Influences of different proton pump inhibitors on the anti-platelet function of clopidogrel in relation to *CYP2C19* genotypes

Takahisa Furuta,¹ Takayuki Iwaki² & Kazuo Umemura²

¹Center for Clinical Research and ²Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan

Correspondence

Dr Takahisa Furuta MD PhD, Center for Clinical Research, Hamamatsu University School of Medicine, 1-20-1. Handayama, Higashi-Ku, Hamamatsu 431-3192, Japan.
Tel.: +81 53 435 2850
Fax: +81 53 435 2851
E-mail: furuta@hama-med.ac.jp

Keywords

clopidogrel, *CYP2C19*, drug–drug interaction, genotype, platelet function, proton pump inhibitor

Received

13 January 2010

Accepted

21 April 2010

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Active metabolism of clopidogrel is mainly mediated by *CYP2C19*. There are genetic differences in the activity of *CYP2C19*. Therefore, active metabolism of clopidogrel is affected by *CYP2C19* genotypes.
- The main metabolizing enzyme of proton pump inhibitors (PPIs) is *CYP2C19*. Therefore, the anti-platelet function of clopidogrel is attenuated by concomitant use of PPIs.
- There are differences in the metabolic disposition among different PPIs. Affinity to *CYP2C19* differs among different PPIs.

WHAT THIS STUDY ADDS

- Whether a PPI attenuates the efficacy of clopidogrel depends on *CYP2C19*. Individuals who are decreased metabolizers, i.e. carriers the allele of *CYP2C19* *2 and/or *3, are more likely to convert from 'responder' to 'non-responder' to clopidogrel when placed on a concomitant PPI.
- We found that rabeprazole, whose affinity to *CYP2C19* has been considered lower, attenuated the efficacy of clopidogrel.
- We tested whether the separate dosing of a PPI and clopidogrel decreased the risk of attenuation of clopidogrel efficacy. We unfortunately found that separate dosing did not avoid the problematic interaction between clopidogrel and a PPI in subject's with *CYP2C19* *2 and/or *CYP2C19* *3.

AIMS

The efficacy of clopidogrel is influenced by *CYP2C19* genotypes and substrates of *CYP2C19*, such as proton pump inhibitors (PPIs). We assessed the influence of three different PPIs on the anti-platelet function of clopidogrel in relation to *CYP2C19* genotype status.

METHODS

Thirty-nine healthy volunteers with different *CYP2C19* genotypes took clopidogrel 75 mg with or without omeprazole 20 mg, lansoprazole 30 mg or rabeprazole 20 mg in the morning for 7 days. The influence of the three PPIs on the anti-platelet function of clopidogrel was determined. A less than 30% inhibition of platelet aggregation (IPA) during clopidogrel dosing was defined as a 'low responder'. We also examined whether evening dosing of omeprazole could prevent the interaction with clopidogrel dosed in the morning.

RESULTS

In rapid metabolizers (RMs, *1/*1, $n = 15$) of *CYP2C19*, omeprazole and rabeprazole significantly attenuated the anti-platelet function of clopidogrel. In decreased metabolizers (DMs, carriers of *2 and/or *3, $n = 24$), there was a large variation in IPA and there was a trend but no significant decrease in IPA when placed on a concomitant PPI. Some DMs became 'low-responders' when placed on a concomitant PPI. Evening omeprazole dose in RMs did not seem to cause a significant decrease in IPA in contrast to morning dosing, but did so in DMs.

CONCLUSIONS

The three PPIs affected the efficacy of clopidogrel to different degrees. Both omeprazole and rabeprazole significantly decreased IPA in RMs but not DMs, although there was a trend towards lower IPA in DMs. Morning and evening dosing of omeprazole were both associated with lower IPA in DMs.

Introduction

Anti-platelet therapy is now widely used in patients with stroke, myocardial infarction or peripheral arterial disease. Clopidogrel and aspirin are the most important cornerstone agents in this therapy. To prevent stent thrombosis after implantation of bare metal and drug-eluting stents, dual anti-platelet therapy with aspirin and clopidogrel is now a standard therapy [1]. However, such antiplatelet therapy increases the risk of gastrointestinal bleeding [2–7]. A proton pump inhibitor (PPI), therefore, is often prescribed with anti-platelet agents. In 2008, the American College of Cardiology Foundation (ACCF), the American College of Gastroenterology (ACG) and American Heart Association (AHA) published their statement on antiplatelet therapy which recommends the prescription of a PPI to patients with a risk of peptic ulcer and/or on two or more antiplatelet agents [8–10].

The drug–drug interaction between clopidogrel and a PPI has been receiving attention lately. Clopidogrel is metabolized by CYP2C19 to form its active metabolites. Therefore, the plasma concentration of the active metabolite of clopidogrel depends on the activity of CYP2C19 [11]. CYP2C19 is also the main metabolizing enzyme of PPIs. Therefore, concomitant use of clopidogrel and a PPI induces a drug–drug interaction, resulting in the decreased activation of clopidogrel, which attenuates its the anti-platelet effect and could lead to an increased risk of re-infarction and/or stent thrombosis. PPIs *per se* are recognized as inhibitors of CYP2C19 [12], which would also contribute to the attenuation of activation of clopidogrel. Juurlink *et al.* [13] reported that concomitant therapy with a PPI other than pantoprazole was associated with an increased risk of re-infarction. Ho *et al.* [14] reported that concomitant use of clopidogrel and a PPI was associated with an increased risk of adverse outcomes compared with the use of clopidogrel alone. Toth *et al.* [15] reported that the efficacy of clopidogrel can also be reduced if patients are receiving concomitant therapy with a PPI such as omeprazole. However, Siller-Matula *et al.* [16] reported that the intake of pantoprazole or esomeprazole is not associated with an impaired response to clopidogrel. Thus, the influence of PPIs on clopidogrel efficacy seems controversial.

Interestingly, the influences of PPIs on CYP2C19 differ among different PPIs. Omeprazole is a substrate with strong affinity to CYP2C19. Lansoprazole is also a substrate for CYP2C19 but with a weak affinity in comparison with omeprazole. The metabolism of rabeprazole has been thought to be less associated with CYP2C19 [17]. There have been several reports on drug–drug interactions between omeprazole and other drugs (e.g. warfarin, phenytoin, diazepam), while incidences of drug–drug interactions of rabeprazole and lansoprazole are not so many as observed with omeprazole [12]. However, it has not been fully elucidated whether this difference in the

affinity to CYP2C19 among different PPIs results in different effects on the anti-platelet function of clopidogrel.

Another problem with clopidogrel is interindividual difference in its anti-platelet activity among the different CYP2C19 genotype groups [11]. Patients with intermediate or poor metabolizer genotype of CYP2C19 are at a higher risk of stent thrombosis and re-infarction because of decreased active metabolism of clopidogrel [18–22]. However, no study has evaluated the influence of CYP2C19 genotype status and different PPIs on the anti-platelet function of clopidogrel simultaneously.

Based on the backgrounds mentioned above, we prospectively examined the effect of three proton pump inhibitors, omeprazole, lansoprazole and rabeprazole, on the anti-platelet function of clopidogrel in relation to CYP2C19 genotype status. In addition, we examined whether the interaction between a PPI and clopidogrel could be avoided by administering the two drugs separately instead of taking them concomitantly.

Methods

Subjects

Thirty-nine healthy volunteers were enrolled in this study. None had taken any drug and smoked for at least 2 weeks before and during this study. Written informed consent was obtained from each of the subjects before their participation in the study. The protocol was approved in advance by the Human Institutional Review Board of Hamamatsu University School of Medicine, Hamamatsu, Japan. Clinical demographic characteristics of the subjects are summarized in Table 1. The study was performed from February 2009 to June 2009.

Genotyping of CYP2C19

DNA was extracted from blood samples obtained from volunteers using a commercially available kit (Genomix,

Table 1

Demographic clinical characteristics of subjects enrolled to the study

Parameters	RMs n = 15	DMs n = 24	P values
CYP2C19 genotypes	*1/*1: n = 15	*1/*2: n = 13 *1/*3: n = 9 *2/*2: n = 0 *2/*3: n = 2 *3/*3: n = 0	
Male/Female	14/1	18/6	>0.2
Age (years)	22.4 ± 2.1	24.0 ± 5.7	>0.2
Height (cm)	172.7 ± 8.2	168.7 ± 6.4	>0.2
BW (kg)	64.9 ± 10.9	61.7 ± 9.3	>0.2
BMI (kg m ⁻²)	21.7 ± 2.4	21.6 ± 2.1	>0.2

DM, decreased metabolizer of CYP2C19; RM, rapid metabolizer of CYP2C19.

Figure 1

Schematic demonstration of the study protocol. Firstly, all 39 subjects took 75 mg clopidogrel at 08.00 h for 7 days. Next, all subjects took clopidogrel 75 mg with a different PPI, 20 mg omeprazole, 30 mg lansoprazole or 20 mg rabeprazole. The order of the three PPIs was randomized. All medications were taken once daily at 08.00 h. There was a washout period of at least 2 weeks between the two study periods. In the second study, 30 of 39 subjects took 20 mg omeprazole (OPZ) at 20.00 h and 75 mg clopidogrel at 08.00 h for 7 days. Platelet aggregation induced by 20 μ M of ADP was measured before the study and at 4 h after the last dose of clopidogrel on the 7th day of each study period

Talent, Trieste, Italy). DNA samples were genotyped for *CYP2C19* as previously reported [23] to identify the *CYP2C19* wild type (*1) gene and two mutant alleles, *CYP2C19* *2 (*2) in exon 5 and *CYP2C19* *3 (*3) in exon 4. Volunteers were then classified into three groups by genotype, namely rapid metabolizers (RMs) (*1/*1), intermediate metabolizers (IMs) (*1/*2 or *1/*3) and poor metabolizers (PMs) (*2/*2, *3/*3, or *2/*3) [23]. The presence of the *CYP2C19* *17 (*17) allele (ultra rapid metabolizer) was also determined for all DNA samples as previously reported [24].

Study protocol

This was the open-label single-arm crossover study. The schematic protocol is shown in Figure 1. Firstly, all 39 subjects took 75 mg of clopidogrel at 08.00 h for 7 days. Platelet aggregation induced by 20 μ M of ADP was measured before the first dose and 4 h (at 12.00 h) after the last dose of clopidogrel for 7 days to calculate the baseline levels of inhibition of platelet aggregation (IPA) (%), a representative index of anti-platelet function of clopidogrel, as described later for each subject. Next, all subjects participated in a crossover study of 7 days dosing of clopidogrel 75 mg with a different PPI. They took 75 mg of clopidogrel with 20 mg of omeprazole (Omepral®, AstraZeneca K.K., Osaka, Japan), 30 mg of lansoprazole (Takepuron®, Takeda Pharmaceutical Co Ltd. Osaka, Japan) or 20 mg of rabeprazole (Pariet®, Eisai Co. Ltd, Tokyo, Japan). The order of the three PPIs was randomized. All medications were taken once daily at 08.00 h. There was a washout period of at least 14 days between different PPI dosings. Compliance was confirmed by sending a reminder e-mail every

morning and by receiving a response from each subject confirming the completion of taking the drugs.

Of 39 subjects, 30 participated in the second study, to examine whether the separate dose of a PPI and clopidogrel could prevent the drug–drug interaction between them. They took 20 mg omeprazole at 20.00 h in the evening and 75 mg clopidogrel at 08.00 h the next morning for 7 days. Platelet aggregation induced by 20 μ M of ADP was measured at 4 h after the last dose of clopidogrel on the 7th day as noted above.

Measurement of platelet aggregation

Platelet aggregation was measured as previously reported [11]. In brief, blood samples were collected in test tubes containing 1/10 volume of 3.2% trisodium citrate, and platelet-rich and platelet-poor plasma were prepared by differential centrifugation at room temperature (150 g [900 rev min⁻¹] for 15 min for platelet-rich plasma and 1710 g [3000 rev min⁻¹] for 15 min for platelet-poor plasma). Maximum platelet aggregation (MPA) was determined in response to 20 μ M ADP by light transmittance aggregometry using MCM hematracr 313-M (SSR engineering Co. LTD., Tokyo, Japan). MPA was measured by an expert technician who was unaware of any of information about the subjects. The inhibition of platelet aggregation (IPA)(%) was calculated from the observed MPA value at each scheduled time point with each treatment where:

$$\text{IPA} = \frac{[(\text{MPA baseline} - \text{MPA postdose}) / \text{MPA baseline}] \times 100\%}{}$$

IPA <30% was defined as a ‘low-responder’ [25, 26].

Data analysis

All numerical data are given as mean $\pm$ standard deviation (SD). Statistically significant differences in means of age and body weight between the two *CYP2C19* genotype groups were assessed by Student’s *t*-test. The male : female ratios between the two genotype groups were assessed by Fisher’s exact test. Statistically significant difference in changes in IPA with different regimens between the two *CYP2C19* genotype groups were assessed by repeated measures ANOVA and Scheffe’s multiple comparison test. All *P* values were two-sided and *P* < 0.05 indicated statistical significance.

Results

CYP2C19 genotype and compliance

The study subjects consisted of 15 rapid metabolizers (RMs *1/*1), 22 intermediate metabolizers (IMs *1/*2: *n* = 13, *1/*3: *n* = 9) and two poor metabolizers (PMs *2/*3: *n* = 2). There were no subjects who had the *17 allele. All subjects completed the study protocol without any adverse events. Because the number of PMs was limited (only two), IMs and

Figure 2

Changes in the inhibition of platelet aggregation (IPA) by clopidogrel with different PPIs in the whole group (A) and in different *CYP2C19* genotype groups (B). The grey zone indicates the low-responders. The anti-platelet function of clopidogrel was not influenced by any of the three PPIs, omeprazole (OPZ), lansoprazole (LPZ) or rabeprazole (RPZ), in the whole group (A). When separately analyzed according to *CYP2C19* genotype groups, statistically significant differences in the inhibition of platelet aggregation by clopidogrel were found (B). This difference was maintained during the concomitant dosing with different PPIs. Omeprazole (OPZ) and rabeprazole (RPZ) significantly attenuated the inhibition of platelet aggregation by clopidogrel in rapid metabolizers (RMs) of *CYP2C19* (B). (B): Rapid metabolizer ($n = 15$) (—○—); Decreased metabolizer ($n = 24$) (—●—)

PMs were combined into one group, named as the decreased metabolizers (DMs) of *CYP2C19*, who had *2 and/or *3 allele (Table 1).

Effect of concomitant dose of PPIs on anti-platelet function of clopidogrel

The mean IPAs induced by clopidogrel alone, clopidogrel with omeprazole, clopidogrel with lansoprazole and clopidogrel with rabeprazole were 45.0%, 40.2% ($P = 0.094$ vs. clopidogrel alone), 44.2% ($P = 0.724$) and 44.4% ($P = 0.825$), respectively. The mean of IPA induced by clopidogrel was not significantly decreased by any of the three PPIs. The effect of omeprazole did not reach the statistical significance. (Figure 2A).

When data were stratified based on *CYP2C19* genotype status to RMs and DMs, the IPA of RMs was significantly higher than in DMs in any of the study regimen (Figure 2B). The mean IPA induced by clopidogrel alone, clopidogrel with omeprazole, clopidogrel with lansoprazole and clopidogrel with rabeprazole in RMs was 58.3%, 51.2% ($P = 0.015$, vs. clopidogrel alone), 56.5% ($P = 0.508$) and 53.5% ($P = 0.035$), respectively, and that in DMs was 36.6%, 33.3% ($P = 0.443$), 36.4% ($P = 0.951$) and 38.7% ($P = 0.635$), respec-

tively. In RMs, omeprazole and rabeprazole significantly decreased the mean IPA induced by clopidogrel. On the other hand, in DMs, the IPA appeared to be decreased by omeprazole, but the difference did not reach statistical significance due to the wide distribution of IPA values.

The incidence of 'responder' (IPA $\geq 30\%$) and 'low responder' (IPA $< 30\%$) in different regimens as a function of *CYP2C19* status is summarized in Table 2. The incidence of low-responders in the RM group ($n = 15$) were 0 (0%), 0 (0%), 0 (0%) and 1 (7%) in the regimens with clopidogrel alone, clopidogrel and omeprazole, clopidogrel and lansoprazole, and clopidogrel and rabeprazole, respectively. Those in the DM group ($n = 24$) were 8 (33%), 10 (42%), 6 (25%) and 6 (25%), respectively.

Changes in IPA by different PPIs in individual subjects in the RM and DM groups are shown in Figure 3A, B, respectively. The IPA did not decrease to the levels of 'low-responder' in RMs except in one case when rabeprazole was dosed (Figure 3A) as noted above, although the mean IPA was significantly decreased by omeprazole and rabeprazole in RMs. On the other hand, the mean IPA at baseline in DMs was close to the threshold line of $< 30\%$ and eight of the 24 DMs (30%) were in the 'low-responder'

Table 2

Incidence of 'responder' and 'low responder' to clopidogrel in different regimens

Regimen		Responder/Non-responder (n) Clopidogrel alone	Clopidogrel + OPZ	Clopidogrel + LPZ	Clopidogrel + RPZ
CYP2C19	RM	15/0 (100%/0%)	15/0 (100%/0%)	15/0 (100%/0%)	14/1 (93%/7%)
	DM	16/8 (67%/33%)	14/10 (58%/42%)	18/6 (75%/25%)	18/6 (75%/25%)

DM, decreased metabolizer of CYP2C19 who has *2 and/or *3 allele of CYP2C19; LPZ, lansoprazole; OPZ, omeprazole; RM, rapid metabolizer of CYP2C19 (*1/*1); RPZ, rabeprazole.

Figure 3

Individual changes in inhibition of platelet aggregation by clopidogrel with or without different PPIs. The connecting lines should be used to trace the IPA values for each subject. The order of three PPIs was randomized. In the rapid metabolizers of CYP2C19 (RM), omeprazole (OPZ) and lansoprazole (LPZ) influenced the efficacy of clopidogrel, but did not decrease it to the level of low-responders except in one case. In the decreased metabolizers (DM), there was a wide distribution in the degree of inhibition of platelet aggregation by clopidogrel. Of 24 decreased metabolizers, 16 were not in the low-responder group. PPIs affected the efficacy of clopidogrel. Of the 16 subjects not in the low-responder group, five, one and two became non-responders after concomitant dosing with omeprazole, lansoprazole or rabeprazole (RPZ), respectively. Dotted lines indicate the IPA of poor metabolizers (*2/*3)

group before concomitant PPI dosing. When clopidogrel was dosed with a PPI, IPA showed a wide variability and some subjects who were in the 'responder' group during the dosing of clopidogrel alone became 'low-responders' after receiving PPIs. Of the 16 DMs who were judged as 'responders' during the dosing with clopidogrel alone, 5 (31%), 1 (6%) and 2 (13%) became 'low-responders' after receiving a concomitant omeprazole, lansoprazole or rabeprazole, respectively. (Figure 3B). Although Table 2 suggests that the incidence of low responders did not change so much with the concomitant use of a PPI, in fact only 10 of them remained in the 'responder group' irrespective of PPI dosing and six of 16 became 'low responders' when dosed with either of three PPIs. This conversion rate (6/16, 38%) in DMs appeared higher than that in RMs (1/15,

7%) ($P = 0.083$). On the other hand, six of eight DMs (75%) who were in the 'low-responder' group with clopidogrel alone converted to 'responders' when either of three PPIs was concomitantly dosed. Thus, the concomitant use of a PPI made the effect of clopidogrel widely variable, especially in DMs.

Effect of separate doses of PPIs on the anti-platelet function of clopidogrel

Of the 39 subjects, 30 completed the second study. IPA induced by clopidogrel alone in the morning, clopidogrel plus omeprazole in the morning, clopidogrel in the morning plus omeprazole in the evening were 49.5%, 41.5% ($P = 0.014$ vs. clopidogrel alone) and 43.4% ($P = 0.044$), respectively. As a whole, the effect of clopidogrel

Figure 4

The effect of morning (Mor) or evening (Eve) dose of omeprazole on the efficacy of clopidogrel in the whole group (A) and in different *CYP2C19* genotype groups (B). Decreased efficacy of clopidogrel by the concomitant dosing of omeprazole was not restored by the separate dosing of omeprazole as a whole (A). When separately analyzed according to *CYP2C19* genotype, the decreased efficacy of clopidogrel with concomitant dosing with omeprazole was restored by a separate dosing of omeprazole in the rapid metabolizers (RMs) of *CYP2C19*. In the decreased metabolizers (DM), omeprazole appeared to decrease the efficacy of clopidogrel, which seemed not to be restored by the separate dosing of omeprazole. (B): Rapid metabolizer (n = 14) (—○—); Decreased metabolizer (n = 16) (—●—)

was significantly attenuated by a concomitant dose of omeprazole, which could not be improved when omeprazole was dosed separately in the evening (Figure 4A).

The data were analyzed according to *CYP2C19* genotype status. In RMs of *CYP2C19* (n = 14), the effect of clopidogrel was attenuated by co-administration of omeprazole (from 59.1% to 51.8%; $P = 0.021$) but appeared to be improved when omeprazole was dosed separately in the evening (56.2%; $P = 0.229$ vs. clopidogrel alone). However, in the DM group (n = 16), the attenuated effect of clopidogrel by omeprazole dosed concomitantly did not appear to be improved by separate dosing of omeprazole in the evening (41.2%, 35.2%; $P = 0.124$ vs. clopidogrel alone, 32.2%; $P = 0.096$) (Figure 4B).

In the RMs, regardless of whether omeprazole was dosed concomitantly in the morning or separately in the evening, none was judged to be a 'low-responder' (Figure 5A). On the other hand, some of the DMs converted to 'responders' after the separate dosing of omeprazole and clopidogrel, while some DMs converted to 'low responders' with the same regimen (Figure 5B).

Discussion

We evaluated the influences of PPIs on the effect of clopidogrel in relation to *CYP2C19* genotypes and found that any of three PPIs (i.e. omeprazole 20 mg, lansoprazole 30 mg and rabeprazole 20 mg) could cause attenuation of the anti-platelet function of clopidogrel. We observed that the influence of a PPI on the effect of clopidogrel differed between the *CYP2C19* genotype groups and that drug–drug interactions between clopidogrel and PPIs resulting in the decrease in IPA to the level of 'low-responder' were likely to occur in DMs of *CYP2C19*, who are carrying the decreased function allele of *CYP2C19* (i.e. *2 and/or *3). We also observed that separate dosing of a PPI (i.e. clopidogrel in the morning and a PPI in the evening) did not prevent the drug–drug interaction between clopidogrel and a PPI. Therefore, we have to reconsider the risk and benefit balance of the concomitant use of clopidogrel and a PPI with reference to *CYP2C19* genotype status.

There appear to be differences in the effects on clopidogrel among the different PPIs. Juurlink *et al.* [13] have indicated that omeprazole, lansoprazole and rabeprazole,

Figure 5

Individual changes in inhibition of platelet aggregation by clopidogrel with morning (Mor) or evening (Eve) dosing of omeprazole in different *CYP2C19* genotype groups. The connecting lines should be used to trace the IPA of each subject. The order of three PPIs was randomized. In the rapid metabolizers of *CYP2C19* (RM), none became a low-responder (A). In the decreased metabolizers (DMs), four of seven subjects classified as low-responders during concomitant dosing of clopidogrel and omeprazole become responders, but two subjects not classed as low-responders during concomitant dosing with clopidogrel and omeprazole became low-responders after the separate dosing of clopidogrel and a PPI. The dotted line indicates the IPA of a poor metabolizer (*2/*3)

but not pantoprazole, attenuate the clinical effect of clopidogrel in patients with cardiovascular disorders. Ho *et al.* [14] have reported that omeprazole and rabeprazole attenuate the clinical effect of clopidogrel. Sibbing *et al.* [27] reported that omeprazole impaired the anti-platelet function of clopidogrel, but pantoprazole and esomeprazole did not. Gilard *et al.* [28] demonstrated that omeprazole impaired the anti-platelet function of clopidogrel [16, 27–29]. Small *et al.* [29] reported that lansoprazole decreased the anti-platelet function of clopidogrel. Siller-Matula *et al.* [16] reported that intake of pantoprazole or esomeprazole was not associated with impaired response to clopidogrel. On the other hand, Zuern *et al.* [30] reported that co-administration of PPIs including pantoprazole and esomeprazole significantly decreased the effect of clopidogrel on platelet aggregation. Together, all PPIs used in clinical practice appear to have the potential to attenuate the efficacy of clopidogrel. However, the recent report by O'Donoghue *et al.* [31] demonstrated that a clinically problematic interaction was not observed between clopidogrel and a PPI, although the *ex vivo* study demonstrated that PPI use attenuated the anti-platelet effect of clopidogrel. Rassen *et al.* [32] did not observe any conclusive evidence of a clopidogrel–PPI interaction of major clinical relevance. Therefore, a drug–drug interaction between clopidogrel and a PPI has been controversial.

In the present study, we tested omeprazole, lansoprazole and rabeprazole and found that the influence of three PPIs on the anti-platelet function of clopidogrel differed among them. However, the levels of attenuation of clopidogrel by PPIs depended on *CYP2C19* genotype status. Although the efficacy of clopidogrel was decreased by a PPI in RMs of *CYP2C19*, the levels of anti-platelet function of clopidogrel after attenuation by a PPI in this group were mostly not problematic (i.e. rarely decreased to the levels of 'low-responder'). Of 15 RMs, only one case became 'low-responder' after concomitant use of rabeprazole 20 mg. On the other hand, the efficacy of clopidogrel appeared to be unstable with concomitant use of any of the PPIs used in the present study especially in the DM group. Six of 16 DMs who were judged as 'responders' during the dosing with clopidogrel alone became 'low responders' when clopidogrel was dosed with either of three PPIs, suggesting that DMs could be at a higher risk of becoming a 'low-responder' to clopidogrel and could easily become 'low responders' when clopidogrel was dosed with a PPI. Therefore, we have to be careful with the concomitant use of clopidogrel and a PPI in DMs of *CYP2C19* in particular.

The drug–drug interactions of PPIs with other drugs through cytochrome P450s have not been considered so problematic. Several drug–drug interactions have been reported with omeprazole, but such reports on lansopra-

zole and rabeprazole have been rare [12]. However, recent reports and our results have demonstrated that omeprazole as well as lansoprazole and rabeprazole attenuate the efficacy of clopidogrel. The following explanations could be offered as the reason why the PPIs that had few reports on drug–drug interactions with many drugs influenced clopidogrel as shown in this study. The major metabolic route of clopidogrel is its metabolism by esterase to the inactive metabolite (SR26334) and the active metabolism of clopidogrel by CYP2C19 is a minor route [33]. Moreover, CYP2C19 catalyzes the two step activation of clopidogrel requires metabolism by CYP2C19 twice [33]. Therefore, in the subjects with decreased activity of CYP2C19, such as DMs, active metabolism of clopidogrel seemed to be easily impaired by concomitant use of a PPI. Moreover, plasma concentrations of PPIs in DMs of CYP2C19 are higher and sustained longer in comparison with RMs [34–36], which could further attenuate the active metabolism of clopidogrel.

However, in our study, some DMs, who were judged to be ‘low responders’, converted to ‘responders’ when a PPI was dosed. There is wide variation in the activity of CYP2C19 in DMs of CYP2C19. The individuals who were judged as the ‘low responders’ might have a lower activity of CYP2C19. However, omeprazole is known to induce CYP1A2 in individuals with lower activity of CYP2C19 [37]. Therefore, the concomitant use of a PPI might induce CYP1A2, which metabolized clopidogrel to the active metabolites, resulting in conversion of some ‘low-responders’ to ‘responders’. Together, concomitant use of a PPI with clopidogrel makes the effect of clopidogrel unstable.

There were wide variation in the IPA in the DMs of CYP2C19, as demonstrated in our previous report [11]. We cannot offer any appropriate explanation for the wide variation in clopidogrel efficacy in DMs. However, we are tempted to hypothesize that most of the DMs are heterozygous for a wild type allele and a mutated allele. Which of the two alleles, a mutated allele or a wild type allele, is dominant, may differ among different individuals. DMs in whom a mutated allele is dominant may show the lower efficacy of clopidogrel and appear ‘low-responders’. On the other hand, DMs in whom the wild type allele is dominant, where clopidogrel is extensively metabolized to the active metabolites, appear ‘responders’.

In the present study, we tested whether the separate dosing of a PPI (i.e. clopidogrel in the morning and omeprazole in the evening) could prevent the drug–drug interaction between clopidogrel and a PPI. However, our study results indicated that separate dosing could not prevent the drug–drug interaction between clopidogrel and a PPI. The results in the RM group suggested that the evening dosing of omeprazole could decrease the influence of omeprazole on the efficacy of clopidogrel dosed in the morning. However, as shown in the present study, the RMs rarely became non-responders even if clopidogrel was

dosed concomitantly with a PPI. Therefore, whichever dosing scheme, concomitant or separate dosing of clopidogrel and a PPI, does not appear to be a problem in RMs.

On the other hand, in the DM group, the separate dosing of clopidogrel and omeprazole could not prevent the attenuation of clopidogrel efficacy by omeprazole. Because the plasma half life and C_{max} of omeprazole in the DM group are increased in comparison with RMs [34] and because activity of CYP2C19 to metabolize clopidogrel to its active metabolite is decreased in comparison with RMs, omeprazole dosed in the evening could last for a long in the systemic circulation and interfere with the efficacy of clopidogrel dosed in the morning in DMs.

The prophylactic use of a PPI for GI bleeding in patients undergoing anti-platelet therapy has been recommended [8–10]. However, our results indicate that prophylactic use of a PPI should be based on CYP2C19 genotype status. In the RMs, clopidogrel is extensively metabolized to its active metabolites, which indicates an increased risk of GI bleeding with clopidogrel. Therefore a PPI should be used, because the effect of a PPI on clopidogrel efficacy in this group may be not so problematic. On the other hand, in the DMs of CYP2C19, efficacy of clopidogrel is decreased in comparison with RMs. Moreover, a PPI has a higher risk of conversion from ‘responder’ to ‘low-responder’ in this group. Therefore, we assume that the CYP2C19 genotyping test could be useful for the optimal prophylactic treatment for patients undergoing anti-platelet therapy.

Recently, a histamine H_2 -receptor antagonist (H_2RA) was reported to be effective for the prevention of aspirin-induced gastric injury [38]. Yasuda *et al.* [39] demonstrated that an H_2RA as well as a PPI completely prevented the GI bleeding and that an H_2RA did not increase the risk of stent thrombosis or new lesions in coronary arteries in patients taking dual antiplatelet therapy after coronary stenting, suggesting that an H_2RA could replace a PPI as the prophylactic agent in patients taking dual antiplatelet therapy. However, this study is retrospective and no prospective study has been performed. Therefore, which anti-secretory agent, a PPI or an H_2RA , is better as the prophylactic agent in dual anti-platelet therapy must be verified from the points of view of safety and efficacy in a future prospective study.

Lastly, our results must be interpreted within the limitations of the study. First of all, our study subjects were all healthy volunteers, not patients. Second, the sample size was small. However, we could not estimate the appropriate sample size because of lack of a preliminary study. Third, we did not use aspirin, although many patients were treated with dual anti-platelet agents such as clopidogrel and aspirin. Therefore, our study results should be considered as preliminary. However, we would like to emphasize that the risk of attenuation of clopidogrel by a PPI depends on CYP2C19 genotype status. Therefore, we believe that the testing of CYP2C19 genotype could contribute to the development of tailored optimal anti-platelet therapy.

However, a further large scale study is necessary to verify the clinical usefulness of CYP2C19 genotyping in antiplatelet therapy.

Competing interests

There are no competing interests to declare.

This work was supported by a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (20590718). We thank the staff at the Translational Research Unit, Ms Takako Toyoda, Ms Yoko Akahori, Ms Yumi Kiyama, Ms Keiko Arasawa, Ms Saori Oikawa and Ms Naomi Hashimoto for their help.

REFERENCES

- 1 Cattaneo M. Aspirin and clopidogrel: efficacy, safety, and the issue of drug resistance. *Arterioscler Thromb Vasc Biol* 2004; 24: 1980–7.
- 2 Derry S, Loke YK. Risk of gastrointestinal haemorrhage with long term use of aspirin: meta-analysis. *BMJ* 2000; 321: 1183–7.
- 3 Chan FK, Ching JY, Hung LC, Wong VW, Leung VK, Kung NN, Hui AJ, Wu JC, Leung WK, Lee VW, Lee KK, Lee YT, Lau JY, To KF, Chan HL, Chung SC, Sung JJ. Clopidogrel versus aspirin and esomeprazole to prevent recurrent ulcer bleeding. *N Engl J Med* 2005; 352: 238–44.
- 4 Hallas J, Dall M, Andries A, Andersen BS, Aalykke C, Hansen JM, Andersen M, Lassen AT. Use of single and combined antithrombotic therapy and risk of serious upper gastrointestinal bleeding: population based case-control study. *BMJ* 2006; 333: 726.
- 5 Awtry EH, Loscalzo J. Aspirin. *Circulation* 2000; 101: 1206–18.
- 6 Taha AS, Angerson WJ, Knill-Jones RP, Blatchford O. Upper gastrointestinal haemorrhage associated with low-dose aspirin and anti-thrombotic drugs – a 6-year analysis and comparison with non-steroidal anti-inflammatory drugs. *Aliment Pharmacol Ther* 2005; 22: 285–9.
- 7 Nakayama M, Iwakiri R, Hara M, Ootani H, Shimoda R, Tsunada S, Sakata H, Fujimoto K. Low-dose aspirin is a prominent cause of bleeding ulcers in patients who underwent emergency endoscopy. *J Gastroenterol* 2009; 44: 912–18.
- 8 Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, Johnson DA, Mahaffey KW, Quigley EM. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. *Circulation* 2008; 118: 1894–909.
- 9 Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, Johnson DA, Mahaffey KW, Quigley EM, Harrington RA, Bates ER, Bridges CR, Eisenberg MJ, Ferrari VA, Hlatky MA, Kaul S, Lindner JR, Moliterno DJ, Mukherjee D, Schofield RS, Rosenson RS, Stein JH, Weitz HH, Wesley DJ. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use. *Am J Gastroenterol* 2008; 103: 2890–907.
- 10 Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, Johnson DA, Mahaffey KW, Quigley EM, Harrington RA, Bates ER, Bridges CR, Eisenberg MJ, Ferrari VA, Hlatky MA, Kaul S, Lindner JR, Moliterno DJ, Mukherjee D, Schofield RS, Rosenson RS, Stein JH, Weitz HH, Wesley DJ. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. *J Am Coll Cardiol* 2008; 52: 1502–17.
- 11 Umemura K, Furuta T, Kondo K. The common gene variants of CYP2C19 affect pharmacokinetics and pharmacodynamics in an active metabolite of clopidogrel in healthy subjects. *J Thromb Haemost* 2008; 6: 1439–41.
- 12 Stedman CA, Barclay ML. Review article: comparison of the pharmacokinetics, acid suppression and efficacy of proton pump inhibitors. *Aliment Pharmacol Ther* 2000; 14: 963–78.
- 13 Juurlink DN, Gomes T, Ko DT, Szmítko PE, Austin PC, Tu JV, Henry DA, Kopp A, Mamdani MM. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. *CMAJ* 2009; 180: 713–18.
- 14 Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, Rumsfeld JS. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA* 2009; 301: 937–44.
- 15 Toth PP, Armani A. Thienopyridine therapy and risk for cardiovascular events in secondary prevention. *Curr Atheroscler Rep* 2009; 11: 364–70.
- 16 Siller-Matula JM, Spiel AO, Lang IM, Kreiner G, Christ G, Jilma B. Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. *Am Heart J* 2009; 157: 148 e1–5.
- 17 Ishizaki T, Horai Y. Review article: cytochrome P450 and the metabolism of proton pump inhibitors – emphasis on rabeprazole. *Aliment Pharmacol Ther* 1999; 13 (Suppl. 3): 27–36.
- 18 Malek LA, Kisiel B, Spiewak M, Grabowski M, Filipiak KJ, Kostrzewa G, Huczek Z, Ploski R, Opolski G. Coexisting polymorphisms of P2Y12 and CYP2C19 genes as a risk factor for persistent platelet activation with clopidogrel. *Circ J* 2008; 72: 1165–9.
- 19 Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009; 360: 363–75.
- 20 Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS. Cytochrome P-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; 360: 354–62.
- 21 Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias WL, Braunwald E,

- Sabatine MS. Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation* 2009; 119: 2553–60.
- 22 Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, Payot L, Brugier D, Cayla G, Beygui F, Bensimon G, Funck-Brentano C, Montalescot G. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 2009; 373: 309–17.
- 23 Furuta T, Sagehashi Y, Shirai N, Sugimoto M, Nakamura A, Kodaira M, Kenmotsu K, Nagano M, Egashira T, Ueda K, Yoneyama M, Ohashi K, Ishizaki T, Hishida A. Influence of CYP2C19 polymorphism and *Helicobacter pylori* genotype determined from gastric tissue samples in response to triple therapy for *H. pylori* infection. *Clin Gastroenterol Hepatol* 2005; 3: 564–73.
- 24 Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 2006; 79: 103–13.
- 25 Geisler T, Langer H, Wydymus M, Gohring K, Zurn C, Bigalke B, Stellos K, May AE, Gawaz M. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. *Eur Heart J* 2006; 27: 2420–5.
- 26 Hoshino K, Horiuchi H, Tada T, Tazaki J, Nishi E, Kawato M, Ikeda T, Yamamoto H, Akao M, Furukawa Y, Shizuta S, Toma M, Tamura T, Saito N, Doi T, Ozasa N, Jinnai T, Takahashi K, Watanabe H, Yoshikawa Y, Nishimoto N, Ouchi C, Morimoto T, Kita T, Kimura T. Clopidogrel resistance in Japanese patients scheduled for percutaneous coronary intervention. *Circ J* 2009; 73: 336–42.
- 27 Sibbing D, Morath T, Stegherr J, Braun S, Vogt W, Hadamitzky M, Schomig A, Kastrati A, von Beckerath N. Impact of proton pump inhibitors on the antiplatelet effects of clopidogrel. *Thromb Haemost* 2009; 101: 714–19.
- 28 Gilard M, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, Mansourati J, Mottier D, Abgrall JF, Bosch J. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole Clopidogrel Aspirin) study. *J Am Coll Cardiol* 2008; 51: 256–60.
- 29 Small DS, Farid NA, Payne CD, Weerakkody GJ, Li YG, Brandt JT, Salazar DE, Winters KJ. Effects of the proton pump inhibitor lansoprazole on the pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel. *J Clin Pharmacol* 2008; 48: 475–84.
- 30 Zuern CS, Geisler T, Lutitsky N, Winter S, Schwab M, Gawaz M. Effect of comedication with proton pump inhibitors (PPIs) on post-interventional residual platelet aggregation in patients undergoing coronary stenting treated by dual antiplatelet therapy. *Thromb Res* 2010; 125: e51–4.
- 31 O'Donoghue ML, Braunwald E, Antman EM, Murphy SA, Bates ER, Rozenman Y, Michelson AD, Hautvast RW, Ver Lee PN, Close SL, Shen L, Mega JL, Sabatine MS, Wiviott SD. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. *Lancet* 2009; 374: 989–97.
- 32 Rassen JA, Choudhry NK, Avorn J, Schneeweiss S. Cardiovascular outcomes and mortality in patients using clopidogrel with proton pump inhibitors after percutaneous coronary intervention or acute coronary syndrome. *Circulation* 2009; 120: 2322–9.
- 33 Hagihara K, Kazui M, Kurihara A, Yoshiike M, Honda K, Okazaki O, Farid NA, Ikeda T. A possible mechanism of the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel. *Drug Metab Dispos* 2009; 37: 2145–52.
- 34 Furuta T, Ohashi K, Kosuge K, Zhao XJ, Takashima M, Kimura M, Nishimoto M, Hanai H, Kaneko E, Ishizaki T. CYP2C19 genotype status and effect of omeprazole on intragastric pH in humans. *Clin Pharmacol Ther* 1999; 65: 552–61.
- 35 Shirai N, Furuta T, Xiao F, Kajimura M, Hanai H, Ohashi K, Ishizaki T. Comparison of lansoprazole and famotidine for gastric acid inhibition during the daytime and night-time in different CYP2C19 genotype groups. *Aliment Pharmacol Ther* 2002; 16: 837–46.
- 36 Sugimoto M, Furuta T, Shirai N, Kajimura M, Hishida A, Sakurai M, Ohashi K, Ishizaki T. Different dosage regimens of rabeprazole for nocturnal gastric acid inhibition in relation to cytochrome P450 2C19 genotype status. *Clin Pharmacol Ther* 2004; 76: 290–301.
- 37 Diaz D, Fabre I, Daujat M, Saint Aubert B, Bories P, Michel H, Maurel P. Omeprazole is an aryl hydrocarbon-like inducer of human hepatic cytochrome P450. *Gastroenterology* 1990; 99: 737–47.
- 38 Taha AS, McCloskey C, Prasad R, Bezlyak V. Famotidine for the prevention of peptic ulcers and oesophagitis in patients taking low-dose aspirin (FAMOUS): a phase III, randomised, double-blind, placebo-controlled trial. *Lancet* 2009; 374: 119–25.
- 39 Yasuda H, Yamada M, Sawada S, Endo Y, Inoue K, Asano F, Takeyama Y, Yoshida M. Upper gastrointestinal bleeding in patients receiving dual antiplatelet therapy after coronary stenting. *Intern Med* 2009; 48: 1725–30.