Pharmacodynamic Evaluation of Pantoprazole Therapy on Clopidogrel Effects
Results of a Prospective, Randomized, Crossover Study

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Background—Safety concerns have recently emerged based on a drug interaction between clopidogrel and proton pump inhibitors leading to reduced pharmacodynamic effects. However, whether such drug interaction is a class effect or a drug effect and if this can be modulated by timing of drug administration remains a matter of debate. The aim of this study was to assess the impact of high-dose pantoprazole therapy, a proton pump inhibitor with low potential to interfere with clopidogrel metabolism, administered concomitantly or staggered, on clopidogrel-mediated pharmacodynamic effects.

Methods and Results—This was a prospective, randomized, crossover study conducted in 20 healthy volunteers. Subjects were randomly assigned to receive pantoprazole (80 mg daily) administered concomitantly (CONC) or staggered by 8 to 12 hours (STAG) for 1 week on a background of clopidogrel therapy (600-mg loading dose followed by a 75-mg maintenance dose during all phases) in a crossover fashion with a 2- to 4-week washout period between treatments. All subjects had a 1-week treatment phase with a clopidogrel-only regimen with a 2- to 4-week washout period from randomization sequence. Platelet function was assessed by flow cytometric analysis of the status of phosphorylation of the vasodilator-stimulated phosphoprotein, light transmittance aggregometry after adenosine diphosphate stimuli, and VerifyNow P2Y12 system at 3 time points: baseline, 24 hours after loading dose, and 1 week after maintenance dose. The primary end point was the comparison of P2Y12 reactivity index assessed by vasodilator-stimulated phosphoprotein at 1 week. After 1 week, there were no significant difference in P2Y12 reactivity index between the CONC and STAG regimens (least-squares mean ± SEM, 56.0 ± 3.9% versus 56.1 ± 3.9%; P = 0.974), as well as when compared with the clopidogrel-only regimen (61.0 ± 3.9%; P = 0.100 versus CONC and P = 0.107 versus STAG). Further, no differences were observed at baseline and 24 hours between regimens. Concordant results were obtained by light transmittance aggregometry and VerifyNow P2Y12. assays.

Conclusions—Pantoprazole therapy used at high doses is not associated with modulation of the pharmacodynamic effects of clopidogrel, irrespective of timing of drug administration.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01170533.

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Key Words: clopidogrel • pantoprazole • proton pump inhibitors • drug interaction

Clopidogrel therapy in addition to aspirin is associated with a significant reduction in recurrent atherothrombotic events in high-risk settings, such as acute coronary syndromes or percutaneous coronary interventions.1–3 However, numerous studies have shown that patients with high on-treatment platelet reactivity remain at increased risk of recurrent ischemic events.4 Several factors have been associated with reduced pharmacokinetic and pharmacodynamic response profiles to clopidogrel.5,6 Among these, a drug interaction between proton pump inhibitors (PPIs), in particular omeprazole, and clopidogrel has recently emerged.7–9 This drug interaction probably is due to the common metabolic pathway of these agents, which involves the cytochrome P450 (CYP) 2C19 enzyme.10,11 The CYP2C19 isoenzyme is
of particular importance because it is involved in both oxidation steps required for clopidogrel prodrug to generate its active metabolite. Therefore, intrinsic (eg, genetic polymorphisms) or extrinsic (eg, drugs) factors modulating the activity of this enzyme may affect active metabolite levels and thus the platelet-inhibitory effects of clopidogrel.

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Although the clinical implications of the clopidogrel-PPI interaction remains highly controversial, pharmacokinetic and pharmacodynamic studies have consistently shown clopidogrel effects to be significantly altered by omeprazole, a PPI that is primarily metabolized by CYP2C19. The concerns surrounding this interaction have prompted a box warning for the concomitant use of these drugs. However, whether the clopidogrel-PPI interaction is a class effect or a drug-specific effect is still a matter of debate. In fact, the effects of other PPIs that are less influential on CYP2C19 activity have not been well explored and often controversial. Further, the impact of timing of administration of these agents on pharmacodynamic effects has also been a topic of debate. Therefore, the aim of this pharmacodynamic study was to evaluate the impact of pantoprazole, a PPI with low potential to inhibit CYP2C19, on clopidogrel-induced antplatelet effects and whether these may be affected by timing of administration of these agents.

Methods

Subject Population and Study Design

This was a prospective, open-label, 2-sequence, 3-period, randomized, crossover study conducted in nonmedicated healthy male subjects between the ages of 18 and 65 years. This investigation expands on a recently reported study by our group evaluating the pharmacodynamic effects of the clopidogrel-omeprazole drug interaction and how this may be affected by timing of drug intake and presents the same study entry criteria. The study design of the present investigation is illustrated in Figure 1. Subjects were randomly assigned in a 1:1 fashion to take pantoprazole concomitantly (CONC regimen) or staggered by 8 to 12 hours (STAG regimen) for 1 week on a background of clopidogrel therapy. After a 2- to 4-week washout period, subjects crossed over treatment regimen. All subjects also had a 1-week treatment phase with clopidogrel alone, without receiving pantoprazole therapy (CLOP regimen), with a 2- to 4-week washout period from randomization sequence. The clopidogrel dosing regimen for all 3 phases was a 600-mg loading dose (LD) and a 75-mg maintenance dose (MD). Clopidogrel doses were chosen to reflect regimens most commonly used in clinical practice. Pantoprazole was used at a dose of 80 mg/daily. Pantoprazole dosing was higher than that conventionally recommended (40 mg/daily) to maximize any of its effects on CYP2C19. Blood sampling for platelet function assessments were performed at all 3 phases of the study at the following time points: (1) baseline, (2) 24 hours after LD (before intake of study medication), and (3) 7 days (24 hours after the last MD). Clopidogrel was administered as 75-mg tablets of Plavix (Bristol-Myers Squibb/Sanofi Aventis, Bridgewater, NJ) and pantoprazole as 40 mg-tablets of Protonix (Wyeth Pharmaceuticals, Philadelphia, PA). In particular, 8 75-mg Plavix tablets were given for the LD and 1 tablet daily during the maintenance phase, and 2 Protonix tablets were given daily. The washout periods were included to minimize carryover effects between treatment regimens. Patient compliance was assessed by interview and pill counting.

The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Florida College of Medicine–Jacksonville. All subjects provided written informed consent. An independent data safety monitoring committee was instituted for adjudication of adverse clinical events.

Sample Collection and Platelet Function Assays

Blood samples for platelet function analyses were collected at scheduled time points before intake of study medication from an antecubital vein. The first 2 to 4 mL of blood was discarded to avoid spontaneous platelet activation. Samples were processed by laboratory personnel blinded to treatment. Platelet function assays included flow cytometric analysis of the status of phosphorylation of the vasodilator-stimulated phosphoprotein (VASP), VerifyNow P2Y12 (VN-P2Y12) system, and light transmission aggregometry ( LT A).

VASP Assay

The VASP assay was used to determine the P2Y12 reactivity index (PRI) according to standard protocols. In brief, VASP phosphorylation (VASP-P) was measured by quantitative flow cytometry using commercially available labeled monoclonal antibodies (Becton Dickinson, Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels after stimulation with adenosine diphosphate (ADP), PGE1 increases VASP-P levels through stimulation of adenyly cyclase; ADP binding to purinergic receptors leads to inhibition of adenyly cyclase; thus, the addition of ADP to PGE1-stimulated platelets reduces levels of PGE1-induced VASP-P. The PRI was calculated as follows: ([MFI PGE1]−[MFI
PGE1/ADP/[MFI PGE1])×100%. A reduced PRI is indicative of greater inhibition of the P2Y12 signaling pathway.17,18

**VN-P2Y12 Assay**
The VN-P2Y12 assay is a rapid whole-blood point-of-care device and was used according to the instructions of the manufacturer (Accumetrics, Inc, San Diego, CA) as previously described.19 In brief, VN-P2Y12 assay mimics turbidometric aggregation and uses disposable cartridges containing 20 μmol/L ADP and 22 nmol/L PGE1. Aggregation testing using ADP as a sole agonist activates P2Y1 and P2Y12 purinergic signaling, whereas adding PGE1 increases the specificity of the test for P2Y12 signaling.20 In a separate channel of the cartridge in which iso-TRAP is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of platelet inhibition without having to wean the patient off antiplatelet treatment. The VN-P2Y12 assay reports the results as P2Y12 reaction units (PRU) and percent inhibition of platelet aggregation (%IPA), which is calculated as [(baseline – PRU)/baseline]×100. In contrast to IPA values, which increase with decreasing platelet function, PRU values decrease with decreasing platelet function.

**Light Transmission Aggregometry**
LTA was performed according to standard protocols as previously described.18 In brief, platelet aggregation was assessed using platelet-rich plasma and platelet-poor plasma by the turbidometric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp, Havertown, PA). Light transmission was adjusted to 0% for platelet-rich plasma and to 100% for platelet-poor plasma for each measurement. Maximal platelet aggregation (MPA) was induced by 5 μmol/L and 20 μmol/L ADP as agonist.

**Study End Points and Sample Size Calculation**
The primary end point of this study was the comparison of the PRI achieved at 1 week between the CONC and STAG treatment regimens. A sample size of 18 patients was required to be able to detect a 10% absolute difference in PRI between both regimens with 80% power and 2-sided significance level of 0.05, assuming a 15% standard deviation for the difference between regimens. Considering an approximate 15% dropout rate, random assignment of up to 22 patients was allowed to ensure that pharmacodynamic data from 18 patients completing both treatment regimens were available. Other end points included (1) comparison of PRU and MPA (assessed by VN-P2Y12 and LTA, respectively) between CONC and STAG at 1 week; (2) comparison of PRI, PRU, and MPA between the 3 regimens (CONC, STAG, and CLOP) at 24 hours and 1 week.

**Statistical Analysis**
Continuous variables are expressed as mean±SD. Normal distribution was evaluated for continuous variables with the Kolmogorov-Smirnov test. Categorical variables are expressed as frequencies and percentages. Only subjects who successfully completed the first 2 treatment periods of the study were considered for analysis. All statistical comparisons of platelet function for the primary and secondary end points were conducted using linear mixed-effects models with treatment, sequence, period, and treatment×period (treatment by period interaction to test for carryover effects) as fixed effects, subject as a random effect, and baseline value of the corresponding platelet function test (PRI, PRU, or MPA) as a covariate. A 2-tailed probability value of <0.05 was considered to indicate a statistically significant difference for all the analyses performed. Results are reported as least-squares mean±SEM. Statistical analysis was performed using SPSS version 16.0 software (SPSS Inc, Chicago, IL).

**Results**
Twenty-two healthy male subjects ages 33.6±5.4 years with body mass index of 25.6±2.9 kg/m² were randomly assigned: 11 starting with the CONC regimen and 11 with the STAG regimen. One patient from each group withdrew consent after random assignment. Therefore, a total of 20 subjects were available for analysis, all of whom completed the 3 periods of the study.

There were no differences in any of the pharmacodynamic measures between the 3 regimens studied at baseline (data not shown) or at 24 hours after clopidogrel LD administration, as summarized in the Table. At 1 week, there were no significant difference in the primary end point, which showed similar PRI values with both CONC and STAG regimens (least-squares mean±SEM, 56.0±3.9% versus 56.1±3.9%; P=0.974;
A numerically higher PRI value was obtained with the CLOP regimen but without reaching statistical significance when compared with both regimens in which pantoprazole was administered irrespective of timing of administration (61.0 ± 3.9%; P = 0.100 versus CONC and P = 0.107 versus STAG) (Figure 2). The lack of significance is also observed because the confidence intervals (CI) of the least-significant differences between the CLOP and CONC regimens and between the CLOP and STAG regimens include the 0 value: 4.9% (95% CI, 1.0% to 10.8%) and 4.8% (95% CI, −1.1% to 10.7%), respectively. Distribution of PRI values over the treatment periods are represented in Figure 3, showing that PRI values did not significantly separate at any time point between regimens. No statistically significant differences were observed by sequence, period, or the treatment-by-period interaction, which suggests no carry-over effect.

Parallel findings were observed with the other platelet function tests performed. No significant difference for any comparison with any assay used was found. Results of the VN-P2Y12 assay, either expressed as PRU or %IPA, are shown in Figure 4A and 4B, respectively. MPA values using 20 μmol/L ADP (CONC, 45.9 ± 4.4; STAG, 44.2 ± 4.4; CLOP, 43.5 ± 4.4; no significant probability values for all comparisons) and 5 μmol/L ADP (CONC, 27.5 ± 3.3; STAG, 31.4 ± 3.3; CLOP, 39.6 ± 3.3; no significant probability values for all comparisons) were also consistent.

**Discussion**

Recent studies have demonstrated a drug interaction between PPIs and clopidogrel.7–9 Although the clinical implications associated with the reduced pharmacodynamic effects in clopidogrel-treated patients as a cause of this drug interaction remain controversial, this has prompted drug-regulating authorities in the United States and in Europe to provide a box warning for the use of these drugs, administered either concomitantly or staggered.13,14 In fact, the impact of any negative interaction between PPIs and clopidogrel is of particular concern because of the high frequency with which these two drugs are coprescribed. Therefore, even a small increase in ischemic risk caused by this drug interaction may have significant consequences.12 The mechanism underlying this drug interaction is a competitive inhibition at the level of the CYP2C19 isoenzyme, a critical step in the hepatic biotransformation of clopidogrel.5,6,10,11 However, PPIs are recommended in patients at high risk for gastrointestinal bleed, such as those taking dual antiplatelet therapy with aspirin and clopidogrel.21 This has prompted expert consensus to consider gastric protection strategies with lower potential to inhibit CYP2C19.21,22 Most of the available pharmacodynamic data on the PPI-clopidogrel interaction is with omeprazole, a moderate CYP2C19 inhibitor.7–9 Limited information is available on the pharmacodynamic effects of
other PPIs, such as pantoprazole, which has lower potential to inhibit CYP2C19. The results of this prospective, randomized, crossover study demonstrate the lack of any significant impairment in clopidogrel-induced pharmacodynamic effects as assessed by a multitude of assays with the use of pantoprazole administered either concomitantly or staggered. Of note, the dose of pantoprazole used in this study was higher than that conventionally used in practice, which provides further support to the conclusions of our investigation.

The results of our study are in line with prior pharmacodynamic investigations assessing the impact of pantoprazole on clopidogrel effects. However, at difference with prior investigations, our study also investigated whether concomitant versus staggered treatment could have an impact on the pharmacodynamic findings. This is noteworthy because it has been suggested that staggering treatment may be a modality to overcome the PPI-clopidogrel drug interaction. However, recent pharmacodynamic studies using omeprazole showed trends toward an increase in platelet reactivity with staggered PPI treatment. These findings support the recommendation of drug-regulating authorities to avoid concomitant use of pantoprazole in clopidogrel-treated patients. Overall, these considerations underscore the importance of also comprehensively investigating the impact of timing of pantoprazole administration, as performed in the current investigation.

Understanding the clinical implications of the clopidogrel-PPI interaction remains a critical unmet need. Several observational studies have shown significant associations between PPI use and cardiovascular events. However, other retrospective analyses (including observational and post hoc analyses of randomized trials) and the only randomized, clinical trial evaluating the potential interaction between clopidogrel and a PPI (omeprazole) failed to show an increased risk of adverse cardiovascular events among PPI users, irrespective of the type of PPI. With regard to pantoprazole, a population-based, nested, case-control study of patients receiving clopidogrel therapy after acute myocardial infarction showed that pantoprazole was not associated with an increase in cardiac events, whereas other PPIs were. On the contrary, recently published retrospective cohort studies have shown that pantoprazole also adversely affects cardiovascular outcomes in clopidogrel users. These conflicting findings suggest that PPI use might be a marker of unmeasured and uncontrolled confounding in observational studies because PPIs might be selectively prescribed to higher-risk patients, thus, potentially biasing the risk of ischemic outcomes. This is in line with a post hoc analysis of the Clopidogrel for the Reduction of Events During Observation (CREDO) trial, in which PPI use was associated with impaired clinical outcomes regardless whether or not the patients were receiving clopidogrel treatment. Similar conclusions also derive from a post hoc analysis of the PLAtel inhibition and patient Outcomes (PLATO) trial, in which the use of a PPI was independently associated with an increased risk of the composite of cardiovascular death, myocardial infarction, and stroke in patients receiving clopidogrel or ticagrelor, suggesting that PPI use is more likely a marker for, rather than a cause of, a higher risk of cardiovascular events.

In conclusion, the present study demonstrates the lack of a pharmacodynamic interaction between clopidogrel and pantoprazole, a PPI with low potential to inhibit CYP2C19, supporting that the pharmacodynamic interaction between clopidogrel and PPIs is a drug-specific (eg, PPIS with moderate-high potential to inhibit CYP2C19) rather than a class effect. The lack of a pharmacodynamic interaction was observed irrespective of timing of administration of pantoprazole, which was given at a higher than standard dosing regimen. These results support recent recommendations suggesting that if a PPI is warranted in a patient at increased risk of a gastrointestinal bleed while receiving dual antiplatelet therapy, pantoprazole may be considered as a safer treatment option.

**Study Limitations**

This study had an open-label design and was performed at a single center and has intrinsic limitations. In addition, the study was performed in healthy volunteers, and it may be argued that the data may not necessarily be extrapolated to patients with coronary artery disease. However, the objective of this study was to elucidate the pharmacodynamic interaction between clopidogrel and pantoprazole, and being performed in nonmedicated subjects precludes any impact of medications commonly prescribed in patients with coronary artery disease that may interfere with the CYP system, which could potentially bias the pharmacodynamic findings. Although this study is supportive of the concept that the clopidogrel-PPI drug interaction is not a class effect and results of prior studies suggest that this is a drug effect, head-to-head investigations comparing the effects of PPIs with different effects on CYP2C19 activity (eg, omeprazole versus pantoprazole) would provide more insights to this topic. In addition, it may be argued that the presence of CYP2C19 polymorphisms could have modified the pharmacodynamic response to clopidogrel. However, the influence of CYP2C19 loss-of-function allelic variations on clopidogrel-mediated antiplatelet effects is known to be relatively small (5% to 12%). In addition, the small sample size of this pilot investigation and the fact that pantoprazole has limited interference, CYP2C19 activity makes it unlikely that CYP2C19 polymorphisms would have emerged as a variable modifying our pharmacodynamic findings. Of note, prior clinical investigations have failed to identify any impact of CYP2C19 polymorphisms on adverse outcomes of PPI-treated patients. Also, a pharmacokinetic evaluation would have provided more insights on the lack of a metabolic interaction between pantoprazole and clopidogrel. Ultimately, whether the results obtained in our study would have been different using a pantoprazole daily dose of 40 mg, which is commonly used in clinical practice, instead of 80 mg cannot be ascertained. However, a pharmacodynamic interaction with a lower dose would be unlikely.

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Disclosures

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**CLINICAL PERSPECTIVE**

The prognostic implication of reduced pharmacodynamic efficacy of clopidogrel therapy as a result of a drug-drug interaction with proton-pump inhibitors (PPI) has not been elucidated fully. The regulatory authorities, in particular the Food and Drug Administration and the European Medicines Agency, have recommended avoidance of the combination of clopidogrel and omeprazole, the most commonly prescribed PPI. However, limited information is available on the effects of other PPIs, such as pantoprazole, which has lower potential to inhibit the CYP2C19 enzyme, on the pharmacodynamics of clopidogrel.

The results of this prospective, randomized, crossover study demonstrate the absence of any significant impairment in clopidogrel-induced pharmacodynamic efficacy as assessed by several assays when pantoprazole is administered either concomitantly or staggered. Notably, this investigation used a dose of pantoprazole (80 mg) higher than that used in clinical practice to maximize any of its adverse effects on CYP2C19. Therefore, it is unlikely that a pharmacodynamic interaction would be observed with the lower dose used more commonly in clinical practice (eg, 40 mg). These observations are in line with the concept that a PPI-clopidogrel interaction is not a class-specific effect but rather a drug-specific effect affecting PPIs metabolized primarily by CYP2C19 (eg, omeprazole) and support recommendations suggesting that if a PPI is warranted in a patient at increased risk of a gastrointestinal bleed while receiving dual antiplatelet therapy, pantoprazole may be considered as a safe treatment option.
Pharmacodynamic Evaluation of Pantoprazole Therapy on Clopidogrel Effects: Results of a Prospective, Randomized, Crossover Study

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