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Influence of Omeprazole on the Antiplatelet Action of Clopidogrel Associated With Aspirin

The Randomized, Double-Blind OCLA (Omeprazole CLopidogrel Aspirin) Study

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Objectives This trial sought to assess the influence of omeprazole on clopidogrel efficacy.

Background Clopidogrel has proved its benefit in the treatment of atherothrombotic diseases. In a previous observational study, we found clopidogrel activity on platelets, tested by vasodilator-stimulated phosphoprotein (VASP) phosphorylation, to be diminished in patients receiving proton pump inhibitor (PPI) treatment.

Methods In this double-blind placebo-controlled trial, all consecutive patients undergoing coronary artery stent implantation received aspirin (75 mg/day) and clopidogrel (loading dose, followed by 75 mg/day) and were randomized to receive either associated omeprazole (20 mg/day) or placebo for 7 days. Clopidogrel effect was tested on days 1 and 7 in both groups by measuring platelet phosphorylated-VASP expressed as a platelet reactivity index (PRI). Our main end point compared PRI value at the 7-day treatment period in the 2 groups.

Results Data for 124 patients were analyzed. On day 1, mean PRI was 83.2% (standard deviation [SD] 5.6) and 83.9% (SD 4.6), respectively, in the placebo and omeprazole groups (p = NS), and on day 7, 39.8% (SD 15.4) and 51.4% (SD 16.4), respectively ($p < 0.0001$).

Results Omeprazole significantly decreased clopidogrel inhibitory effect on platelet P2Y12 as assessed by VASP phosphorylation test. Aspirin-clopidogrel antiplatelet dual therapy is widely prescribed worldwide, with PPIs frequently associated to prevent gastrointestinal bleeding. The clinical impact of these results remains uncertain but merits further investigation. (OCLA: Influence of Omeprazole on the Antiplatelet Action of Clopidogrel Associated to Aspirin; http://www.clinicaltrials.gov/ct2/show/NCT00349661; NCT00349661) (J Am Coll Cardiol 2008;51: 256–60) © 2008 by the American College of Cardiology Foundation

Platelet activation and aggregation play an important role in the pathogenesis of arterial thrombosis and lead to acute coronary syndrome and complications during and after percutaneous coronary intervention. Clopidogrel, a thienopyridine, inhibits platelet activation induced by adenosine diphosphate (ADP). Alone or in association with aspirin, clopidogrel has successfully proved its benefit in the treatment of atherothrombotic disease (1,2). It also decreases the incidence of coronary artery stent thrombosis (2).

Clopidogrel is a prodrug, and must be metabolized in the liver to acquire its antiaggregation properties. Clopidogrel requires several biotransformation steps, mediated mainly by cytochrome P-450 isoenzymes, to generate an active metabolite. It exerts its antiplatelet effect by forming an inactivating disulfide bond with the platelet P2Y12 ADP receptor. Clopidogrel inhibits the effect of

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ADP on the P2Y12 receptor. This is associated with dephosphorylation of intraplatelet vasodilator-stimulated phosphoprotein (VASP). Vasodilator-stimulated phosphoprotein phosphorylation provides an index of platelet reactivity to clopidogrel: the higher the platelet reactivity index (PRI), the more frequently thrombosis occurs under clopidogrel (3).

Several functional polymorphisms have been found in genes encoding cytochrome P-450 isoforms involved in the

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metabolic activation of clopidogrel upstream of P2Y12 (4,5). The isoenzyme CYP2C19 seems to be one of the determinants of the pharmacodynamic response to clopidogrel (6), and is also involved in the metabolism of proton pump inhibitors (PPIs), such as omeprazole (7). Patients receiving clopidogrel and aspirin dual therapy after coronary stenting are commonly treated with PPIs.

We previously showed in an observational study of 105 consecutive patients that PPI users had significantly higher PRI values (8). Our hypothesis is that PPIs reduce the biological action of clopidogrel, probably by competitive metabolic effects on CYP2C19. The aim of the present prospective, randomized, double-blind study was to assess whether the action of clopidogrel would be reduced in patients receiving associated omeprazole treatment.

Methods

Patients and study protocol. We conducted a prospective, double-blind, placebo-controlled, randomized trial. All consecutive patients undergoing elective coronary stent implantation were considered for inclusion. They received aspirin (75 mg/day) and clopidogrel (loading dose 300 mg, followed by 75 mg/day). After written informed consent was obtained, patients were randomized to 2 treatment groups: associated 20 mg/day omeprazole or placebo, for 7 days.

Exclusion criteria were previous treatment with clopidogrel or PPI, history of thrombocytopenia (<150,000 platelets/ml) or bleeding disorder, liver disease, gastrointestinal ulcer, or pregnancy.

Patients were documented for atherosclerosis risk factors. Concomitant cardiovascular medication—statins, beta-blockers, or angiotensin-converting enzyme inhibitors—at inclusion was also recorded. The institutional review board approved the study protocol.

Platelet reactivity. Blood samples were collected on sodium citrate on Day 1 before the loading dose of clopidogrel, and 7 days afterward. Laboratory physicians performed VASP phosphorylation analysis blinded to treatment group and to whether the sample was from Day 1 or Day 7. Platelet reactivity was assessed by measuring platelet-phosphorylated VASP in whole blood using a new commercially available Platelet VASP kit (Biodis-Stago, Asnières, France) adapted from the method of Schwartz et al (9). Briefly, after initial incubation with prostaglandin E1 (PGE1) with or without 10 μmol/l ADP, platelets were fixed. The second stage consists of coupling phosphorylated VASP with a monoclonal fluorescein isothiocyanate–labeled antibody specific to the phosphorylated form of VASP. Platelet mean fluorescence intensity (MFI) was then determined using a flow cytometer counting 10,000 platelets. Platelet reactivity was expressed as a PRI calculated as:

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

This ratio is expressed as mean percentage platelet reactivity, inversely correlated with clopidogrel treatment efficiency. According to the criteria of Barragan et al. (3), patients are good responders to clopidogrel if PRI is <50% and poor responders if PRI is >50%.

Abbreviations and Acronyms

ADP = adenosine diphosphate
MFI = mean fluorescence intensity
PPI = proton pump inhibitor
PRI = platelet reactivity index
SD = standard deviation
VASP = vasodilator-stimulated phosphoprotein

Statistics. The number to treat was estimated on the basis of our previous observational study (8). We estimated that a study sample size of 120 would enable a one-half standard deviation (SD) difference (i.e., a 10% difference in PRI between groups) to be detected, with an 80% statistical power and a 5% alpha risk. To ensure that this sample size would be available for analysis, 20 extra patients were randomized and included.

The characteristics of the 2 groups were compared using chi-square tests for qualitative and *t* tests for continuous variables. The main end point compared the PRI value at the 7-day treatment period in the 2 groups by a Student *t* test. The secondary end points were the PRI variation during the 7-day treatment period in the 2 groups and a chi-square comparison of the proportion of patients with

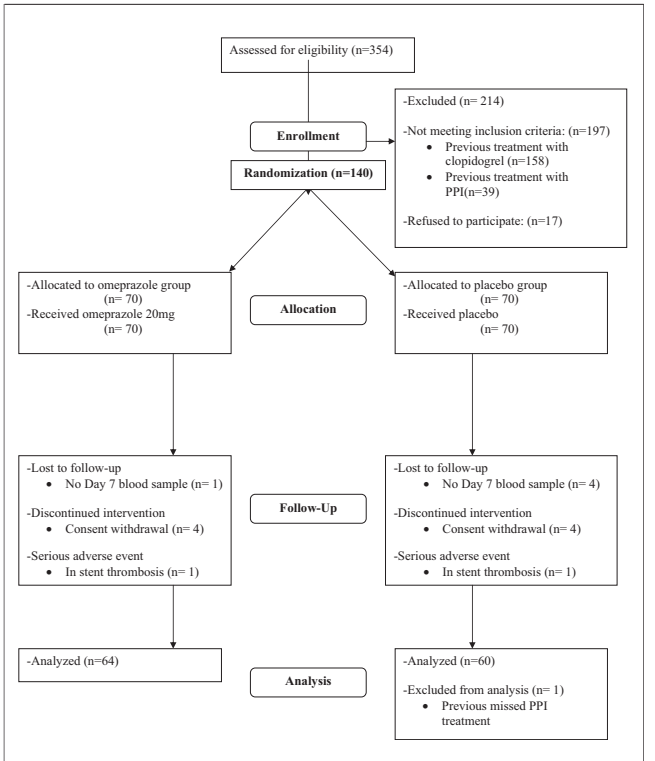

Figure 1 Consort Statement and Flow Diagram

From 354 eligible patients, 140 were included. Finally, 64 patients were analyzed in the omeprazole group and 60 in the placebo group. PPI = proton pump inhibitor.

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Table 1 Patient Baseline Characteristics and Treatments

	Placebo Group (n = 60)	Omeprazole Group (n = 64)	p
Characteristics			
Age (yrs)	63.67 ± 12.22	62.28 ± 15.04	NS
Male gender	45 (75%)	52 (81.3%)	NS
Smoker	43 (71.7%)	41 (64.1%)	NS
Hypertension	32 (47.1%)	36 (52.9%)	NS
Family history of CAD	26 (43.3%)	22 (34.4%)	NS
Diabetes mellitus	11 (18.3%)	6 (9.4%)	NS
Body mass index (kg/m^2)	27.51 ± 4.3	26.66 ± 4.7	NS
Dyslipidemia	43 (71.6%)	42 (65.6%)	NS
Previous MI	5 (8.3%)	7 (10.9%)	NS
Treatment			
Beta-blocker	58 (90.6%)	50 (83.3%)	NS
ACE inhibitor	32 (50%)	29 (48.3%)	NS
Atorvastatin	41 (68.3%)	43 (67.2%)	NS
Other statin	18 (28.1%)	14 (23.3%)	NS

ACE = angiotensin-converting enzyme; CAD = coronary artery disease; MI = myocardial infarction; NS = not significant.

PRI below 50% in the 2 groups (3). A value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed blind to randomization group.

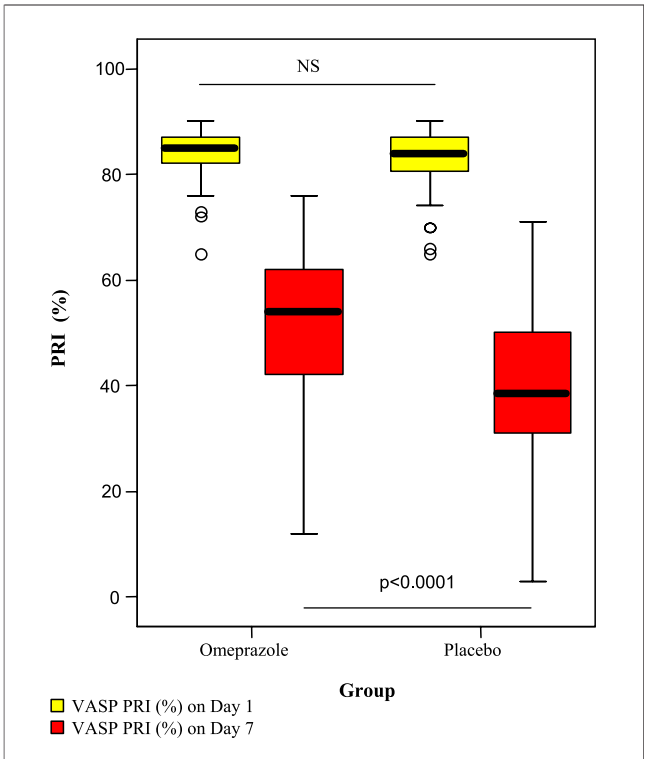

Figure 2 Mean PRI on Days 1 and 7 in the Two Groups

On Day 1, mean platelet reactivity index (PRI) was 83.2% and 83.9%, respectively, in the placebo and omeprazole groups (nonsignificant). On Day 7, mean PRI was 39.8% and 51.4%, respectively, in the placebo and omeprazole groups ($p < 0.0001$). VASP = vasodilator-stimulated phosphoprotein.

Results

Between July 2006 and November 2006, 354 patients were assessed for eligibility. Following predetermined exclusion criteria, 140 patients were included and randomized, 70 in the omeprazole group and 70 in the placebo group. The patient flowchart is shown in Figure 1. No patient was lost to follow-up. Data for 16 of the 140 patients were not analyzed: 1 was receiving PPIs at inclusion, 5 failed to return on Day 7 for blood sampling (they had no adverse events); 8 excluded themselves from the study, using their right to withdraw consent; and 2 experienced a serious adverse event (2 in-stent thromboses, both on Day 4, 1 in each group). No other serious adverse event was noted. Finally, 64 patients were analyzed in the omeprazole group and 60 in the placebo group.

Baseline characteristics were similar in the 2 groups (Table 1). On Day 1 the mean PRI was similar, 83.2% (SD 5.6) and 83.9% (SD 4.6), and on Day 7 the mean PRI was 39.8% (SD 15.4) and 51.4% (SD 16.4), respectively, in the placebo and omeprazole groups ($p < 0.0001$) (Fig. 2). The mean PRI variation was −43.3% (SD 15.9) and −32.6% (SD 16.4), respectively, in the placebo and omeprazole groups ($p < 0.0001$) (Fig. 3).

On Day 7, 16 patients (26.7%) were poor responders in the placebo group compared with 39 (60.9%) in the omeprazole group ($p < 0.0001$). The odds ratio of being a poor responder to clopidogrel when concomitantly treated with omeprazole was 4.31 (95% confidence interval 2.0 to 9.2).

Discussion

In this double-blind, placebo-controlled, randomized trial, omeprazole significantly decreased the effect of clopidogrel on platelet activation as tested by VASP phosphorylation.

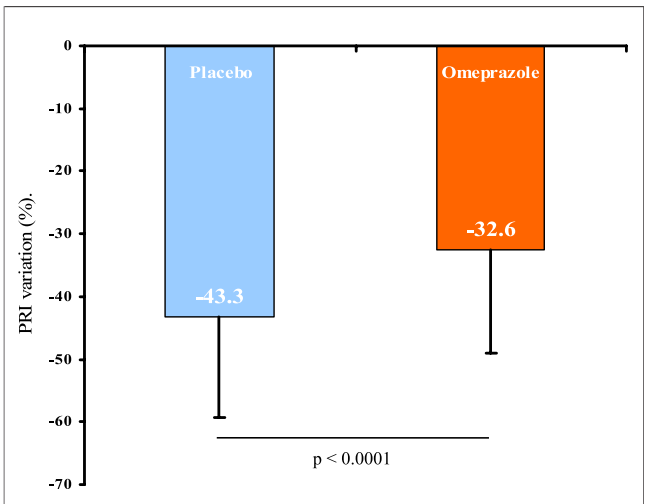

Figure 3 Variation of Platelet Reactivity Index

The platelet reactivity index (PRI) decrease was −43.3% in the placebo group and −32.6% in the omeprazole group after 7 days of treatment ($p < 0.0001$).

Platelet function test. After percutaneous coronary intervention or acute coronary syndrome, clopidogrel–aspirin dual therapy has become the standard antiplatelet management approach (1,2). Previous studies indicated that clopidogrel resistance might indicate increased risk of cardiovascular event recurrence (3,10). The mechanisms underlying clopidogrel resistance are still controversial. Possible explanations include increased reactivity in remaining platelets, genetic polymorphisms of the ADP receptor, differences in resorption, or a combination of these factors (4,5,11). Dysfunctional cytochrome P450 metabolism has also been shown when clopidogrel was associated with various drugs such as atorvastatin (4,12). However, no such significant effect emerged in further placebo-controlled studies (13,14).

Standard platelet aggregometry measures the inhibition of ADP-induced aggregation (15). Other methods include measuring fibrinogen binding or platelet adhesion, monitoring platelet activation. These general platelet function indices are relatively unspecific and are of limited clinical use because patients typically receive several drugs affecting platelet function (e.g., aspirin) concomitantly. Analysis of VASP phosphorylation is more reliable than previous assays for quantifying clopidogrel effects, with a high measurement precision and reproducibility (16,17). Because the VASP assay directly measures the function of the clopidogrel target (the P2Y12 receptor), it is selective for thienopyridine effects and is not affected by other commonly used platelet inhibitors such as aspirin (16). Potential benefits of VASP measurements would be helpful for multicenter trials and contrast with unspecific platelet function measurements that are usually performed.

Potential clinical impact. The clinical implications of our results remain uncertain but merit further investigation. The VASP assay or other techniques showed wide variability in the inhibitory platelet response to clopidogrel, with one-third of the patients appearing to be unprotected (15,17,18). Such heterogeneity may have important clinical implications (3,10). In patients undergoing percutaneous coronary intervention, Matetzky et al. (10) found ADP-induced platelet activation to be highly predictive of secondary acute cardiovascular events: 40% of patients in the first quartile of reduction experienced a recurrent cardiovascular event during 6-month follow-up, versus only 1 patient (6.7%) in the second and none in the third and fourth quartiles. Barragan et al. (3) showed that patients were protected if they had a VASP phosphorylation rate (PRI) <50%. A strong correlation between VASP test results and subacute coronary stent thrombosis was found. In our study, 60.9% of patients undergoing omeprazole therapy showed a PRI >50% (poor responders), compared with only 26.7% in the placebo group (odds ratio 4.31).

Clopidogrel, PPIs, and cytochrome P4502C19. The active metabolite of clopidogrel, which irreversibly blocks platelet ADP P2Y12 receptors, arises from complex biochemical reactions involving several CYP isoforms (19). Lau et al. (11) reported that CYP3A4 metabolic activity correlated with between-subject variability in clopidogrel responsiveness.

The PPIs are eliminated by the hepatic route, and the polymorphically expressed CYP2C19 is involved in the metabolism of omeprazole, lansoprazole, rabeprazole, esomeprazole, and pantoprazole (7). As with clopidogrel, genotypic differences in CYP2C19 status affect the action of PPIs (20).

Conclusions

In this randomized study, omeprazole significantly decreased the effect of clopidogrel on platelet as tested by VASP phosphorylation. Aspirin–clopidogrel antiplatelet dual therapy is widely prescribed worldwide, with PPIs frequently associated to prevent gastrointestinal bleeding. The clinical impact of these results must be assessed by further investigations, but we recommend not adding systematically a PPI treatment to the antiplatelet dual therapy without formal indication.

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