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Coronary blood flow volume change is negatively associated with platelet aggregability in patients with non-obstructive ischemic heart disease who have no anti-platelet agents

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ABSTRACT

Background: Thrombus formation is one of the main pathogeneses of acute coronary syndrome with atherosclerotic rupture. Previous studies have reported that atherosclerosis increases platelet aggregability and that vascular endothelial dysfunction reflects early change of atherosclerosis. However, the relationship between coronary endothelial dysfunction and platelet reactivity remains unclear. Therefore, in this study, we investigated the relationship between them in non-obstructive ischemic heart disease (IHD) patients.

Methods: Three hundred sixty-eight consecutive stable patients with suspected angina presenting non-obstructive coronary arteries (<50% diameter) in coronary angiography were investigated with the intracoronary acetylcholine provocation test and measured adenosine triphosphate-induced coronary flow reserve. Finally, 25 non-obstructive IHD patients who had no anti-platelet agents were assessed for the relationship between coronary blood flow volume (CBFV) change and platelet aggregability as P2Y12 reaction unit (PRU) by VerifyNow P2Y12 assay system.

Results: CBFV change by intracoronary 20 μg/kg per minute acetylcholine provocation showed a significant negative correlation with platelet aggregability as PRU ($r = 0.44$, $P = 0.03$). Conversely, there was no significant correlation between PRU and endothelial function as coronary flow reserve. Furthermore, multivariable linear regression analysis indicated that an incremental CBFV change was independently associated with PRU ($\beta = 0.63$, $P < 0.001$) in non-obstructive IHD patients.

Conclusions: In patients with non-obstructive IHD, CBFV change was significantly associated with platelet aggregability, indicating that coronary endothelial dysfunction might mediate higher platelet aggregability.

1. Introduction

Platelets interacting with circulating cells and coagulation factors in different vascular beds modify several complex pathologies including atherosclerosis, leading to vicious cycles of atherothrombosis [1]. Platelet aggregability can be evaluated as P2Y12 reaction unit (PRU) using VerifyNow P2Y12 assay system. VerifyNow P2Y12 assay has made it possible to clinically quantify residual platelet reactivity. VerifyNow is a potentially useful predictor of periprocedural bleeding events [2].

Progression of atherosclerosis and thrombus formation are well known as crucial pathogeneses of various cardiovascular diseases [3]. Early detection and management of atherosclerosis can prevent various cardiovascular events. Detecting endothelial dysfunction is attracting attention as an early marker for atherosclerosis and a prognostic factor of cardiovascular diseases [4].

Reactive hyperemia-peripheral arterial tonometry, which is used to measure the digital hyperemic response, is a noninvasive, automatic, and less operator-dependent test that is clinically used to evaluate endothelial function [5,6]. We previously reported that the reactive hyperemia-peripheral arterial tonometry index was useful for identifying high risk of ischemic heart disease and cardiovascular events [7–10].

Acute coronary syndrome results from coronary atherosclerosis, generally with superimposed coronary thrombosis caused by rupture or erosion of an atherosclerotic lesion [11,12]. Plaque disruption exposes substances that promote platelet activation and aggregation, thrombin generation, and ultimately thrombosis formation [13,14]. Plaque erosion and rupture can accelerate platelet activity.

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The vascular endothelial function also regulates platelet function [15,16], and platelet aggregation is closely associated with the integrity and condition of the vascular endothelium in vivo [17]. However, the evidence for the relationship between coronary endothelial dysfunction and platelet reactivity is limited. We previously reported that the relationship between peripheral endothelial dysfunction and platelet aggregability in patients with stable coronary artery disease who underwent dual antiplatelet therapy with aspirin and clopidogrel [18]. Clopidogrel is a prodrug activated in the liver by cytochrome P450 (CYP) enzymes [19], and it has been demonstrated that patients with variants in CYP2C19 have lower levels of the active metabolite of clopidogrel and less inhibition of platelet aggregation [20,21].

Although our previous study tried to exclude the effects of variants of CYP2C19 and clinical factors, that study was not enough to exclude the effect of metabolic activity of aspirin and clopidogrel due to various factors associated with the metabolism of clopidogrel. Hence, we conducted this study to investigate the association between platelet aggregability and endothelial function in patients without obstructive coronary artery disease who have no anti-platelet agents. The aim of this study was to investigate the relationship between endothelium-dependent coronary blood flow volume (CBFV) change and platelet aggregability in these patients.

2. Methods

2.1. Study design

This study was a single center, retrospective study and was conducted to clinically evaluate the relationship between endothelial function and platelet aggregability.

2.2. Study population

The study screened three hundred sixty-eight consecutive stable patients with suspected angina who were admitted to Kumamoto University Hospital between January 2002 and April 2011. We performed coronary angiography (CAG), intracoronary acetylcholine-provocation test and measured coronary flow reserve (CFR) to diagnose angina, and excluded patients with ischemic heart disease (IHD) and spastic angina. We also excluded patients who were taking anti-platelet agents. We finally enrolled 25 patients without obstructive or spastic angina and assessed the relationship between CBFV and platelet aggregability (Fig. I in the online-only Data Supplement).

2.3. Ethics statement

All procedures were conducted in accordance with the Declaration of Helsinki and its amendments. The study protocol was approved by the institutional review board of Kumamoto University (approval number, Senshin 2313). This study is registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN000031390). Opt-out materials are available through the website: http://www.kumadai-junnai.com/home/wp-content/uploads/houkatsu.pdf.

2.4. CBFV measurement

CBFV was measured during the CAG procedure. The detailed methods of CBFV measurement were described previously [22]. In brief, we placed doppler flow wire at the left anterior descending coronary artery and measured average peak velocity (APV). APV was measured 1 min after intra-coronary injection of Acetylcholine (Ach) 20 μg/kg per minute. Coronary artery diameter at the tip of doppler wire was measured by quantitative CAG. Myocardial ischemia was evaluated by blood lactic acid of aorta and coronary sinus. CFR was measured in a reaction coronary extension by loaded ATP. CBFV was calculated by following the formula CBFV (ml/min) = 0.236 × Diameter (mm)^2 × APV (cm/s) [22].

2.5. CFR measurement

Adenosine triphosphate-induced CFR (ATP-CFR) is a non-endothelium-dependent coronary reactivity test [23].

ATP (150 μg/kg per minute) was administered via the central vein until maximal hyperemia was achieved for the calculation of CFR [24]. CFR was calculated with the following formula: Hyperemia APV/Post-Isosorbide dinitrate (ISDN) APV [25].

2.6. P2Y12 reaction unit (PRU) measurement

Early morning fasting blood samples were collected for baseline laboratory tests from the antecubital vein using standard phlebotomy techniques. Platelet function was assessed before elective CAG. Blood samples for platelet function were collected using the double-syringe technique, in which the first 2 to 4 ml of blood is discarded to avoid spontaneous platelet activation. Platelet aggregability was evaluated as PRU by VerifyNow P2Y12 assay system. VerifyNow P2Y12 assay has made it possible to clinically quantify residual platelet reactivity at bed side. This test measures adenosine diphosphate-induced platelet agglutination as an increase in light transmittance and uses a proprietary algorithm to report values in PRU. To measure PRU, insert the measurement tool and withdraw a blood sample.

2.7. Definition of risk factors for cardiovascular disease

Risk factors for cardiovascular disease were defined as the following; hypertension (>140/90 mm Hg or taking antihypertensive medication), dyslipidemia (high-density lipoprotein cholesterol <40 mg/dl, low-density lipoprotein cholesterol ≥140 mg/dl, or triglycerides >150 mg/dl or taking medication for dyslipidemia defined as guidelines of the Japanese Society of Atherosclerosis.), and diabetes mellitus (symptoms of diabetes plus casual plasma glucose concentration ≥ 200 mg/dl, fasting plasma glucose concentration ≥ 126 mg/dl, 2 h plasma glucose concentration ≥ 200 mg/dl during 75-g oral glucose tolerance test, or taking medication for diabetes mellitus), and current smoking (smoking within 1 year). Estimated glomerular filtration rate (eGFR) was calculated by following formula; Male; eGFR(ml/min/1.73 m^2) = 194 × Cr$^{-1.094}$ × age$^{-0.287}$, Female; eGFR(ml/min/1.73 m^2) = 194 × Cr$^{-1.094}$ × age$^{-0.287}$ × 0.739.

2.8. Laboratory data

We measured various laboratory tests, such as high-sensitivity C-reactive protein and B-type natriuretic peptide (BNP) in stable patients with non-obstructive and spastic angina before CAG. Blood samples were collected for baseline laboratory tests from the vein in the early morning.

2.9. Statistical analysis

The Shapiro-Wilk test was used to assess the normal distribution of continuous data. Data are expressed as mean ± standard deviation, whereas those with skewed distributions were expressed as the median value with interquartile range. Categorical data were presented as frequencies and percentages. Differences in continuous variables were analyzed by the unpaired *t*-test or Mann-Whitney *U* test as appropriate. Pearson's correlation coefficient was used to evaluate the association between endothelium function and the parameters of platelet aggregation. Spearman's rank correlation coefficient was used if variables were not normally distributed. Significant clinical parameters associated with PRU in univariate analysis and several factors reported previously to affect platelet reactivity were evaluated by multivariable linear regression analysis with principal component analysis to simplify the complexity in various clinical factors. A P value of <0.05 denoted statistical significance. Statistical analyses were performed using The Statistical Package for the Social Sciences version 20 (SPSS Inc., Tokyo, Japan).

3. Results

3.1. Baseline characteristics

Table 1 lists the baseline characteristics of the enrolled patients. The average age was 64 years and 36% of the population were male. Four patients (24%) had diabetes, 11 (44%) hypertension, and 11 (44%) dyslipidemia. The use of angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, statins, and hypoglycemic agents in these patients was 24%, 24%, and 4%, respectively. The rate of calcium channel blockers and nitrates use were 17 (68%) and 3 (12%), respectively. However, we discontinued calcium blockers or nitrates at least 5 times or more of $T_{1/2}$ of these drugs before intracoronary acetylcholine-provocation test. The rates of calcium blockers or nitrates use did not affect the results.

3.2. Correlation of PRU and CBFV and CFR

PRU was significantly negatively correlated to sex (r = −0.48, P = 0.014), smoking (r = −0.58, P = 0.002), statin use (r = −0.53, P = 0.006), and hemoglobin (r = −0.42, P = 0.035). It was positively correlated to LDL-C (r = 0.46 P = 0.018). However, there was no correlation between PRU and platelet count, age, and hypoglycemic agent (Table 2). CBFV change showed the significant negative correlation to PRU (r = 0.44, P = 0.028, Fig. 1). Conversely, there was no significant correlation between CFR and PRU (Fig. II in the online-only Data Supplement). Multivariable linear regression analysis with principal component analysis indicated that an incremental CBFV change was independently associated with PRU (β = −0.43, P = 0.038; Table 3).

Table 1
Baseline characteristics.

	Total n = 25
Age (yrs)	64.5 ± 9.0
Man, n (%)	9 (36)
Body mass index (kg/m^2)	23.7 ± 3.0
Diabetes, n (%)	5 (24)
Hypertension, n (%)	11 (44)
Dyslipidemia, n (%)	11 (44)
Smoking, n (%)	10 (40)
Hemoglobin (g/dl)	13.6 ± 1.6
Platelet count (×10^4/mm^3)	21.8 ± 4.2
eGFR (ml/min/1.73 m^2)	74.4 ± 17.5
Hemoglobin A1c (%)	5.8 [5.6–5.9]
Total cholesterol (mg/dl)	189 [168–212]
HDL cholesterol (mg/dl)	54 [51–67]
Triglycerides (mg/dl)	128 [88–149]
High sensitivity CRP (mg/dl)	0.5 [0.3–0.8]
BNP (pg/ml)	18.4 [8.5–31.8]
LVEF (%)	66.5 [61.3–70.0]
β blockers, n (%)	1 (4)
ACE inhibitors or ARBs, n (%)	6 (24)
Calcium channel blockers, n (%)	17 (68)
Nitrates, n (%)	3 (12)
Statins, n (%)	5 (24)
Hypoglycemic agents, n (%)	1 (4)
CBFV ratio	2.1 ± 0.8
CFR	3.1 ± 1.1
PRU	367.5 ± 58.7

Data are presented as mean ± SD or median (IQR).
Abbreviations are following: eGFR, estimated glomerular filtration rate, CRP, C-reactive protein, BNP, B-type natriuretic peptide, LVEF, left ventricular ejection fraction, ACE, angiotensin converting enzyme; and ARB, angiotensin II receptor blockers.

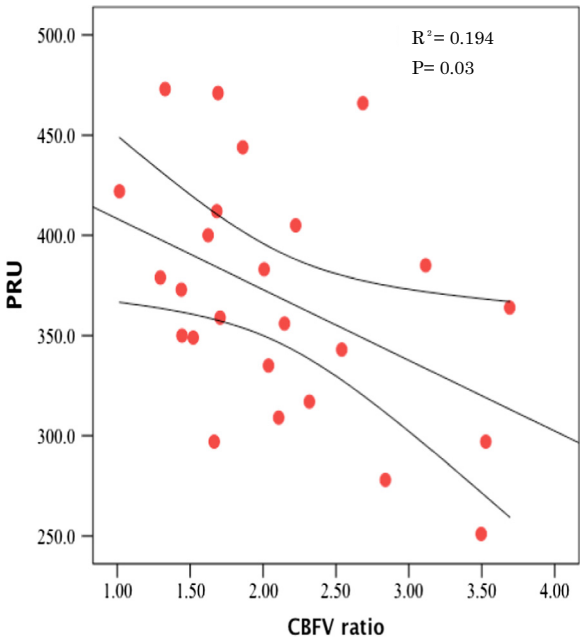

Fig. 1. Negative correlation between CBFV ratio and PRU.

4. Discussion

A previous study reported that high platelet aggregability has a significant correlation with vascular endothelial dysfunction in stable coronary artery disease patients with aspirin and clopidogrel [18] and that a correlation exists between high platelet aggregability and endothelial dysfunction, independent of risk factors for cardiovascular disease, medications, and genetic polymorphisms. However, accumulating evidence of the association between coronary endothelial dysfunction and platelet reactivity remains insufficient. Therefore, to examine the close association between platelet aggregability and coronary endothelial dysfunction, we assessed coronary endothelial function by measuring CBFV and platelet activity by measuring PRU by VerifyNow. In the population of this study, PRU was significantly correlated to platelet aggregability assessed by the value of light transmittent aggregometry, which is the established methods to evaluate adenosine diphosphate-induced platelet function (r = 0.39). Previous study reported the change of PRU after clopidogrel discontinuation [26], indicating PRU reflects platelet function in the clopidogrel free condition. These data show PRU can reflect platelet aggregability in subjects who do not have any anti-platelet agent.

Because we needed to determine the difference between endothelial function and vascular function to platelet aggregability, we assessed the correlation between CFR and PRU. In this study, PRU was significantly associated with CBFV, but not with CFR. In the clinical setting, the association between coronary endothelial dysfunction and platelet aggregability is still unclear. Furthermore, the association between the whole vascular condition and platelet aggregabitliy remains unclear. In addition to assessment of endothelial function by CBFV and platelet aggregatilibty, we also assessed the relation between the whole vascular condition and platelet aggregabitliy. CVFB represents acetylcholine-induced coronary endothelial function. CFR is one of the clinically available modality to represent the vasodilator capacity of the coronary vascular bed. CFR is affected by the whole vascular tonus, vascular resistance, and myocardial metabolic demands such as heart rate or blood pressure [27]. We assessed both CBFV and CFR to show which factor affects platelet aggregability. This study showed that PRU was significantly associated with CBFV, but not associated with CFR. Although we could not demonstrate the precise molecular mechanisms, endothelial dysfunction was correlated with high platelet aggregability in patients with non-obstructive ischemic heart disease who have no anti-platelet agents.

Although the precise molecular mechanism of endothelial dysfunction in patients with high platelet aggregability remains unknown, the impaired endothelial function reflects early atherosclerotic changes. Endothelial function plays important roles not only in the maintenance of vascular tone, and vasculature-blood cell homeostasis, but also thrombosis formation and platelet adhesion. Endothelial cells play important role in the regulation of platelet aggregation, and platelet adhesion to endothelium by producing biologically active substances such as NO and prostacyclin (PGI2). Endothelium derived NO and PGI2 inhibit platelet activation via cyclic guanosine monophosphate (cGMP) or cyclic adenosine monophosphate (cAMP). Endothelial dysfunction could

Table 2
Correlation coefficient of PRU.

	Correlation coefficient	P value
Age	0.355	0.082
Sex (man)	−0.485	0.014
Body mass index	0.073	0.727
Diabetes	−0.247	0.233
Hypertension	−0.186	0.374
Dyslipidemia	0.077	0.714
Smoking	−0.585	0.002
Hemoglobin	−0.424	0.035
Platelet count	−0.172	0.411
eGFR	−0.218	0.295
Hemoglobin A1c	−0.032	0.878
HDL cholesterol	−0.087	0.678
LDL cholesterol	0.469	0.018
Triglyceride	−0.153	0.464
High sensitivity CRP	−0.108	0.608
BNP	0.255	0.218
LVEF	−0.289	0.161
β blocker	0.186	0.373
ACE inhibitors or ARBs	0.007	0.972
Statins	−0.535	0.006
Hypoglycemic agents	−0.208	0.317

See Table 1 for abbreviations.

Table 3
Multivariable linear regression analysis of PRU.

	Non-standardized coefficients		Standardized coefficients	95% CI		
Variable	β value	Standard error	β value	Lower limit	Upper limit	P value
Principal component scores	−2.97	11.65	−0.05	−27.13	21.19	0.80
CBFV ratio	−34.51	15.65	−0.43	−66.97	−2.05	0.038

Abbreviations are following: CBFV, coronary blood flow volume; and CI, confidence interval.

decrease the production of NO and PGI2, leading to platelet activation. Previous studies demonstrated that NO and PGI2 produced by endothelial cells, directly inhibit platelet aggregation [28,29]. Our previous in vitro experiment also showed that human platelet incubated with dysfunctional human coronary artery endothelial cells treated by nitro-L-arginine methyl ester had significant higher reactivity than that incubated with vehicle control [18]. These studies support that endothelial dysfunction are associated with increased platelet aggregability. von Willebrand factor (vWF) plays a central role in primary hemostasis where it mediates platelet adhesion to damaged vascular subendothelium and subsequently platelet aggregation. vWF binding to platelet membrane glycoprotein Ib initiates intracellular pathways of platelet activation. Vascular injury results in the exposure of collagen and vWF in the vessel wall. Circulating platelets adhere and form a monolayer of activated platelets on the collagen matrix, which drives the release of adenosine diphosphate (ADP) and thromboxane (Tx) A2 from the adherent platelets. Secretion of ADP and TxA2 promotes changes in platelet shape and amplification of platelet activation. Thrombin, generated by locally produced tissue factor (TF), is the most potent platelet activator. In the perpetuation phase, platelet contacts promote growth and stabilization of the platelet plug [30]. Platelet-derived NO is also considered to regulate the platelet reactivity [31,32].

In the present study, however, we could not prove the close association of endothelial cell-derived NO with platelet-derived NO in the regulation of platelet reactivity because of not measuring NO levels in patients. Further investigations are needed to examine of correlation between NO and platelet activity derived coronary endothelial cells.

4.1. Study limitation

Interpretation of the results of the present study is limited by the small sample size and single-center study. Because the population was small, we could not conclusively confirm a significant correlation between PRU and CBFV in the clinical outcome in the present study. Second, this study was a post hoc study. Further clinical trials in larger population and prospective study are required.

5. Conclusions

In patients with non-obstructive IHD, CBFV change was significantly associated with platelet aggregability (Fig. III in the online-only Data Supplement). This indicates that coronary endothelial dysfunction is an important pathogenesis for increased platelet aggregability.

Conflict of interest statement

None.

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None.

Disclosure

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.08.068.

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