately (4 °C, 3000 rpm, 15 min) and stored at −80 °C until analyzed.

Table 1
Comparisons of baseline characteristics of study patients.

Antiplatelet therapy	With CAD			Controls
	Dual drugs (n=262)	Aspirin alone (n=69)	No drug (n=74)	No drug (n=59)
Age (yrs)	66±11	66±10	63±13	64±16
Male (%)	53	59	60	51
Current Smoking (%)	20	22	22	21
Past Smoking (%)	38*	36*	31*	16
Hypertension (%)	59*	45	57*	32
Previous MI (%)	35*†	42*†	0	0
Previous PCI (%)	100*†#	77*†	0	0
Multivessel disease (%)	55*	63*	43*	0
HDL-C (mg/dl)	43±13*	43±14*	47±13*	55±14
LDL-C (mg/dl)	144±35*	136±24*	136±26*	115±28
Hb A1c (%)	6.1±1.5*	5.8±1.1*	5.8±0.8*	5.2±0.6
BMI (kg/m^2)	23.8±3.3	23.3±2.2	24.8±2.1	23.2±3.8
CRP (mg/dL)	0.21±0.3*	0.22±0.2*	0.23±0.2*	0.09±0.1
BNP (pg/mL)	42.2±55*	48.5±56*	39.8±15*	13.3±8
vWF Ag (U/L)	243±44†#	272±30*	252±27*	226±33
Medication use (%)				
Aspirin	100	100	0	0
Ticlopidine	79	0	0	0
Clopidogrel	21	0	0	0
Warfarin	22*	10*	12*	0
ACE-I	19*	22*	16	6
ARB	50*	42*	40*	19
β-blocker	19*	14	15	9
Ca-blocker	53*	55*	46	32
Statin	44*	45*	48*	24

Data are expressed as the mean ± SD or the percentage of patients. *p<0.05 vs. control subjects, †p<0.05 vs. CAD with no antiplatelet drug, #p<0.05 vs. CAD with aspirin alone. Dual drugs, aspirin plus ticolpidine or clipidogrel; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; BMI, body mass index; CRP, C-reactive protein; BNP, brain natriuretic peptide; vWF Ag, von Willebrand factor antigen; ACE-I, angiotensin- converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Measurement of platelet retention rate in a column of collagen-coated beads

The platelet retention rate was measured in all study patients according to the methods described by Ozaki et al [10,15]. Briefly, co-polymer plastic beads (0.125 – 0.212 mm of diameter) coated with porcine type I collagen were packed into a polyvinyl tubing with an internal diameter of 2 mm and a length of 80 mm. This Pla-Bead® column was disposable and commercially available (ISK Co., Ltd., Tokyo, Japan). The citrated whole blood samples were mixed by twirling and drawn into a plastic syringe (2.5 mL) (Terumo, Tokyo, Japan). A collagen-bead column was connected to the syringe and was placed in the holder of an injection pump (Fig. 1). The blood samples in the syringes were passed through small-sized collagen-bead columns at a fixed flow rate of 2.25 mL/min. The samples before and after passage through the columns were collected into plastic tubes, and platelet counts were measured by an automated hematology analyzer, NE-8000 (Sysmex, Kobe, Japan). Platelet retention rates (%) were calculated as: 100×[(platelet count before passage)−(platelet count after passage)]/(platelet count before passage). An increase in the retention rate reflects an increase in the platelet activity. The entire procedure required <10 min. The intra-assay and inter-assay coefficients of variation for platelet retention rates were within 5.8% and 6.9%, respectively.