Original Article

Optimal Timing of Clopidogrel Discontinuation in Japanese patients: Platelet Aggregation Test Using the VerifyNow® system

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ABSTRACT

Objective

The Japanese Circulation Society recommends discontinuation of antiplatelet therapy 7–14 days before major surgery. However, reports on the relationship between the timing of clopidogrel discontinuation and the risk of postoperative bleeding in Japanese subjects are lacking. We assessed the optimal timing of clopidogrel discontinuation before elective surgery using the VerifyNow® P2Y12 assay. Additionally, the relationship between preoperative platelet function and risk of postoperative bleeding was evaluated.

Methods

Study 1: Between June 2012 and December 2014, Platelet function was examined by the VerifyNow P2Y12 assay in patients scheduled for cardiac surgery, every other day after clopidogrel cessation.

Study 2: We compared the preoperative platelet function, measured by the Verify Now, with the postoperative bleeding.

Results

Study 1: Twenty-four patients were included in this study. The mean P2Y12 reaction units (PRU) on Day-0 was 186, and increased significantly to 283 PRU on Day-7 in a time-dependent manner after clopidogrel discontinuation (p=0.001). The mean PRU value significantly exceeded the cutoff of 230 on Day-5.

Study 2: Correlation between the preoperative aspirin reaction units (ARU) and postoperative bleeding showed a slight inverse correlation in patients undergoing aortic valve replacement (rS= –0.363, p= 0.013), mitral valve plasty (rS= –0.300, p= 0.085) and off-pump coronary artery bypass (rS= –0.176, p= not significant).

Conclusion

Platelet aggregation had already recovered at 5 days after clopidogrel cessation. Surgeons could consider decreasing the interval from clopidogrel discontinuation to surgery from the recommended 7–14 days. The VerifyNow assay can be used to predict the risk of perioperative bleeding.

INTRODUCTION

The antiplatelet agent clopidogrel is used widely for the prevention and treatment of peripheral, coronary and cerebrovascular arterial thrombotic diseases. With the spread of percutaneous coronary interventions using drug-eluting stents, cardiac surgeons increasingly encounter patients on long-term clopidogrel therapy.

The American College of Cardiology and American Heart Association recommends ≥5 days from clopidogrel discontinuation to cardiac surgery because clopidogrel has been shown to increase the risk of peri-procedural bleeding and surgical re-exploration, transfusion frequency, and resource utilization in patients undergoing surgery. In the USA, patients on maintenance clopidogrel scheduled for a surgical procedure are, in general, instructed to discontinue the drug 5–7 days before the procedure.[1-6]

In Japan, discontinuation of clopidogrel is recommended >14 days before the surgical procedure. It is mentioned that if a sufficient period between clopidogrel discontinuation and surgery is not possible, special care is essential because of the increased risk of severe bleeding. However, reports on the relationship between the timing of clopidogrel discontinuation before surgery and bleeding risk in Japanese subjects are lacking.

Historically, platelet reactivity has been measured by light-transmittance aggregometry (LTA) but this method is technically complex and time-consuming. Several more practical, point-of-care tests are being used to assess platelet reactivity to clopidogrel. VerifyNow® (Accumetrics, San Diego, CA, USA) is a reliable point-of-care assay that measures platelet aggregation. Results are expressed in platelet P2Y12 reaction units (PRU) and aspirin reaction units (ARU). VerifyNow has been reported to be easy to use and rapid to measure.[7-9] The VerifyNow P2Y12 Test is designed to measure the platelet P2Y12 receptor, which is blocked by clopidogrel. PRU detects platelet aggregation with adenosine-5-diphosphate, and does not influence the arachidonic acid cascade. VerifyNow is a point-of-care, rapid, cartridge-based, platelet-function assay that is used to measure platelet aggregation in a system containing fibrinogen-coated beads. The instrument measures the rate of platelet aggregation based on changes in light transmission through whole blood. Results of the VerifyNow P2Y12 assay are expressed in PRU, with higher PRU values indicating a lower degree of inhibition of the platelet P2Y12 receptor by clopidogrel, and thus less platelet inhibition. Residual platelet reactivity as measured by the VerifyNow P2Y12 assay has been reported to be correlated with that measured by LTA, which is considered to be the “gold standard” for determination of the effects of antiplatelet therapy upon platelet function. [10,11]

The aim of this prospective observational study is to assess the optimal timing of discontinuation of clopidogrel before elective cardiovascular surgery by examining recovery of platelet function after clopidogrel cessation using the VerifyNow P2Y12 assay. Additionally, we investigated the relationship between the preoperative platelet function, assessed by Verify Now assay system, and the risk of the postoperative bleeding.

METHODS

Study 1: We undertook this prospective, observational study to determine the optimal timing of discontinuation of clopidogrel before cardiovascular surgery by monitoring changes in platelet reactivity after clopidogrel discontinuation using the VerifyNow P2Y12 assay. This study was conducted in patients scheduled to undergo surgery at Kawasaki Medical School in Okayama, Japan between June 2012 and December 2014.

After providing written informed consent, 24 patients were enrolled. Fourteen patients (58.3%) received dual-antiplatelet therapy with clopidogrel (75 mg) and aspirin (100 mg) daily. Low-dose aspirin was not discontinued for coronary artery bypass surgeries. The demographic data reviewed comprised the age, body mass index, sex, history of smoking, and comorbidities (diabetes mellitus, hypertension, hyperlipidemia, coronary artery disease, peripheral vascular disease).

A whole-blood sample (designated as the Day-0 sample) was obtained from each patient immediately before clopidogrel discontinuation, and tested by the VerifyNow P2Y12 assay to determine the degree of platelet inhibition. Then, clopidogrel was discontinued on the same day (Day-0), about 7 days before the scheduled surgical procedure. Whole-blood samples were obtained on Days 1, 3 and 5 or 2, 4 and 6, followed by collection of a pre-procedural blood sample on Day-7. To observe changes in the activity of platelet aggregation, we measured changes in percent inhibition of the P2Y12 receptor relative to that at baseline. This measurement was based on the PRU values at the time-point of determination and the BASE PRU (BASE), which is an independent measurement based on the rate and extent of platelet aggregation in the BASE channel, using the following equation:

$$(\mathrm{BASE} - \mathrm{PRU})/\mathrm{BASE} \times 100.$$

The BASE PRU result serves as an estimate of platelet function at baseline, and is independent of inhibition of the P2Y12 receptor.

Study 2:

We measured preoperative PRU and ARU by the VerifyNow and postoperative amount of bleeding over 24 hours after admission to the intensive care unit (ICU) undergoing the cardiac surgery between November 2011 and December 2014. Then, the correlation between the preoperative platelet aggregation and postoperative bleeding was examined.

Statistical analyses

Results of the VerifyNow P2Y12 assay for individual patients were plotted over time. Descriptive statistics (mean, median, quartiles, mean ± standard error, ranges) were used to construct summary plots covering all subjects. Statistical analyses were carried out using Stat Flex v6.0 (Artech, Osaka, Japan). Two-specimen Student's *t*-test was carried out to determine the significance of changes over

time of VerifyNow P2Y12 assay results and percent inhibition in subjects with data for all periods. $p<0.05$ was considered significant. Spearman's rank correlation coefficient was calculated for the preoperative platelet function and risk of postoperative bleeding. P values of less than 0.05 were considered to denote statistical significance.

RESULTS

Study 1

Demographics

Twenty-four patients on long-term clopidogrel therapy were enrolled. However, four patients were excluded because Day-0 PRU was >230. **Table 1** shows the baseline demographic data of study subjects. Mean age of patients was 70.4±8.4 years, and 15 (75%) patients were male. Comorbidities comprised diabetes mellitus (40%), hyperlipidemia (55%), hypertension (65%), coronary artery disease (15%), and peripheral vascular disease (30%); 65% of patients had a history of smoking.

VerifyNow Assay

After discontinuation of clopidogrel, the PRU returned to baseline level in a time-dependent manner (**Fig. 1**). Mean platelet reactivity in subjects on Day-0 (immediately before clopidogrel discontinuation) was 186 (range, 83–256) PRU, which increased to 283 (range, 187–364) PRU on Day-7. Thus, significant differences recognized between Day-0 *vs* Day-3 (p=0.02353), Day-0 *vs* Day-5 (p=0.00134) and Day-0 *vs* Day-7 (p=0.00146) were recorded using the Student's *t*-test. The mean PRU value exceeded the cutoff value of 230 at 5 days after clopidogrel cessation. **Figure 2** shows the percent inhibition of the platelet receptor P2Y12 after clopidogrel discontinuation. Mean percent inhibition (standard error) on Day-0 was 38% (16.7%) and decreased to 6.5% (9.7%) on Day-7. Thus, percent inhibition decreased significantly in a time-dependent manner after clopidogrel discontinuation. We considered that the activity of platelet aggregation had already recovered by Day-5.

Study 2:

Demographics

Table 2 shows the clinical characteristics of the cardiac surgery patients. The proportion of females was relatively higher among patients undergoing aortic valve replacement (AVR), and several of these patients were not taking antiplatelet agents. The proportion of males was relatively higher among patients undergoing mitral valve plasty (MVP), and these patients were taking neither antiplatelet agents nor warfarin. Several patients were taking aspirin in the off-pump coronary artery bypass (OPCAB) group.

VerifyNow Assay

While a slight correlation was observed between the ARU value and the amount of bleeding showed inverse correlation in the patients undergoing AVR ($rS= -0.363$, $p= 0.0131$), MVP (rS=

−0.300, p= 0.0851) and OPCAB (rS= −0.176, p= not significant) groups (**Fig.3**). Furthermore, no correlation was observed between the PRU and the amount of bleeding in AVR (rS= −0.044, p= 0.7638), MVP (rS= –0.008, p= 0.9630) and OPCAB (rS= 0.074, p= not significant) groups (**Fig. 4**).

DISCUSSION

Several guidelines focus on the care of patients taking antiplatelet medications in the perioperative period, with variations in perioperative antiplatelet management evident for cardiac, peripheral vascular, coronary intervention, and other types of surgery. The guideline of the Japanese Circulation Society recommends discontinuation of antiplatelet therapy 7–14 days before major surgery (class IIa′). However, the evidence level for the optimal timing of discontinuation before surgery is very low (level C).[12] Use of a simple point-of-care test, such as the VerifyNow P2Y12 assay, to monitor the platelet response to clopidogrel discontinuation may provide useful information for making therapeutic decisions relating to management of antiplatelet drugs in the perioperative period, and for prediction of adverse events. Thus, we undertook this study to evaluate prospectively the interval to recovery of platelet function after clopidogrel discontinuation before elective surgery.

In our study, 80% of subjects showed PRU values >230 PRU and percent inhibition of the P2Y12 receptor <20% by 5 days after discontinuation of clopidogrel. Furthermore, mean platelet inhibition was only 8.3% by Day-5. These findings may allow surgeons to consider reducing the interval from clopidogrel discontinuation to surgery from the generally accepted interval of 7–14 days between withdrawal of antiplatelet drug and carrying out surgery. Further prospective studies are required to determine the optimal threshold at which bleeding and thrombotic complications can be minimized.

Median interval from the final clopidogrel dose to coronary artery bypass graft (CABG) was 4 (range, 1–9) days. Patients who withheld clopidogrel intake <4 days before surgery had lower PRU values at the time of the CABG than those who withheld the drug ≥4 days (622 ± 220 *vs*. 1028 ± 676 mL; p=0.026) before surgery in Transcatheter Cardiovascular Therapeutics 2012. Thresholds of ≥208 PRU and ≥275 PRU were correlated with a lower amount of bleeding but the differences did not reach significance (p=0.20 and p=0.39, respectively). Patients with PRU values ≥230 had significantly less chest-tube drainage over the 24 h after CABG than those with PRU values of <230 (622 ± 220 *vs*. 1028 ± 676 mL; p=0.026).[13]

We expected to observe an inverse correlation between preoperative platelet function and the amount of bleeding, but the inverse correlation was small. This observation could be explained by the small number of study subjects. Our study was conducted in patients scheduled to undergo surgical procedures; each patient had a different comorbidity, different primary disease that necessitated initiation of clopidogrel administration, and underwent a distinct surgical procedure. Each of the comorbidities may have had a different effect on platelet reactivity as measured by the VerifyNow P2Y12 assay. In addition, concomitant drugs, genetics, and the time of day at which

platelet function is measured may have different effects on the rate of production of new platelets, their activity, the rate of platelet turnover, and residual activity of platelets as measured by the VerifyNow P2Y12 assay. Further studies are needed to better define the relationship between the level of platelet inhibition measured by the VerifyNow P2Y12 assay and clinical endpoints such as bleeding and thrombotic complications.

Study Limitations

Our study had several limitations. First, there was a small number of cases in the study of clopidogrel cessation. Hence, we could not examine the relationship between the amount of bleeding and period of clopidogrel discontinuation before surgery. In future studies by our research team, the number of patients will increase, so we will review the correlation between discontinuation dates and amount of postoperative bleeding. Second, the relationship between the amount of bleeding and ARU platelet function on the day of surgery had a slight inverse correlation. Because of the small number of cases and various influencing factors that affect the extent of postoperative bleeding, the correlation did not reach significance. With a larger study cohort, it may be possible to reach a significant inverse correlation.

CONCLUSIONS

Useful information for making therapeutic decisions related to management of antiplatelet drugs during the perioperative period may be obtained by monitoring the platelet response to discontinuation of clopidogrel using the VerifyNow P2Y12 assay.

We conclude that platelet aggregation had already recovered 5 days after clopidogrel cessation, and that surgeons could shorten the period from the recommended 7–14 days after clopidogrel discontinuation before the surgical procedure. The VerifyNow platelet-function assay can also be used to predict the risk of perioperative bleeding.

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Conflict of interest: Authors declare no conflict of interest.

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Table 1. Baseline Demographic Data of Patients on Long-term Clopidogrel Therapy (n = 20)

Age	**70.4 ± 8.4 years**
Body mass index	**22.4 ± 3.1 kg/m^2**
Male	**75%** (15)
Diabetes mellitus	**40%** (8)
Hypertension	**65%** (13)
Hyperlipidemia	**55%** (11)
Smoking	**65%** (13)
Coronary artery disease	**15%** (3)
Peripheral vascular disease	**30%** (6)
Aspirin	**65%** (13)

Table 2. Clinical characteristics of cardiac surgery patients (n=114)

		AVR (n = 50)	MVP (n = 34)	OPCAB (n = 30)
Gender	Male	22	23	26
	Female	28	11	4
Age (years)		73 ± 9	66 ± 13	69 ± 11
Drug	Aspirin (A)	7	0	11
	Clopidogrel (Cl)	1	1	0
	Cirostazol (Ci)	2	0	0
	Warfarin (W)	6	7	1
	A+Cl	0	0	11
	A+Ci	1	0	1
	A+W	1	0	1
	A+Cl+W	0	1	0
	Cl+W	1	1	0
	Other	1	1	1
	None	30	23	4
PreOP Ht (%)		36.0 ± 5.7	41.3 ± 4.0	38.2 ± 6.0
PreOP Plt (x10^4/μl)		18.3 ± 6.3	20.3 ± 12.4	19.4 ± 4.9
PRU		351 ± 64	323 ± 55	325 ± 69
ARU		604 ± 66	629 ± 33	515 ± 73
Bleeding (ml)		401 ± 240	368 ± 181	473 ± 277

Fig 1. Distribution of PRU values after clopidogrel discontinuation. The dotted line represents the PRU cutoff point (230). Significant differences were observed between Day-0 *vs* Day-3, Day-0 vs Day-5 and Day-0 *vs* Day-7 (Student's t test).

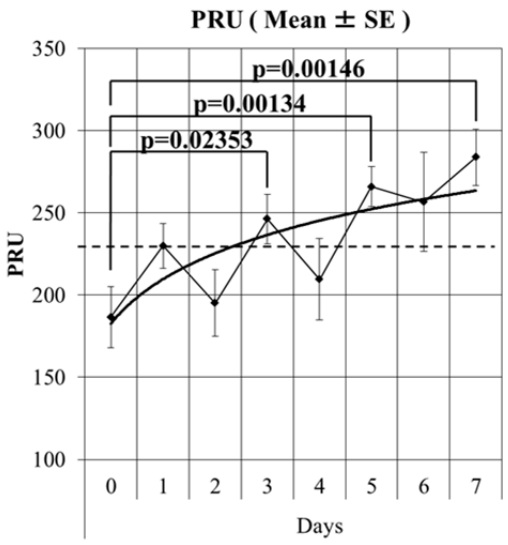
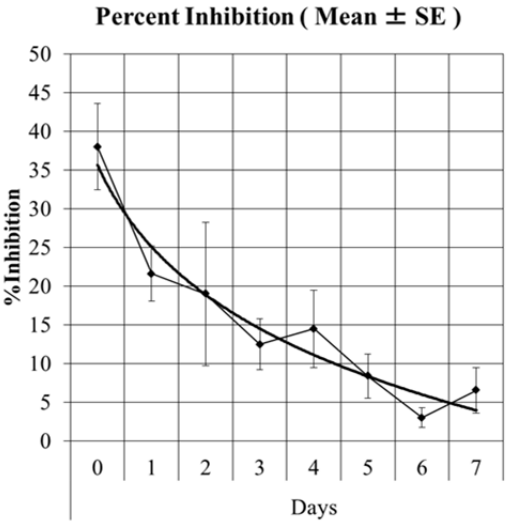


Fig 2. Percent inhibition over time after clopidogrel discontinuation. Data are the mean ± standard error.



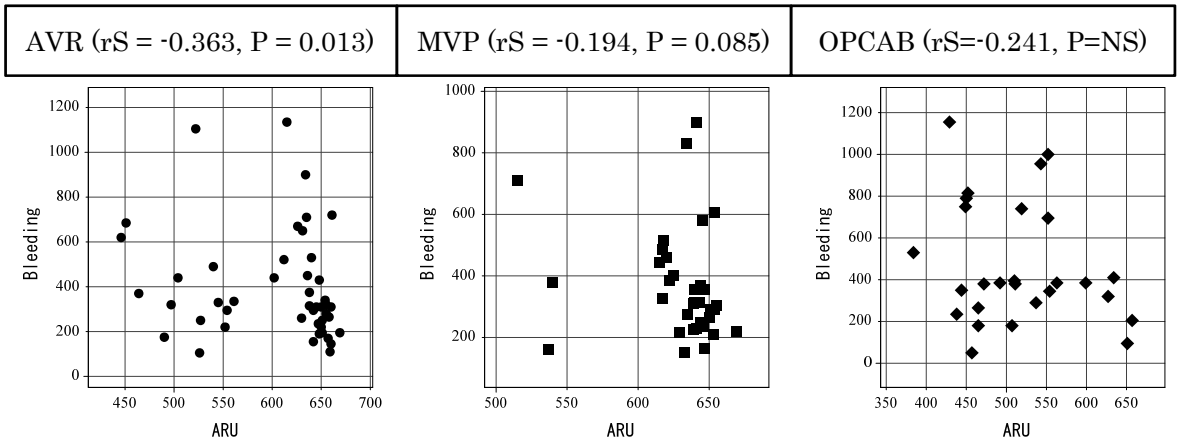


Fig 3. Correlation between preoperative platelet function (ARU) and amount of bleeding (mL) after AVR, MVP and OPCAB.

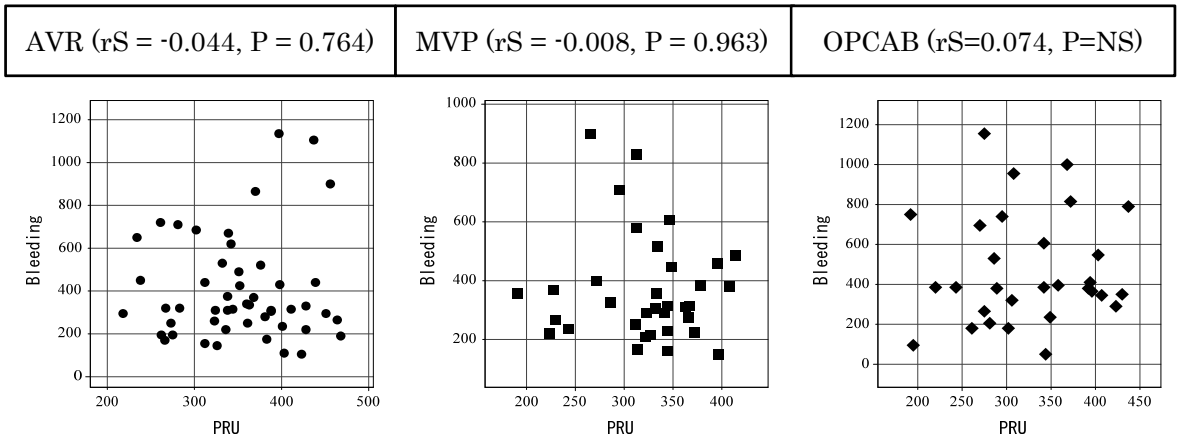


Fig 4. Correlation between preoperative platelet function (PRU) and the amount of bleeding (mL) after AVR, MVP and OPCAB.