Original Article

Effects of PPIs and an H2 blocker on the Antiplatelet Function of Clopidogrel in Japanese Patients under Dual Antiplatelet Therapy

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Aim: Dual antiplatelet therapy (DAPT) with aspirin and clopidogrel is essential after percutaneous coronary intervention (PCI). Clopidogrel is a prodrug and changed into active metabolite by cytochrome p450 enzymes (CYPs), especially CYP2C19. Proton pump inhibitors (PPIs) are used for the prevention of aspirin-induced gastrointestinal bleeding. PPIs are also metabolized by CYP2C19, although the degree of its contribution is dependent on the kind of PPI. Omeprazole, a PPI, has been reported to weaken the antiplatelet effects of clopidogrel. Famotidine, a histamine receptor type 2 (H2) blocker, could also be an alternative to PPIs. The aim of this study was to evaluate the effects of PPIs and an H2 blocker on the antiplatelet function of clopidogrel.

Methods: Patients receiving DAPT due to prior PCI, who took either omeprazole or rabeprazole, were enrolled ($n=25$). The initial PPI was changed to the other PPI as a crossover study. In another study, patients undergoing DAPT without taking PPIs or H2 blockers were enrolled ($n=30$) and famotidine was added.

Results: Platelet aggregability when taking omeprazole was higher than when taking rabeprazole, evaluated by an optical aggregometer using collagen as a stimulus ($p=0.0051$) and by the VerifyNow P2Y12 assay ($p=0.0060$). Platelet aggregability when taking rabeprazole was comparable to that in control patients ($n=15$). Concomitant use of famotidine had no effect.

Conclusion: Omeprazole significantly reduced the antiplatelet effect of clopidogrel and this effect on clopidogrel was stronger than that of rabeprazole. Concomitant use of famotidine had no effect on the antiplatelet effect of clopidogrel.

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Introduction

Currently, percutaneous coronary intervention (PCI) with stent implantation is widely performed as

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standard therapy for ischemic heart disease. In Japan, more than 200,000 patients undergo this treatment per year, as described in the surveillance report from the Japan Circulation Society. The most severe complication after successful PCI is stent thrombosis since mortality rates associated with this complication are very high (up to 45%)¹⁾, although its incidence is low. For prevention, dual antiplatelet therapy (DAPT) with aspirin and an ADP-receptor blocker such as clopidogrel is most effective²⁻⁶⁾, and almost all patients undergo this therapy after coronary stent implanta-

tion.

Clopidogrel is a prodrug that requires metabolism by liver cytochrome p450 enzymes (CYPs) to become an active metabolite. Among the CYPs, CYP2C19 is now considered to play the most important role⁷⁾; however, variants of CYP2C19 arising through single nucleotide polymorphism (SNP) lose enzymatic activity. It has been demonstrated that the antiplatelet effects of clopidogrel are weakened in patients with CYP2C19 SNPs, which are associated with increased cardiovascular events, including stent thrombosis after PCI^{7, 8)}. Studies by us⁹⁾ and others¹⁰⁾ examined the antiplatelet effects of clopidogrel in Japanese patients and detected clear effects related to CYP2C19 SNPs. The percentage of people lacking enzymatic activity, designated as poor metabolizers (PM), is 3-5% in Western populations, while approximately 20% in Asians, including Japanese^{11, 12)}; therefore, CYP2C19 SNPs have a bigger impact on Asians¹³⁾, including Japanese.

It is well known that antiplatelet therapy increases the risk of bleeding complications. In particular, aspirin is associated with a high risk of gastrointestinal tract bleeding. In 2008, American professional societies in the fields of cardiology and gastroenterology (ACCF/AGC/AHA) together published an expert consensus on the prevention of gastrointestinal tract bleeding due to the intake of nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin¹⁴⁾. As a result, proton pump inhibitors (PPIs) were recommended for patients with a high risk of gastrointestinal tract bleeding, such as those receiving DAPT with aspirin and clopidogrel.

Although PPIs used in today's clinical setting are inactivated by CYP2C19, the extent of inactivation by CYP2C19 is dependent on the type of PPI¹⁵⁾; therefore, concomitant use of clopidogrel and a PPI might weaken the antiplatelet effect of clopidogrel through drug interaction. Several studies have reported that the antiplatelet effect of clopidogrel was reduced by the concomitant use of omeprazole, a PPI¹⁶⁻¹⁸⁾, and the ACCF/AGC/AHA expert consensus was revised as follows: it is noted that the antiplatelet effect of clopidogrel is consistently reduced by omeprazole although this may not apply to other PPIs because of the absence of large-scale, randomized, experimental studies that directly compare PPIs with different pharmacokinetic properties¹⁹⁾.

Histamine receptor type 2 (H2) blockers also reduce gastric acid secretion and are used for the treatment of gastroduodenal ulcers. Famotidine, an H2 blocker, has been shown recently to reduce gastric mucosal injury caused by aspirin therapy²⁰⁾, although

the protective effects of PPIs are much stronger than those of H2 blockers²¹⁾.

Considering these data, ACCF/AGC/AHA have recently revised the expert consensus¹⁹⁾. Therein it was noted that omeprazole affected the antiplatelet activity of clopidogrel consistently in several studies, but that the effect was unclear for other PPIs. It was also noted that H2 blockers could be an alternative to PPI in patients treated with clopidogrel who are at low risk of gastrointestinal tract bleeding, although the effect of H2 blockers on the prevention of gastric mucosal injury is weaker than PPIs.

In this study, we prospectively examined the effect of two PPIs, rabeprazole and omeprazole, on the antiplatelet function of clopidogrel in relation to CYP2C19 genotypes, and also evaluated the effect of famotidine in an actual clinical setting in Japan.

Aim

The aim of this study was to evaluate the effects of PPIs and an H2 blocker on the antiplatelet function of clopidogrel.

Methods

Study Population and Protocol

Both studies (PPI and H2 blocker) were approved by the Ethics Committee of Kyoto University, and written informed consent was given by the patients who participated in the studies. Common entry criteria were a platelet count of $100-350 \times 10^9/\text{mL}$ and hemoglobin $\geq 10\text{g/dL}$. Common exclusion criteria were (i) recent bleeding diathesis, (ii) hematologic or malignant disorder, (iii) oral anticoagulation with coumarin derivatives, (iv) glycoprotein IIb/IIIa inhibitor or fibrinolytics administered during the preceding 14 days, (v) antiplatelet therapy with ticlopidine, cilostazole or dipyridimole within the preceding 28 days, and (vi) alanine transaminase and aspartate transaminase > 3 fold of the upper limit of normal.

PPI Study

Twenty-five Japanese patients with prior coronary stent implantation who had received DAPT with 82-162 mg/day aspirin and 75 mg/day clopidogrel for more than 6 months were enrolled between September 2009 and May 2011. Patients had also been taking a PPI, either omeprazole or rabeprazole, for at least 2 weeks. The landmark analysis of the J-Cypher Study, a registry of patients who had undergone PCI with a sirolimus-eluting stent in Japan, revealed that discontinuation of clopidogrel did not increase stent throm-

Fig. 1. Schematic presentation of the study protocol of the PPI study.

bosis in patients with prior sirolimus-eluting stent implantation performed more than 6 months before²²⁾, suggesting that aspirin alone could effectively prevent stent thrombosis for 6 months after stenting. Therefore, we enrolled patients who had undergone coronary stent implantation more than 6 months prior to the study to avoid an increased risk of stent thrombosis because of the possible reduced antiplatelet effect of clopidogrel due to concomitant use of omeprazole or rabeprazole. The enrolled patients continued taking the PPI (omeprazole or rabeprazole) until the next outpatient department (OPD) visit (more than 2 weeks later, the 2nd visit), where the current PPI was switched to the other PPI, omeprazole to rabeprazole, or vice versa, without changing the other concomitant medications, and continued until the 3rd visit more than 2 weeks later (**Fig. 1**). Fasting blood samples were collected at the 2nd and 3rd visits, and general blood chemistry and platelet function tests were performed as described below (**Fig. 1**). The tests were performed in the morning prior to taking clopidogrel. Patients who met the inclusion criteria but were taking no PPI or H2 blocker ($n=15$) were used as controls in this study.

H2 Blocker Study

Thirty Japanese patients with previously implanted coronary stents undergoing DAPT with 82-162 mg/day aspirin and 75 mg/day clopidogrel for more than 14 days without taking a PPI or an H2 blocker

were enrolled between June 2010 and February 2011. They continued on the same medications until the next OPD visit (more than 2 weeks later, the 2nd visit), where famotidine (20 mg bid), an H2 blocker, was added without changing the other medications and continued until the 3rd visit more than 2 weeks later. Fasting blood samples were collected at the 2nd and 3rd visits, and analyzed for general blood chemistry and platelet function tests as described below (**Fig. 5A**).

Sample Size Calculation

PPI Study

From the study by Gilard *et al.*¹⁶⁾, the mean platelet reactivity index after receiving clopidogrel in the omeprazole and placebo groups was $51.4 \pm 16.4\%$ and $39.8 \pm 15.4\%$, respectively. With this information, we calculated that approximately 33 patients should be recruited to each group, the control group and the PPI group, with a 2-sided α value of 0.05 at the power of 80%.

H2 Blocker Study

We could not estimate the appropriate sample size because of the lack of a preliminary study.

Analysis with Optical Aggregometer

Blood samples were collected, using a tourniquet, with a 21G needle, into a glass tube containing a solution of 0.313% sodium citrate (final concentration).

Platelet-rich plasma (PRP) was prepared by centrifugation at 150 x g at 25°C for 15 min and platelet-poor plasma (PPP) was prepared by centrifugation at 1,740 x g at 25°C for 10 min. The PRPs were stimulated with 20 μ mol/L adenosine diphosphate (ADP; Chronolog, Havertown, PA, USA) and 2 μ g/mL collagen (Hormon-Chemie, Munich, Germany) at 37°C, and the aggregation was analyzed with stirring using a 12-channel light transmission aggregometer (MCM HEMA TRACER 313; MC Medical, Tokyo, Japan). The degree of light transmission of the PRP was defined as 0% of the aggregation rate and that of PPP as 100%^{23, 24}. The degree of light transmission was monitored for 10 minutes after agonist stimulation and all the procedures were completed within 2 hours after blood sampling. We evaluated the maximal aggregation rates (MARs).

VerifyNow Assay

The VerifyNow P2Y12 assay (Accumetrics, San Diego, CA, USA) is a whole-blood, point-of-care, light transmission-based optical detection assay specialized for evaluation of the antiplatelet effects of ADP-receptor blockers. This system measures platelet aggregation in the presence of ADP and prostagrandin E1 in a single-use disposable cartridge containing fibrinogen-coated beads. Results from the device are reported as P2Y12 reaction units (PRUs) on a continuous scale from 0 upward.

Genotyping of CYP2C19

Genotypes of CYP2C19, comprising combinations of a wild-type (*1) gene and 2 mutant alleles, CYP2C19 *2 (*2) and CYP2C19 *3 (*3), were determined as previously reported.⁹ Patients were then classified into 3 groups by genotypes, namely extensive metabolizers (EMs) (*1/*1), intermediate metabolizers (IMs), (*1/*2 or *1/*3) and poor metabolizers (PMs) (*2/*2, *3/*3, *2/*3).

Statistical Analysis

Continuous variables were expressed as the means $\pm$ standard deviation (SD). Categorical variables were expressed as frequencies and percentages. Statistical comparisons between categorical variables were performed using the 2-tailed Fisher's exact test or Pearson's chi-square test. Student's *t*-test was used to compare continuous variables. Changes in MARs and PRUs with different PPIs, and comparisons of measurements between pre- and post-H2 blocker treatment were assessed by the Wilcoxon matched-pairs signed-ranks test. *P* < 0.05 was defined as significant. Statistical analysis was performed using JMP 8.02

software (SAS Institute).

Results

Effect of PPIs on the Antiplatelet Function of Clopidogrel

The baseline characteristics of the enrolled patients (25 patients in the PPI group (67.4 $\pm$ 9.8 years old, 84% male) and 15 in the control group (68.5 $\pm$ 10.3 years old, 80% male)) are shown in **Table 1**. None of the parameters were significantly different between the control group and the PPI group. Moreover, none were significantly different between the rabeprazole group (*n*=21) and the omeprazole group (*n*=4) (data not shown). No serious adverse drug-related side effects were observed. No cardiovascular events occurred throughout the study period. In the PPI group, the two PPIs used were crossed over at the 2nd OPD visit (**Fig. 1**). Namely, rabeprazole was switched to omeprazole in 21 patients (84%) and omeprazole was switched to rabeprazole in 4 patients (16%). We compared the platelet aggregability in patients undergoing DAPT during rabeprazole use with that during omeprazole use. Platelet function was evaluated at the 2nd visit, 63 (28.5-77) (median [interquartile range]) days after the 1st visit, and at the 3rd visit, 63 (38.5-77) days after the 2nd visit.

The MARs induced by 20 μ mol/L ADP, measured by the optical aggregometer, was 52.2 $\pm$ 12.1%, 52.1 $\pm$ 11.9%, and 54.1 $\pm$ 10.0% in the control patients, patients taking rabeprazole and patients taking omeprazole, respectively (rabeprazole vs. control, *p*=0.99; omeprazole vs. control, *p*=0.59) (**Fig. 2A**).

During DAPT, the antiplatelet effect of clopidogrel could be detected more sensitively using collagen as a stimulus²⁵, so we evaluated the aggregation induced by collagen. The MARs induced by 2 μ g/mL collagen were 30.5 $\pm$ 15.7%, 27.1 $\pm$ 15.0%, and 35.7 $\pm$ 15.8% in the control patients, patients taking rabeprazole and those taking omeprazole, respectively (rabeprazole vs. control, *p*=0.50, omeprazole vs. control, *p*=0.33) (**Fig. 2C**).

Fig. 2B showed that ADP-induced MARs during omeprazole therapy were not different from those during rabeprazole therapy (*p*=0.14). On the other hand, collagen-induced MARs during omeprazole therapy were higher than those during rabeprazole therapy (*p*=0.0051) (**Fig. 2D**). Thus, the platelet aggregability analyzed by the optical aggregometer was significantly higher during omeprazole intake than during rabeprazole intake using collagen as a stimulus for aggregation.

Then, we separately analyzed collagen-induced

Table 1. Patient characteristics in the PPI study

	Control (<i>n</i> =15)	PPI (<i>n</i> =25)	P (control vs. PPI)
General characteristics			
Age (years)	68.5 ± 10.3	67.4 ± 9.8	0.75
Male	12 (80)	21 (84)	1.00
Risk factors			
Hypertension ¹	15 (100)	22 (88)	0.28
Diabetes ²	10 (67)	14 (56)	0.74
Hyperlipidemia ³	15 (100)	22 (88)	0.28
Current smoker	4 (27)	5 (20)	0.71
Prior myocardial infarction	6 (40)	14 (56)	0.51
Hematologic data			
Hb (g/dL)	14.2 ± 1.6	13.2 ± 1.7	0.08
Plt (×10 ⁹ /L)	187.7 ± 37.7	196.0 ± 48.0	0.57
AST (IU/L)	27.7 ± 8.1	24.4 ± 8.4	0.22
ALT (IU/L)	26.8 ± 11.1	24.0 ± 15.9	0.55
eGFR (mL/min/1.73 m ²)	66.5 ± 19.7	57.5 ± 22.6	0.21
Medications			
β-blocker	6 (40)	11 (44)	1.00
Calcium channel blocker	10 (67)	14 (56)	0.74
ACE inhibitor/ARB	12 (80)	20 (80)	1.00
Statin	15 (100)	23 (92)	0.52

Defined as ¹under medical treatment or systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg, ²under medical treatment or HbA_{1c} >6.5%, ³under medical treatment or total cholesterol level >220 mg/dL or low-density cholesterol level >140 mg/dL. PPI, proton pump inhibitor; H2 blocker, Histamine receptor type 2 blocker; ACE inhibitor, angiotensin-converting enzyme inhibitor; ARB, angiotensin II-receptor blocker.

MARs in the group of patients taking omeprazole and changing to rabeprazole (*n*=4) and the group of patients taking rabeprazole and changing to omeprazole (*n*=21). In the former group, the MARs during omeprazole therapy were 30.9 ± 19.8% and those during rabeprazole therapy were 20.2 ± 14.1% (*p*=0.25). In the latter group, the MARs during omeprazole therapy were 36.6 ± 15.4% and those during rabeprazole therapy were 27.9 ± 15.5% (*p*=0.0157). Thus, although the differences were not statistically significant in the former group, the MARs during omeprazole therapy tended to be higher than those during rabeprazole therapy in both groups.

VerifyNow P2Y₁₂ Reaction Units (PRUs)

Recently, the VerifyNow P2Y₁₂ assay has been widely used as a point of care assay to evaluate clopidogrel efficacy because the measurement can be performed easily and rapidly. The P2Y₁₂ reaction units (PRUs), measured by the VerifyNow system, were 205.1 ± 71.6, 216.6 ± 71.5, and 252.2 ± 64.0 in the control patients, patients taking rabeprazole and those taking omeprazole, respectively (rabeprazole vs. control, *p*=0.63, omeprazole vs. control, *p*=0.0374)

(**Fig. 3A**). The PRUs during omeprazole intake were significantly higher than in the control group, while the PRUs during rabeprazole intake were similar to the control group. **Fig. 3B** showed that PRUs during omeprazole intake were significantly higher than those during rabeprazole intake (*p*=0.0060). Thus, using the VerifyNow assay, platelet aggregability while taking omeprazole was higher than while taking rabeprazole and in the control patients.

Effect of CYP2C19 SNPs and PPIs on the Antiplatelet Function of Clopidogrel

We determined the CYP2C19 genotypes in all 25 patients in the PPI group. They were 8 EMs, 14 IMs (*1/*2: *n*=12, *1/*3: *n*=2), and 3 PMs (*2/*2: *n*=2, *2/*3: *n*=1). The Wilcoxon matched-pairs signed-ranks test showed that ADP-induced MARs during omeprazole therapy were not significantly different from those during rabeprazole therapy in both EM (omeprazole 53.8 ± 5.4% vs. rabeprazole 46.9 ± 15.1%, *p*=0.0797) and IM (omeprazole 55.8 ± 11.5% vs. rabeprazole 54.3 ± 10.3%, *p*=0.33) genotype groups. On the other hand, in the EM group, collagen-induced MARs during omeprazole therapy

Fig. 2. Effect of PPIs on the antiplatelet functions of clopidogrel evaluated by the optical aggregometer using 20 μ mol/L ADP (A and B) and 2 μ g/mL collagen (C and D) as stimuli.

A and C, MARs while undergoing dual antiplatelet therapy and taking either rabeprazole or omeprazole were compared with those not taking PPI (control) using Student's *t*-test. Data shown are the mean $\pm$ SD. B and D, MARs while undergoing dual antiplatelet therapy during rabeprazole intake or omeprazole intake are shown, and were compared using the Wilcoxon matched-pairs signed-ranks test. Box-and-whisker plots are also shown to indicate median values, the 25th and 75th percentiles (box) and minimum and maximum range of data (whisker).

($35.6 \pm 15.2\%$) tended to be higher than those during rabeprazole therapy ($23.9 \pm 13.8\%$) ($p=0.0547$) (**Fig. 4A**). In the IM group, collagen-induced MARs during omeprazole therapy ($36.9 \pm 16.5\%$) were significantly higher than during rabeprazole therapy ($27.6 \pm 16.2\%$) ($p=0.0156$) (**Fig. 4B**). In addition, PRUs measured by the VerifyNow system during omeprazole intake (251.5 ± 59.5) were significantly higher than during rabeprazole intake (197.1 ± 69.1) in the EM group ($p=0.0142$) (**Fig. 4D**). In the IM group, PRUs during omeprazole intake (269.5 ± 62.0) tended to be higher than during rabeprazole intake (232.9 ± 78.1) ($p=0.0516$) (**Fig. 4E**). As there were only 3 PM patients, we did not evaluate platelet functions.

We then analyzed platelet aggregability in the

carriers of CYP2C19 SNPs combining both IM and PM patients. In the carrier group, the MARs during omeprazole therapy ($35.7 \pm 16.6\%$) were significantly higher than during rabeprazole therapy ($28.7 \pm 15.7\%$) ($p=0.0351$) (data not shown) and PRUs during omeprazole intake (252.5 ± 67.8) tended to be higher than during rabeprazole intake (225.7 ± 72.8), although this was not statistically significant ($p=0.14$) (data not shown).

Thus, it was most likely that clopidogrel effectiveness was decreased by concomitant intake of omeprazole, and that the effect of omeprazole was relatively strong in the EM group compared to carrier patients with CYP2C19 SNPs.

Fig. 3. Effect of PPIs on the antiplatelet functions of clopidogrel evaluated by the VerifyNow P2Y12 assay.

A, PRUs while undergoing dual antiplatelet therapy and taking either rabeprazole or omeprazole were compared with those not taking PPI (the control) using Student's *t*-test. Data shown are the mean $\pm$ SD. B, PRUs while undergoing dual antiplatelet therapy and taking either rabeprazole or omeprazole are shown, and compared using the Wilcoxon matched-pairs signed-ranks test. Box-and-whisker plots are also shown to indicate median values, the 25th and 75th percentiles (box) and minimum and maximum range of data (whisker).

Fig. 4. Effect of CYP2C19 genotypes on the antiplatelet functions of clopidogrel during PPI therapy.

Platelet aggregability while undergoing dual antiplatelet therapy and taking either rabeprazole or omeprazole was evaluated by the optical aggregometer using 2 μ g/mL collagen (A-C) as stimulus and by the VerifyNow P2Y12 assay (D-F), and compared using the Wilcoxon matched-pairs signed-ranks test. Individual data are shown according to CYP2C19 genotypes; the EM (A and D), IM (B and E), and PM (C and F). Data are also shown as the mean $\pm$ SD.

Effect of Famotidine, an H2 Blocker, on the Antiplatelet Function of Clopidogrel

Famotidine, an H2 blocker, has been demonstrated to prevent gastrointestinal bleeding during aspirin therapy²⁰, and is recommended as an alternative drug for PPIs, especially for patients at low risk of gastrointestinal bleeding¹⁹. We therefore studied the effect of famotidine on the antiplatelet function of clopidogrel in another study. Thirty patients (64.2 ± 9.9 years old, 83% male) undergoing DAPT due to prior coronary stent implantation who were not receiving gastrointestinal protective therapy with PPI or H2 blocker were enrolled (**Table 2**). After platelet function tests were performed at the 2nd visit (**Fig. 5A**), famotidine was started in addition to their usual medications. Their platelet function was again evaluated after 37.5 (33.25–56) days on the 3rd visit during famotidine therapy. As shown in **Fig. 5B** and **5C**, famotidine had little effect on platelet aggregability while taking clopidogrel according to the optical aggregometer using ADP or collagen as a stimulus. Famotidine might even ameliorate the antiplatelet effect of clopidogrel since the MARs during famotidine therapy were significantly lower than before famotidine was given, evaluated with $20 \mu\text{mol/L}$ ADP as a stimulus (**Fig. 5B**). The VerifyNow study also revealed that famotidine had no effect on platelet aggregability during clopidogrel intake (**Fig. 5D**). Thus, famotidine did not adversely affect the antiplatelet function of clopidogrel.

Discussion

Here we evaluated the influences of two kinds of PPIs, omeprazole and rabeprazole, and an H2 blocker, famotidine, on the antiplatelet effect of clopidogrel in patients undergoing DAPT with aspirin and clopidogrel. We found that platelet aggregability when taking omeprazole was higher than when taking rabeprazole, evaluated by an optical aggregometer and the VerifyNow P2Y12 assay. Furthermore, we showed that platelet aggregability during rabeprazole intake was comparable to that of controls who were undergoing DAPT without taking a PPI or H2 blocker. Therefore, we concluded that omeprazole significantly reduced the antiplatelet effect of clopidogrel and that the effect of omeprazole on antiplatelet function was stronger than that of rabeprazole. We further found that concomitant use of famotidine had no effect.

In this study, we showed that omeprazole significantly reduced the antiplatelet effect of clopidogrel in Japanese patients, which was consistent with previous reports in Western patients^{16, 26}. On the other hand,

Table 2. Patient characteristics of the H2 blocker study

	H2 blocker (<i>n</i> =30)
General characteristics	
Age (years)	64.2 ± 9.9
Male (% of total)	25 (83)
Risk factors	
Hypertension ¹	17 (57)
Diabetes ²	13 (43)
Hyperlipidemia ³	27 (90)
Current smoker	20 (67)
Prior myocardial infarction	13 (43)
Hematological data	
Hb (g/dL)	13.8 ± 1.7
Plt ($\times 10^9/\text{L}$)	215.0 ± 56.4
AST (IU/L)	24.1 ± 15.3
ALT (IU/L)	25.4 ± 16.3
eGFR (mL/min/1.73 m ²)	67.1 ± 13.0
Medications	
β -blocker	9 (30)
Calcium channel blocker	13 (43)
ACE inhibitor/ARB	19 (63)
Statins	28 (93)

Definitions and abbreviations are as described in Table 1.

Furuta *et al.*²⁷ have shown that omeprazole tended to reduce the antiplatelet function of clopidogrel in Japanese healthy volunteers ($p=0.094$), but the inhibition was not statistically significant. This could be due to a difference in the study design since Furuta *et al.* evaluated the effect of clopidogrel in healthy volunteers who had just started to take clopidogrel alone, whereas we evaluated patients undergoing long-term DAPT by the cross-over method.

We showed that platelet aggregability under DAPT when taking rabeprazole was comparable to that in control patients and lower than when taking omeprazole by both the VerifyNow P2Y12 assay and the optical aggregometer using collagen as a stimulus. This could be due to the lower contribution of CYP2C19 to the metabolism of rabeprazole than omeprazole²⁸.

In our study, the inhibitory effect of omeprazole on the antiplatelet function of clopidogrel was detected in both EM and IM patients. Among the two patient groups, the omeprazole effect tended to be stronger in EM patients than IM patients. Furuta *et al.*²⁵ have reported that both omeprazole and rabeprazole decreased the antiplatelet function of clopidogrel in only EM patients. These data suggested that the effect of PPIs on clopidogrel efficacy could be more marked in CYP2C19 EM patients than in IM patients,

Fig. 5. Effect of famotidine, an H2 blocker, on the antiplatelet functions of clopidogrel.

A, Schematic presentation of the study protocol. B-D, Platelet aggregability while undergoing dual antiplatelet therapy before and after receiving famotidine was evaluated by the optical aggregometer using 2 μ g/mL collagen (B) or using 20 μ mol/L ADP (C) as stimulus and by the VerifyNow P2Y12 assay (D), and compared using the Wilcoxon matched-pairs signed-ranks test. Data shown are the mean $\pm$ SD. Box-and-whisker plots are also shown to indicate the median values, the 25th and 75th percentiles (box), and minimum and maximum range of data (whisker).

although theoretically, IM patients with approximately half-normal enzyme activity⁸⁾ might be affected most strongly.

In the VerifyNow P2Y12 assay developed specifically for the evaluation of clopidogrel efficacy using ADP as stimulus, we showed that omeprazole significantly reduced the antiplatelet effect of clopidogrel and that the effect of omeprazole on antiplatelet function was stronger than that of rabeprazole.

The PPI effect on clopidogrel-induced antiplatelet function was observed more clearly when assessed by the optical aggregometer using collagen than ADP as a stimulus in our study, though the precise reason is not known. We previously reported that the antiplate-

let effect of clopidogrel during DAPT could be detected more sensitively using collagen than ADP as a stimulus by the same method²⁵⁾, suggesting that the intraplatelet signaling pathway might be shifted to the P2Y12 ADP-receptor pathway under aspirin therapy, in which platelets cannot adequately generate thromboxane A₂. This could partially explain why the effect of omeprazole was more sensitively detected by the optical aggregometer using collagen as a stimulus.

In the present study, omeprazole significantly reduced the antiplatelet effect of clopidogrel, although the degree of the reduction was not high, as shown in previous studies^{16, 26)}. Accordingly, the effects of these drug interactions might be subclinical since it was

shown in clinical studies such as TRITON-TIMI 38²⁹⁾ and COGENT³⁰⁾ that concomitant use of omeprazole did not increase cardiovascular events in patients undergoing DAPT after coronary stent implantation. Nevertheless, the package insert box warning recommends “to avoid concomitant use of clopidogrel and omeprazole” in the US, and “to take care when omeprazole is used with clopidogrel” in Japan. Further study is needed to evaluate the clinical outcomes of the concomitant use of clopidogrel and a PPI in Japanese patients.

Famotidine, which is not metabolized theoretically by CYP2C19, has been demonstrated to prevent gastric mucosal injury during aspirin intake²⁰⁾, although this preventive effect is weaker than that of PPIs²¹⁾. Here, we prospectively examined the effect of famotidine on the antiplatelet function of DAPT with aspirin and clopidogrel. The results clearly demonstrated that famotidine had no effect on the antiplatelet function of clopidogrel in patients in a real clinical setting.

Finally, this study has some limitations. First of all, the sample size was small due to the difficulty of finding suitable patients for the crossover study. Second, only 4 patients (16%) switched omeprazole to rabeprazole. Third, further investigation is needed, increasing the number of PM patients.

Conclusion

Omeprazole significantly reduced the antiplatelet effect of clopidogrel and the effect of omeprazole on the antiplatelet function of clopidogrel was stronger than that of rabeprazole in a real clinical setting in Japan. Concomitant use of famotidine as a gastrointestinal protective agent had no effect on the antiplatelet effect of clopidogrel.

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Disclosures

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