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CAD

Non-Carriers of Reduced-Function CYP2C19 Alleles are Most Susceptible to Impairment of the Anti-Platelet Effect of Clopidogrel by Proton-Pump Inhibitors: A Pilot Study

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Background: The phenomenon of CYP2C19 polymorphism affects the metabolism of both clopidogrel and proton-pump inhibitors (PPI). However, concomitant use of both drugs may reduce the desired therapeutic effects. In this study, we evaluated whether individuals with different numbers of reduced-function CYP2C19 alleles were equally affected and whether PPIs with different dependencies on CYP2C19 metabolism were equally involved.

Methods: Thirty healthy volunteers were recruited to a six-week regimen of clopidogrel. Three PPIs with different metabolic dependencies on CYP2C19 were included and separately administered in this order. Each PPI was given for a week, followed by a one-week washout period before the intervention of the next PPI. The anti-platelet effect was examined by Thromboelastography Platelet Mapping™ (TEG®) and vasodilator-stimulated phosphoprotein (VASP) assays.

Results: Both TEG® and VASP tests showed the same general qualitative trend, but TEG® detected a statistically significant fluctuation of platelet aggregation in response to different drug interventions. The TEG® results also demonstrated that non-carriers experienced the most significant impairment of anti-platelet effect of clopidogrel after concomitant use of PPIs. This impairment was closely related to the metabolic dependence on CYP2C19 of PPI.

Conclusions: Our study indicated that non-carriers of reduced-function CYP2C19 alleles are most susceptible to impairment of the anti-platelet effect of clopidogrel after concomitant PPI use. Individual subjects are not equally affected, and PPIs are not equally involved. However, large-scale randomized clinical trials are needed to evaluate the clinical outcome.

Key Words: Clopidogrel • CYP2C19 polymorphism • Platelet aggregation • Proton pump inhibitors • TEG • VASP

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Optimizing anti-platelet therapy in patients with coronary artery disease (CAD) subsequent to coronary stenting is a major issue within the field of cardiology. Therefore, understanding how to maximize the anti-platelet effect and minimize the adverse side-effects of clopidogrel will have a tremendous impact on CAD, which remains a leading cause of mortality worldwide. Recently, the issue of concomitant use of clopidogrel and proton-pump inhibitor (PPI) increasing adverse cardiovascular events in CAD patients has drawn much contro-

versy.[1] Since both drugs share the same metabolism pathway via cytochrome P450 (CYP2C19) enzymes, it is likely a potential drug interaction exists.[2] While current data showed reduced-function alleles of CYP2C19 are associated with poor anti-platelet effects of clopidogrel;[3,4] it is unclear whether CYP2C19 is relevant if clopidogrel and PPI are taken concurrently. Although the impact of concomitant use of clopidogrel and PPI on CAD has received various interpretations due to different study designs,[5-7] it is important to know whether individuals with different numbers of reduced-function CYP2C19 alleles are equally affected and whether PPIs with different dependence on CYP2C19 metabolism are equally involved.

The use of PPI remains the drug of choice in patients with cardiovascular diseases and undergoing anti-platelet therapy.[8] There are six different PPIs available, including omeprazole, esomeprazole, lansoprazole, dexlansoprazole, pantoprazole and rabeprazole. All of them are comparable in terms of their therapeutic effects but slightly different in their metabolic dependence on CYP2C19, where pantoprazole > esomeprazole > rabeprazole.[9,10] Additionally, it is also necessary to clarify whether the metabolic dependence of PPI on CYP2C19 has any impact on the anti-platelet effect of clopidogrel.

This study evaluated whether concomitant use of different PPIs impairs the anti-platelet effect of clopidogrel to the same extent in individuals with different numbers of reduced-function CYP2C19 alleles.

MATERIALS AND METHODS

Patients

The protocol of this study was reviewed and approved by the Research Ethics Committee of National Taiwan University Hospital (ClinicalTrials.gov number, NCT01023360) and written informed consents were provided by all the subjects. Thirty healthy Taiwanese were randomly recruited in this study. Participants with the following conditions and medications which may affect platelet function were excluded, namely smoking, abnormal renal function, diabetes, hypertension, asthma, peptic ulcer disease, bleeding tendency, drug history of aspirin, warfarin, and non-steroid anti-inflammatory drugs. Complete blood cell counts, creatinine, aspartate aminotransferase, prothrombin time, and activated partial thromboplastin time were examined in all participants to confirm normal platelet function and coagulation profile. All participants were requested to keep the similar dietary schedule and content during the study as well.

Study design

All the participants received clopidogrel during this 6-week study, including a loading dose of 300-mg on the first day of this study followed by a maintenance dose of 75-mg. After the first week use of clopidogrel, weekly introduction of PPI was initiated. The design of this study is open-labeled and single blinded. Three different PPIs were included in this study, namely rabeprazole 20-mg, pantoprazole 40-mg, and esomeprazole 40-mg. Each was given once daily for one week, followed by a one-week withdrawal period to wash out the residual concentration of previous PPIs. We sampled blood from each participant at the baseline of the study and on the last day of every week during this study to measure the platelet aggregation function (Figure 1A).

TEG® Platelet Mapping™ assay

Blood samples were collected in tubes containing sodium citrate and heparin. The TEG® Platelet Mapping™ assay (Haemoscope Corporation, Niles, Illinois, USA) relies on evaluation of clot strength to enable a quantitative analysis of platelet function. The maximal haemostatic activity is measured by a kaolin-activated whole blood sample treated with citrate. The following measurements are performed with heparin to eliminate thrombin activity: Reptilase and Factor XIII (Activator F) generate a cross-linked fibrin clot to isolate the fibrin contribution to the clot strength.[11] The platelet aggregation was calculated from the formula:

$$\% \text{ Aggregation} = [(MA_{ADP} - MA_{fibrin}) / (MA_{thrombin} - MA_{fibrin})] \times 100.$$

Vasodilator-stimulated phosphoprotein (VASP) assay

Blood samples were collected in tubes containing sodium citrate. Thereafter, VASP phosphorylation analysis was performed with the samples blinded to the

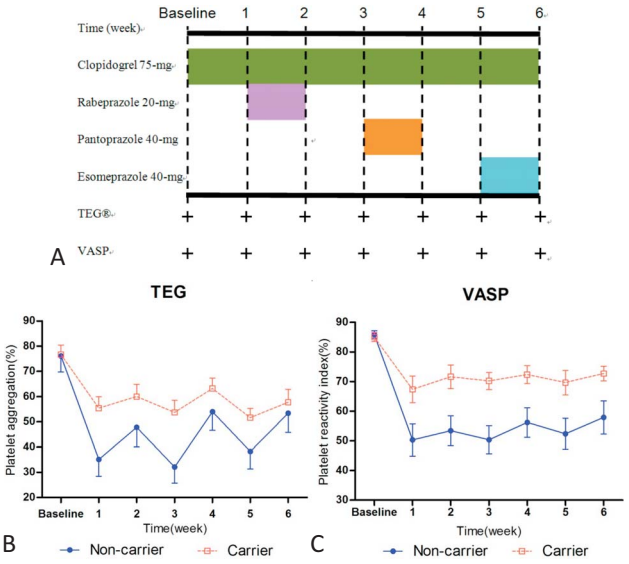

Figure 1. *(A) The design of this study. A one-week washout phase is between every PPI given. The time point for evaluation platelet function by TEG® and VASP is labeled as "+" in the figure. (B) Platelet function of non-carriers and carriers with a reduced-function CYP2C19 allele in each time-assessing point by TEG®, and (C) by VASP. PPI, proton-pump inhibitor; VASP, vasodilator-stimulated phosphoprotein.*

treatment group. Platelet reactivity was assessed by measuring platelet-phosphorylated VASP in whole blood using a commercially available Platelet VASP kit (Biodis-Stago, Asnières, France) adapted from the method of Schwartz et al.[12] Platelet reactivity was expressed as a PRI calculated as:

$$PRI\ (\%) = [(MFI_{PGE1} - MFI_{PGE1+ADP}) / MFI_{PGE1}] \times 100\%.$$

CYP2C19 genotyping

DNA was isolated from 200 μl peripheral potassium EDTA anticoagulated blood. Genotypes were determined with a TaqMan SNP genotyping assay. Pursuant to prior classification,[4] CYP2C19 reduced-function alleles were CYP2C19*2 (681G>A; rs4244285)[13] and CYP2C19 *3 (636G>A; rs4986893).[13] The assay reagent for the single nucleotide polymorphism in the gene was supplied by Applied Biosystem (Foster City, CA, USA). The number of reduced-function alleles were calculated; those individuals without reduced-function alleles were defined as (*1A/*1A) in the observed alleles, whereas individuals with one and two reduced-function alleles were defined as (*1A/*2A or *1A/*3) and (*2A/*2A or *2A/*3), respectively.

Sample size calculation

Given that clopidogrel might reduce platelet reactivity index to 50%,[14] we assumed that PPI would increase it to 75%. When alpha error is set at 0.05 and power at 90%, 30 subjects are needed in this paired study.

Statistics

Demographic and clinical characteristics were summarized at baseline as means [±standard deviation (SD)] for normally distributed continuous variables. For comparisons of the baseline characteristics, the between-group data were compared using Student's unpaired *t* test for continuous data, and using the $\chi 2$ or Fisher's exact test for categorical data [degree of freedom (df) = 1]. Paired-sample *t*-tests were used to evaluate the absolute change in platelet aggregation, and PRI was measured by TEG® and VASP test before and after PPIs were administered, in addition to clopidogrel in subjects after each crossover. To compare the absolute change between different groups of CYP2C19 polymorphisms other than PPIs, statistical analysis was performed with unpaired-sample *t*-tests and analysis of variance. We also performed post analysis of trend according to the numbers of reduced-function CVP2C19 alleles. All statistical tests (performed with SAS software, version 9.1) were two-sided. Here, $p < 0.05$ was considered statistically significant.

RESULTS

The distribution of CYP2C19 genetic polymorphism in this study was compatible with the general population

Thirty subjects, including 15 men and 15 women, were enrolled in this study (Table 1), and all subjects were of Chinese Han descent. The mean (± SD) age was 39.4 ± 10.5 years (range: 27-63 years). The average subject height was 168.6 ± 5.7 cm (155-186 cm), and average weight was 66.3 ± 10.5 kg (50-84 kg). In this study cohort, CYP2C19 genotyping revealed 13 non-carriers of reduced-function CYP2C19 alleles (43.3%), and 17 carriers (56.7%), including 11 subjects with one reduced-function allele (36.7%), and 6 subjects with two reduced-function alleles (20%). The genotype distribution did not deviate from the Hardy-Weinberg equilibrium and was consistent with previous reports.[15,16] In total, all

Table 1. Demographic and laboratory data of all the participants

Variables	Total	Non-carriers	Carriers	p value
Number, n	30	13	17	
Age, y	39.4 ± 10.5	41.6 ± 7.5	38.2 ± 9.4	0.15
Gender (male/female), n	15/15	9/4	11/6	0.14
Body height, cm	168.6 ± 5.7	169.8 ± 5.6	167.2 ± 5.8	0.85
Body weight, kg	66.3 ± 10.5	61.3 ± 8.7	64.8 ± 8.2	0.27
Basic laboratory data				
WBC, k/μL	6724 ± 1721	6490 ± 1850	6904 ± 1651	0.52
Hb, g/dL	14.3 ± 1.40	14.4 ± 1.6	14.2 ± 1.3	0.71
PLT, k/μL	270 ± 36	266 ± 25	273 ± 42	0.65
Cre, mg/dL	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	0.93
AST, U/L	22 ± 9	26 ± 13	20 ± 4	0.11
PT, sec	10.6 ± 2.3	11.1 ± 1.8	10.2 ± 0.9	0.67
aPTT, sec	27.8 ± 3.1	28.4 ± 2.3	27.3 ± 2.6	0.73
Baseline platelet function				
TEG®, %	76.4 ± 18.6	76.1 ± 22.9	76.7 ± 15.3	0.43
VASP, %	85.4 ± 5.5	85.9 ± 4.6	85.0 ± 6.3	0.55

Data were presented as mean ± standard deviation.
aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; Cre, creatinine; Hb, hemoglobin; PLT, platelet; PT, prothrombin time; RBC, red blood cell; VASP, vasodilator-stimulated phosphoprotein; WBC, white blood cell.

thirty subjects completed this six-week study. None of them suffered allergic reactions, gastrointestinal discomfort, or any adverse drug reaction after use of clopidogrel and three different PPIs use.

Both TEG® and VASP tests showed the same trend, but TEG® reflected a fluctuation of platelet aggregation in response to different drug interventions.

Both TEG® and VASP tests were used in this study to measure platelet aggregation. The baseline percent platelet aggregation and PRI were 76.4 ± 18.6% and 85.4 ± 5.5% measured by TEG® and VASP, respectively. The baseline percent platelet aggregation and PRI in non-carriers and carriers of a reduced-function CYP2C19 allele were comparable (TEG, 76.1 ± 22.9% vs. 76.7 ± 15.3%, p = 0.43; VASP, 85.9 ± 4.6% vs. 85.0 ± 6.3%, p = 0.55). Changes in platelet function detected by either TEG® (Figure 1B) or VASP (Figure 1C) at all of the evaluated time points showed the same trend which suggested concomitant use of PPI reduced the anti-platelet effect of clopidogrel. However, TEG® detected a significant fluctuation of platelet aggregation in response to different drug interventions in this study (Figure 1B and 1C).

The anti-platelet effect of clopidogrel was impaired by concomitant use of PPIs

Generally speaking, the anti-platelet effect of clopidogrel was impaired by concomitant use of PPIs (TEG® Figure 2A, p < 0.0001; VASP Figure 2B, p = 0.02). The average reduction was 11% by TEG® and 3% by VASP. This impairment was consistent among all three PPIs. Again, this impairment was more clearly observed when measured by TEG® (Figure 2C; rabeprazole, p = 0.03; pantoprazole, p = 0.002; esomeprazole, p = 0.008). However, VASP results did not show a statistically significant difference ((Figure 2D; rabeprazole, p = 0.42; pantoprazole, p = 0.06; esomeprazole, p = 0.10). In addition, the PPI effect was washed out after the one week withdrawal period, and the anti-platelet effect of clopidogrel was restored accordingly (TEG, Figure 1B, p = 0.44; VASP, Figure 1C, p = 0.79).

Non-carriers were most susceptible to the impaired anti-platelet effect of clopidogrel by concomitant use of PPI

Consistent with previous studies, the anti-platelet effect of clopidogrel was found to be the worst in carriers[3,4] (Figure 1B and 1C). However, non-carriers were found most susceptible to the impairment by concomitant use of PPI (TEG® Figure 3A, p = 0.02; VASP Figure 3B, p = 0.59). If we further separated the subjects according to the number of reduced-function alleles they bear, statistical significance was obtained in the TEG® results (TEG® Figure 3C, p = 0.038; VASP Figure 3D, p =

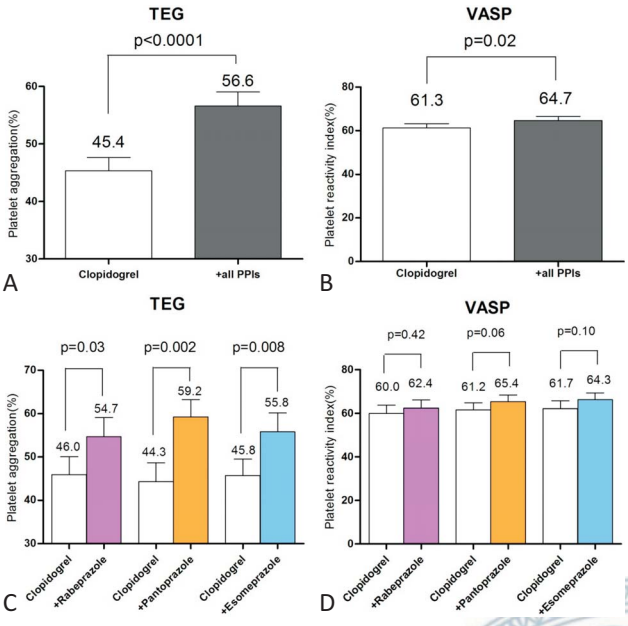

Figure 2. *Measurement of platelet function change after adding PPIs to clopidogrel. Overview (A) by TEG® and (B) by VASP. Individual PPI measurement (C) by TEG® and (D) by VASP. (The average data and p value were shown.) PPI, proton-pump inhibitor; VASP, vasodilator-stimulated phosphoprotein.*

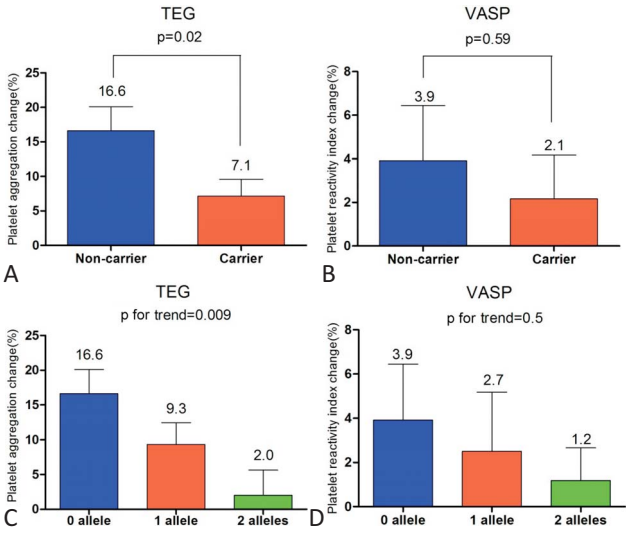

Figure 3. *Comparison of platelet aggregation change in either non-carriers or carriers taking both clopidogrel and PPIs (A) by TEG® and (B) by VASP. An inverse relationship between the numbers of reduced-function CYP2C19 alleles and platelet aggregation change affect by PPIs was shown by (C) by TEG® and (D) by VASP. (The average data and p value were shown.) VASP, vasodilator-stimulated phosphoprotein.*

0.8). A significant linear trend in the TEG® results (TEG®, Figure 3C, post-hoc analysis, p = 0.009) was also shown, suggesting that the impairment by concomitant use of PPI was inversely related to the number of reduced-function CYP2C19 alleles. Although the VASP measurement did not reach the required statistic significance, the same trend was observed.

The impairment by concomitant use of PPIs was closely related to the metabolic dependence on CYP2C19 of PPI

Although we found concomitant use of PPIs significantly impaired the anti-platelet effect of clopidogrel in non-carriers, this impairment was quite different between different PPIs (Table 2). The TEG results showed that in the most affected non-carrier group, the most significant impairment was found in PPIs with high metabolic dependence on CYP2C19 (TEG® Table 2; rabeprazole, p = 0.07; pantoprazole, p = 0.005; esomeprazole, p = 0.01). In contrast, concomitant use of PPI did not significantly impair the anti-platelet effect of clopidogrel in carriers. In those carriers with one reduced-function allele, this impairment was observed when taking pantoprazole (TEG® Table 2; rabeprazole, p = 0.35; pantoprazole, p = 0.02; and esomeprazole, p = 0.16), suggesting that PPIs with high metabolic dependence on CYP2C19 significantly affected the anti-platelet effect of clopidogrel. Although the VASP measurements did not attain the required statistic significance, the same trend was observed.

DISCUSSION

The laboratory platelet function test is helpful in evaluating the impact of the potential interaction between clopidogrel and PPI. However, one of the major drawbacks of these previous investigations is the choice of platelet function test used in each study. A test which can reflect the most likely scenario *in vivo* is the particular test needed. Traditionally, light transmission aggregometry (LTA) with adenosine diphosphate (ADP) as an agonist is the most widely used test for assessing the anti-platelet effect of clopidogrel. However, LTA is time-consuming, technically demanding, suffers from poor reproducibility, and is not available in most centers. This limits its broad scale application.[17,18] Several new, easy-to-use platelet-function tests have been introduced to clinical practice, including TEG® and VASP. Although

Table 2. Platelet aggregation measured by TEG® in response to clopidogrel and three different PPIs use

PPI	No. of CYP2C19 reduced-function allele	Clopidogrel	Clopidogrel w/PPIs	p value
All PPIs	0 allele (13 × 3)	35.1 ± 23.5	51.8 ± 26.8	< 0.001
	1 allele (11 × 3)	50.8 ± 15.7	60.1 ± 20.0	0.005
	2 alleles (6 × 3)	57.6 ± 18.5	60.7 ± 18.5	0.43
	Carrier (17 × 3)	52.8 ± 17.2	60.3 ± 19.3	0.005
Rabeprazole	0 allele (13)	35.1 ± 24.1	47.8 ± 28.0	0.07
	1 allele (11)	51.1 ± 17.2	56.7 ± 15.4	0.35
	2 alleles (6)	63.4 ± 20.1	65.9 ± 27.6	0.28
	Carrier (17)	55.4 ± 18.7	60.0 ± 20.2	0.18
Pantoprazole	0 allele (13)	32.2 ± 23.1	54.0 ± 26.7	0.005
	1 allele (11)	52.2 ± 16.7	66.3 ± 18.6	0.02
	2 alleles (6)	56.3 ± 26.4	57.6 ± 13.4	0.9
	Carrier (17)	53.7 ± 19.9	63.2 ± 17.0	0.07
Esomeprazole	0 allele (13)	38.2 ± 24.7	53.4 ± 27.3	0.01
	1 allele (11)	49.1 ± 4.3	57.2 ± 25.2	0.16
	2 alleles (6)	56.3 ± 16.6	58.6 ± 13.3	0.61
	Carrier (17)	51.6 ± 15.0	57.7 ± 21.3	0.13

PPI, proton-pump inhibitor.

these two assays are both effective in the evaluation of anti-platelet effect after clopidogrel use, they have a basic difference in design.[19,20] While VASP phosphorylation evaluates the platelet activation from the P_2Y_{12} ADP receptor, TEG® uses the whole blood to evaluate the clot strength and ensures a quantitative analysis of platelet function. Technically, TEG® is more likely to mirror platelet behavior in human blood vessels. In addition, we showed that TEG® reflected the fluctuation of platelet aggregation in response to the selected drug interventions. Consequently, TEG® is an ideal laboratory test to evaluate platelet aggregation and should be considered in studies which aim to simulate clinical scenarios. Although most studies regarding the interaction between clopidogrel and PPIs failed to reach a consistent conclusion by using VASP or LTA alone, the answer may be clearer if more laboratory tests are included.[14,21-23] Despite our findings, we could not exclude the possibility that the impairment caused by PPI was modest.

This study is also the first investigation to point out that the anti-platelet effect of clopidogrel is significantly reduced by PPI in non-carriers of reduced-function CYP2C19 alleles. Our finding did not support our previous assumption that carriers may be the candidates for the impairment conferred by concomitant use of PPI. Given that the highest prevalence of carriers of reduced-function CYP2C19 alleles is found in East Asian populations, including Han Chinese, Japanese, and Korean,[15,24,25] our study provides a comprehensive analysis regarding CYP2C19 genetic polymorphism. Our results suggest that the majority of the general population may be at risk for the drug interaction between clopidogrel and PPI. Our finding also portrays the significance of CYP2C19 polymorphism of individuals and metabolic dependence on CYP2C19 of PPI, which should be seriously considered when evaluating clinical outcomes of concomitant clopidogrel and PPI use. Recently, the FDA notified healthcare professionals about the drug interaction between clopidogrel and omeprazole.[26] Other drugs that potently inhibited the CYP2C19 enzyme when combined with clopidogrel were also included in that notification. Our study showed that, when taking clopidogrel and PPI concurrently, each individual is not equally affected. The number of reduced-function CYP2C19 alleles is inversely related to the impairment of anti-platelet effect of clopidogrel with concomitant use of PPI. Since the anti-platelet effect in carriers taking clopidogrel is modest, it is less likely to see the obvious drug-drug interaction compared in non-carriers. The drug-drug interaction change of anti-platelet effect after taking PPIs must be within the change of anti-platelet effect in patients taking clopidogrel, which may lead to our finding. The most significant insight derived from this study is that CYP2C19 polymorphism should be seriously consid-

ered and tested in clinical trials designed to detect the potential interaction and the clinical outcome, given that the prevalence of non-carriers may approach 75% in western countries. In addition, our study showed that each PPI is not equally involved. The polymorphisms of CYP2C19 influence the metabolism of PPIs mentioned in many scientific reviews, whether in Caucasians or Asians. Most scientific papers discussed the effect of CYP2C19 homozygous or heterozygous polymorphisms on the metabolism of PPIs, but the respective role, *2 and *3, on the metabolism of PPIs is rarely discussed. Given that PPIs are recommended for the therapy and prophylaxis of aspirin-associated gastrointestinal injury, particularly in patients on dual anti-platelet therapy,[8] health care professionals should be careful about their choice of drugs. Our results show that PPI with high metabolic dependence on CYP2C19 have greater impairment on the anti-platelet effect of clopidogrel. Consequently, our results suggest that drug choice should be adjusted according to both the CYP2C19 genotype of patients and the metabolic dependence of the PPI on CYP2C19.

There were several limitations to this study. First, the study sample was small in size. Although the sample size was carefully calculated and the TEG results are statistically significant, we should only cautiously generalize the result because of its relatively small sample size. Second, we did not test PPIs in a randomized fashion. Nonetheless, the anti-platelet effect recovered to the initial clopidogrel response after one-week wash-out phase. This could minimize the concern of any possible former drug-accumulating effect. Finally, this was a pharmacodynamic study. A large-scale clinical outcome trial is warranted to verify the relationship between clopidogrel, PPIs, and CYP2C19 polymorphism.

CONCLUSIONS

Concomitant use of PPIs contributes to the most significant impairment of the anti-platelet effect of clopidogrel in non-carriers. The most significant impairment of the anti-platelet effect of clopidogrel by concomitant use of PPIs was found in non-carriers, and was closely related to the metabolic dependence on CYP2C19 of PPI. This impairment caused by PPI was negatively related to the number of reduced-function CYP2C19 alleles, but positively related to the metabolic dependence on CYP2C19 of PPI. Therefore, our results suggest that CYP2C19 plays a significant role in the drug interaction of clopidogrel and PPI.

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DISCLOSURES

None.

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SUPPLEMENT

Supplemental Table 1. p value for platelet function test (TEG®) change in carriers and non-carriers at each time point

Time point	Carrier	Non-carriers
Baseline → 1	0.001	< 0.001
1 → 2	0.18	0.07
2 → 3	0.16	0.06
3 → 4	0.07	0.005
4 → 5	0.06	0.004
5 → 6	0.13	0.01

Supplemental Table 2. p value for platelet function test (VASP) change in carriers and non-carriers at each time point

Time point	Carrier	Non-carriers
Baseline → 1	< 0.001	< 0.001
1 → 2	0.58	0.66
2 → 3	0.72	0.73
3 → 4	0.61	0.57
4 → 5	0.59	0.57
5 → 6	0.52	0.45

Supplemental Table 3. p value for difference of platelet function test (TEG® and VASP) between carriers and non-carriers at each time point

Time point	TEG®	VASP
Baseline	0.93	0.68
1	0.01	0.02
2	0.17	0.009
3	0.01	0.004
4	0.25	0.01
5	0.07	0.02
6	0.63	0.02