Pharmacodynamic Effects of Concomitant Versus Staggered Clopidogrel and Omeprazole Intake

Results of a Prospective Randomized Crossover Study

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Background—A drug interaction between clopidogrel and omeprazole resulting in impaired platelet inhibition has been reported. It has been suggested that staggering administration of clopidogrel and omeprazole may overcome this pharmacodynamic (PD) interaction.

Methods and Results—This prospective, open-label, 3-period, randomized crossover study was performed in 20 healthy volunteers. Subjects were randomly selected to receive omeprazole (40 mg daily) concomitantly (CONC) or staggered by 8 to 12 hours (STAG) for 1 week on a background of clopidogrel therapy in a crossover fashion, with a 2- to 4-week washout period between treatments. After another 2- to 4-week washout period, all subjects were treated for 1 week with clopidogrel alone. Clopidogrel was administered as a 600-mg loading dose followed by a 75-mg maintenance dose during all phases. PD effects were assessed by vasodilator-stimulated phosphoprotein phosphorylation assay, VerifyNow P2Y₁₂ system, and light transmittance aggregometry at baseline, 24 hours, and 1 week. The primary end point was the comparison of P2Y₁₂ reactivity index assessed by vasodilator-stimulated phosphoprotein phosphorylation assay at 1 week between CONC and STAG regimens. No significant difference in the primary end point was observed (least squares mean ± SEM, 56.1 ± 3.5% for CONC versus 61.6 ± 3.4% for STAG; P = 0.08). P2Y₁₂ reactivity index values were significantly lower in the clopidogrel regimen (48.8 ± 3.4%) than in the CONC (P = 0.02) and STAG (P = 0.001) regimens. No PD differences were observed between regimens at baseline and 24 hours. Concordant results were obtained by P2Y₁₂-specific assessments using VerifyNow but not with light transmittance aggregometry.

Conclusions—Omeprazole impairs clopidogrel-induced antiplatelet effects in the maintenance phase of treatment irrespective of timing of their administration. (Circ Cardiovasc Interv. 2010;3:436-441.)

Key Words: clopidogrel ± omeprazole ± drug interactions

Numerous studies have shown a broad range in antiplatelet response profiles following treatment with clopidogrel, and patients with poor platelet inhibitory effects have an increased risk of recurrent atherothrombotic events.¹⁻⁴ Several mechanisms have been identified to explain the interindividual variability in clopidogrel-induced antiplatelet effects.⁵⁻⁶ Among these, that secondary to a drug interaction between clopidogrel and proton pump inhibitors (PPIs) has been recently implicated.⁷ In particular, pharmacodynamic (PD) studies have shown that omeprazole, which is the most broadly used PPI and primarily metabolized by the cytochrome P450 (CYP) 2C19 isoenzyme,⁸ is associated with reduced platelet inhibitory effects induced by clopidogrel.⁹,¹⁰ Because the CYP2C19 isoenzyme is involved in both oxidation steps required for clopidogrel prodrug to generate its active metabolite, which is responsible for irreversible blockade of the P2Y₁₂ receptor on the platelet surface,¹¹ any interference at this level may compromise the efficacy of the drug.

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Outcome studies have yielded conflicting results on the prognostic implications of concomitant clopidogrel and PPI use.¹²⁻¹⁸ However, given the high frequency with which both drugs are prescribed, even effects of limited magnitude can affect a large number of patients. For these reasons, the Food and Drug Administration and the European Medicines Agency mandated that clopidogrel product information be updated to recommend avoidance of omeprazole.¹⁹,²⁰ This warning is in conflict with a recent expert consensus document on gastrointestinal risks for patients on antiplatelet therapy, which supports the coadministration of these 2 drugs.²¹ Because both clopidogrel and omeprazole are rapidly
metabolized, it has been hypothesized and recommended that staggering administration of these drugs may overcome their interaction. However, despite this recommendation, to date no studies have validated this hypothesis. Therefore, the aim of this study was to evaluate whether the PD interaction between clopidogrel and omeprazole can be overcome by separating the intake of both drugs.

Methods

Subject Population and Study Design

This prospective, open-label, 2-sequence, 3-period, randomized crossover study was conducted in nonmedicated healthy male subjects aged 18 to 65 years. The study design is illustrated in Figure 1. Subjects were randomly selected in a 1:1 fashion to take omeprazole (40 mg daily) concomitantly (CONC regimen) or staggered by 8 to 12 hours (CLOP regimen) for 1 week without taking omeprazole. In particular, in the CONC regimen, both drugs were taken in the morning, whereas in the CLOP regimen, clopidogrel was taken in the morning and omeprazole in the evening. After a 2- to 4-week washout period, subjects crossed over treatment regimen. After completing these 2 treatment phases, subjects underwent another washout period of 2 to 4 weeks and were treated for 1 week with clopidogrel alone, without receiving omeprazole therapy (CLOP regimen). The clopidogrel dosing regimen for all 3 phases was a 600-mg loading dose (LD) and a 75-mg maintenance dose (MD). 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 omeprazole as 20-mg tablets of Prilosec OTC (Proctor & Gamble, Cleveland, Ohio). In particular, 8 75-mg clopidogrel tablets were given for the LD and 1 tablet daily during the maintenance phase; 2 omeprazole 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 were 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 (LTA).

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 (Biocytex Inc, Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels following challenge with prostaglandin E1 (PGE1) and PGE1+ADP. PGE1 increases VASP-P levels through stimulation of adenylate cyclase; ADP binding to purinergic receptors leads to inhibition of adenylate 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+ADP)/(MFI PGE1)]×100%. A reduced PRI is indicative of greater inhibition of the P2Y12 signaling pathway.

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, Calif) as previously described. 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. In a separate channel of the cartridge in which iso-TRAP (thrombin receptor activating peptide) 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 PRI (reaction units) 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, PRI values decrease with decreasing platelet function.

LTA

LTA was performed according to standard protocols as previously described. 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, Penn). Light transmission was adjusted to 0% for platelet-rich plasma and 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 a 2-sided significance level of 0.05, assuming a 15% SD for the difference between regimens. Considering an approximate 25% dropout rate, randomization of up to 24 patients was allowed to ensure that PD data from 18 patients completing both treatment regimens were available. Other end points were (1) comparison of PRI and MPA (assessed by VN-P2Y12 and LTA, respectively) between CONC and STAG at 1 week and (2) comparison of PRI, PRU, and MPA among the 3 regimens (CONC, STAG, and CLOP) at 24 hours and 1 week.
**Results**

Twenty-four healthy male subjects aged 34.0 ± 6.3 years with a body mass index of 24.9 ± 2.9 kg/m² were randomly assigned as follows: 12 starting with the CONC regimen and 12 with the STAG regimen. Two subjects in each group were assigned as follows: 12 starting with the CONC regimen and 24 hours (after clopidogrel LD administration), there were no differences in any of the PD measures among the 3 regimens studied (data not shown). At 1 week, PRI values were numerically lower in the CONC regimen than both the STAG regimen and the CLOP regimen. Two subjects in each group were assigned as follows: 12 starting with the CONC regimen and 12 with the STAG regimen. Two subjects in each group withdrew consent after randomization; therefore, 20 subjects were available for analysis, all of whom completed the 3 periods of the study.

At baseline, there were no differences in any of the PD measures among the 3 regimens studied (data not shown). At 24 hours (after clopidogrel LD administration), there were also no differences in PD measures as summarized in Table. At 1 week, PRI values were numerically lower in the CONC regimen than in the STAG regimen but without reaching statistical significance (LSM ± SEM, 56.1 ± 3.5% versus 61.6 ± 3.4%; P = 0.08 [primary end point]). The PRI was significantly lower following the CLOP regimen (48.8 ± 3.4%) than both regimens in which omeprazole was administered irrespective of timing of administration (CONC, P = 0.02; STAG, P = 0.001). The least significant difference in PRI between the CLOP and CONC regimens and between the CLOP and STAG regimens was 7.3% (95% CI, 1.2% to 13.5%) and 12.8% (95% CI, 6.9% to 18.7%), respectively. Distribution of PRI values over the treatment periods is represented in Figure 2. PRI values separated after 24 hours, and PRI was decreased at 1-week in the CLOP regimen compared with the CONC and STAG regimens. No statistically significant differences were observed by sequence, period, or treatment-by-period interaction, thus suggesting no carryover effect.

Parallel findings were observed with the VN-P2Y12 assay, either expressed as PRU or %IPA as shown in Figure 3A and 3B, respectively. In particular, PRU was significantly lower and %IPA significantly higher following the CLOP regimen than both regimens in which omeprazole was administered irrespective of timing of intake (CONC or STAG). Of note, compared with concomitant administration, staggering the intake of the drugs impaired clopidogrel-induced platelet inhibition measured as %IPA (least significant difference, 6.2%; 95% CI, 0.4% to 12.0%). However, this difference was not significant when VN-P2Y12 assay values were expressed as PRU.

**Discussion**

Recent investigations have shown a PD interaction between clopidogrel and omeprazole, which translates into reduced platelet inhibition.9,10 Although the clinical consequences of this interaction remain controversial,7,12–18 this has led the Food and Drug Administration and European Medicines Agency to update the clopidogrel product information with a warning to avoid omeprazole therapy.19,20 It has been hypothesized and recently recommended that staggering the admin-

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**Table. Pharmacodynamic Measures 24 Hours After Clopidogrel LD**

<table>
<thead>
<tr>
<th>Assay</th>
<th>CONC</th>
<th>STAG</th>
<th>CLOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTA</td>
<td>36.1 ± 4.9</td>
<td>35.8 ± 4.9</td>
<td>36.8 ± 4.9</td>
</tr>
<tr>
<td>MPA (ADP 20 μmol/L)</td>
<td>21.0 ± 3.5</td>
<td>19.3 ± 3.6</td>
<td>19.2 ± 3.6</td>
</tr>
<tr>
<td>VN-P2Y12</td>
<td>128.7 ± 18.2</td>
<td>129.6 ± 18.2</td>
<td>129.8 ± 18.3</td>
</tr>
<tr>
<td>%IPA</td>
<td>57.6 ± 5.7</td>
<td>56.9 ± 5.7</td>
<td>58.2 ± 5.7</td>
</tr>
<tr>
<td>VASP</td>
<td>57.9 ± 4.7</td>
<td>61.7 ± 4.7</td>
<td>58.7 ± 4.7</td>
</tr>
</tbody>
</table>

Data are expressed as LSM ± SEM. CONC indicates clopidogrel 600-mg LD followed by 75-mg MD for 1 week in addition to omeprazole 40 mg daily, taking both drugs at the same time; STAG, clopidogrel 600-mg LD followed by 75-mg MD for 1 week in addition to omeprazole 40 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg LD followed by 75-mg MD for 1 week without taking omeprazole.
CYP2C19 are involved in this interaction.31 In fact, it cannot be excluded that an increase in gastric pH may alter clopidogrel absorption and decrease its bioavailability. Therefore, clopidogrel absorption could potentially be higher when both drugs are taken concomitantly instead of staggered because changes in gastric pH caused by PPIs might have not been fully achieved by the time clopidogrel is absorbed into the bloodstream. Although our study showed trends toward greater impairment in clopidogrel-induced antiplatelet effects with staggered versus concomitant treatment, this did not reach statistical significance for the primary end point and, therefore, cannot fully support this theory. However, our study findings clearly demonstrate the presence of a PD interaction between clopidogrel and omeprazole irrespective of timing of drug administration, which fall in favor of the precautions warranted by drug regulatory authorities on the use of these agents.19,20 Whether the results obtained in our study would have been different using an omeprazole daily dose of 20 mg, which is commonly used in clinical practice, instead of 40 mg cannot be ascertained. However, the degree of impairment of clopidogrel-induced platelet inhibition associated with omeprazole at a dose of 40 mg, as used in our study, is similar to that obtained in other studies using a 20-mg daily dose of omeprazole.9,10

Although gastric pH is important in the drug absorption processes, if this was particularly relevant in modulating clopidogrel effects, it would be expected that other gastric-protecting agents could impair clopidogrel response as well. However, this is not fully supported by PD studies using PPIs other than omeprazole or with the histamine H2-receptor antagonists.32–34 These findings suggest that the PD interaction between clopidogrel and PPIs may be drug specific rather than a class effect and may imply several underlying contributing mechanisms. Indeed, interference at the level of the 2C19 isoenzyme represents one of the most accountable of these mechanisms. In fact, hepatic conversion of clopidogrel into its active metabolite, which occurs through a double oxidation process, is a critical step to achieving its

![Figure 3. VerifyNow P2Y12 testing across study time points. PRU (A) and %IPA (B) determined by the VerifyNow P2Y12 assay. Values are expressed as LSM. Error bars indicate SEMs. CONC indicates clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week in addition to omeprazole 40 mg daily, taking both drugs at the same time; STAG, clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week in addition to omeprazole 40 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week without taking omeprazole. *Nonsignificant P for all comparisons at this time point. †CONC versus STAG, P=0.23; CONC versus CLOP, P=0.05; STAG versus CLOP, P=0.01. ‡CONC versus STAG, P<0.001; CONC versus CLOP, P=0.01; STAG versus CLOP, P<0.001.](image-url)
antiplatelet effects. Several CYP isofoms are involved in clopidogrel metabolism. In particular, CYP3A4, CYP3A5, CYP2C9, and CYP1A2 are involved in 1 oxidation step, whereas CYP2B6 and CYP2C19 are involved in both.11 Thus, the pivotal role of CYP2C19 in both oxidation steps explains why substances, such as omeprazole, that interfere with its activity can modulate clopidogrel-induced antiplatelet effects.11,31 This is also supported by the fact that genetic variants of the CYP2C19 enzyme associated with reduced functional activity have been associated with impaired platelet inhibition and clinical events in clopidogrel-treated patients.35–38

The major concern of the PD interaction described with omeprazole and clopidogrel is its potential to translate into an increased risk of atherothrombotic events. Although the specific thresholds of platelet reactivity associated with an increased risk of adverse events are not fully determined, absolute changes in platelet reactivity similar to that observed in our study have been shown to be associated in other PD studies with incremental cardiovascular risk.5,6 However, studies evaluating the prognostic implications of clopidogrel and PPI use have shown conflicting findings.12–15 This may be largely explained by the fact that most of these studies were retrospective in nature or based on post hoc assessments of clinical trials and, thus, are inadequate to draw definitive conclusions on the clinical implications of this interaction. Nevertheless, it is well established that patients who present with heightened platelet reactivity have an increased risk of ischemic events.5,6 Indeed, further outcome studies, ideally integrated with PD assessments, are warranted to further elucidate the safety concerns surrounding the clopidogrel-omeprazole drug interaction.

Study Limitations
This study had an open-label design and was performed at a single center, which has its intrinsic limitations. It may be argued that the study was performed in healthy volunteers and that the data may not necessarily be extrapolated to patients with coronary artery disease. However, the objective of this study was to elucidate the PD interaction between clopidogrel and omeprazole in nonmedicated subjects because of the fact that many medications commonly prescribed in patients with coronary artery disease may interfere with the CYP system, thus leading to a potential bias in the PD findings. In addition, the lack of a pharmacokinetic evaluation limits the mechanistic interpretation of the study. Therefore, a study evaluating both pharmacokinetics and PD is needed to confirm the findings of our study.

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Disclosures
Dr. Ferreiro reports honoraria for lectures from Eli Lilly Co and Daiichi Sankyo, Inc. Drs Capodanno, Charlton, Darlington, Desai, Dharmashankar, Ferreiro, and Ueno have no conflicts of interest to report.

References
A growing body of evidence has shown a broad variability in interindividual pharmacodynamic response profiles to the platelet inhibitor clopidogrel, and patients with reduced platelet inhibition have an increased risk of recurrent atherothrombotic events. Numerous factors may contribute to poor clopidogrel response. Among these, that secondary to a drug interaction with the proton pump inhibitor omeprazole has emerged. The prognostic implications associated with clopidogrel and omeprazole use are not fully elucidated. However, given the high frequency with which both these drugs are prescribed, even a small and limited impairment in clinical outcomes can potentially affect a large number of patients. The Food and Drug Administration and the European Medicines Agency have recently recommended avoidance of this drug combination. Nevertheless, because both clopidogrel and omeprazole are rapidly metabolized, many experts have hypothesized and proposed to stagger clopidogrel and omeprazole intake to minimize or even overcome their interaction. The Food and Drug Administration and the European Medicines Agency have recently recommended avoidance of this drug combination.

**CLINICAL PERSPECTIVE**

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**Clopidogrel and Omeprazole Drug Interaction**

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