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**Effects of Rabeprazole on the Antiplatelet Effects and Pharmacokinetics
of Clopidogrel in Healthy Volunteers**

**Étude pharmacodynamique et pharmacocinétique de l'interaction entre le rabeprazole et
le clopidogrel chez le volontaire sain**

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ABSTRACT

Background: Several studies have suggested that proton-pump inhibitors (PPIs), mostly omeprazole, interact with clopidogrel efficacy by inhibiting the formation of its active metabolite via CYP2C19 inhibition. Whether this occurs with all PPIs remains however, a matter of debate. Since rabeprazole is a less potent CYP2C19 inhibitor than other PPIs, we studied the interaction between rabeprazole and the antiplatelet actions and pharmacokinetics of clopidogrel.

Aims: Primary objective was to demonstrate non-inferiority of rabeprazole over placebo using the change in platelet reactivity index (VAsodilator-Stimulated Phosphoprotein, VASP assay) in the predefined population of clopidogrel good responders. Omeprazole was used as the positive control.

Methods: Randomized, 3-period cross-over study in healthy volunteers. Thirty six healthy male subjects received clopidogrel (75mg/day for 7 days) together with placebo, omeprazole (20mg/day) or rabeprazole (20mg/day). Clopidogrel antiplatelet effect and disposition kinetics were assessed on day 7 of combination therapy. Non-inferiority threshold was predefined as an upper limit of the 90% CI for the difference of change in platelet reactivity index between placebo and rabeprazole <10% in good clopidogrel responders.

Results: In the predefined group of good clopidogrel responders (inhibition of VASP index >30%), clopidogrel antiplatelet effect remained non-inferior to placebo during rabeprazole (difference 3.4% (-1.7, 8.5)) but not during omeprazole co-administration (difference 7.5% (2.5, 12.6)). AUC₀₋₂₄ and C_{max} of active clopidogrel metabolite decreased with both omeprazole and rabeprazole and conditions of bioequivalence were not met except for AUC₀₋₂₄ with rabeprazole.

Conclusion: Rabeprazole does not interact with clopidogrel to the same extent as omeprazole does. However, under our experimental conditions and proton-pump inhibitors doses, there was

no significant pharmacodynamic interaction between rabeprazole and clopidogrel despite a significant decrease in the formation of clopidogrel active metabolite.

Clinical Trial Registry n° NCT00989300

Key Words (MeSH): Clopidogrel; CYP2C19 protein, human; Cytochrome P-450 Enzyme System; Drug Interactions; Proton Pump Inhibitors; Vasodilator-stimulated Phosphoprotein

RÉSUMÉ

Justification. Plusieurs études, principalement menées avec l'oméprazole, ont suggéré une interaction entre inhibiteurs de la pompe à protons (IPPs) et clopidogrel, via l'inhibition du CYP2C19 impliqué dans la transformation de la pro-drogue clopidogrel en métabolite actif. L'importance de cette interaction avec les autres inhibiteurs de pompes à protons est discutée. Cette étude avait pour objectif l'analyse de l'interaction pharmacodynamique et pharmacocinétique entre le rabéprazole, un inhibiteur plus faible du CYP2C19 que l'oméprazole, et le clopidogrel.

Objectifs. L'objectif primaire était de démontrer la non-infériorité du rabéprazole par comparaison au placebo en utilisant l'index de réactivité plaquettaire (test VASP) dans une population de volontaires sains bon répondeurs au clopidogrel. L'oméprazole a été utilisé comme contrôle positif.

Méthodes. Étude croisée, randomisée, en trois périodes, menée chez 36 hommes volontaires sains recevant du clopidogrel (75mg/jour pendant 7 jours) avec du placebo, de l'oméprazole (20mg/jour) ou du rabéprazole (20mg/jour). L'effet anti-plaquettaire du clopidogrel et ses données pharmacocinétiques ont été mesurés au 7^{ème} jour de traitement. Le seuil de non-infériorité a été défini *a priori* comme une limite supérieure de l'intervalle de confiance à 90% <10% pour la différence entre la diminution de l'index de réactivité plaquettaire (test VASP) entre le placebo et le rabéprazole chez les bons répondeurs au clopidogrel.

Résultats. Dans le groupe de bons répondeurs (inhibition du VASP PRI >30%), l'effet antiplaquettaire du clopidogrel était non inférieur à celui du placebo avec le rabéprazole (différence 3,4% (-1,7 ; 8,5)) contrairement à l'oméprazole (différence 7,5% (2,5 ; 12,6)). Toutefois, l'AUC₀₋₂₄ et la Cmax du métabolite actif du clopidogrel étaient significativement diminuées avec l'oméprazole et le rabéprazole et les conditions de bioéquivalence n'étaient pas remplies, excepté pour l'AUC₀₋₂₄ avec le rabéprazole.

Conclusion. L'interaction pharmacodynamique entre le rabéprazole et le clopidogrel n'a pas le même degré d'intensité que celle entre l'oméprazole et le clopidogrel. Cependant, dans nos conditions expérimentales, l'interaction entre rabéprazole ou oméprazole et le clopidogrel n'était pas significative malgré une inhibition significative de la génération du métabolite actif du clopidogrel.

Clinical Trial Registry n° NCT00989300

Mots-clés: clopidogrel, CYP2C19, cytochrome P450, interactions médicamenteuses, inhibiteurs de pompes à protons, protéine VASP

INTRODUCTION

Dual antiplatelet therapy with aspirin and clopidogrel is associated with a significant reduction in cardiovascular ischemic events after acute coronary syndromes or percutaneous coronary interventions and is recommended in US [1] and European [2] guidelines. Clopidogrel is an inactive prodrug which undergoes two oxidative steps involving multiple cytochrome P-450 enzymes in its bioactivation to its pharmacologically active metabolite. Among them, CYP2C19, a cytochrome P-450 enzyme whose activity is genetically-determined, contributes predominantly to this bioactivation [3, 4] and modulates the antiplatelet and therapeutic response to clopidogrel. Patients with loss of function polymorphism in the CYP2C19 gene are less responsive to clopidogrel [5, 6] although the importance of this phenomenon remains controversial [7-10] and may be limited to the risk of stent thrombosis [11].

Proton-pump inhibitors (PPIs) are recommended in patients treated with dual antiplatelet therapy who are at high risk of gastrointestinal bleeding [12]. PPIs are metabolized primarily via the cytochrome P-450 (CYP) 2C19 and CYP3A4 isoenzymes [13] and are competitive inhibitors of CYP2C19 activity [14]. However, the contribution of the CYP2C19 isoenzyme to PPI biotransformation, and to *H. pylori* eradication rates [15] and the potency for inhibition of CYP2C19 activity [14] varies among different PPIs. CYP2C19 activity appears to affect the response to omeprazole, esomeprazole and lansoprazole [16-18] and to be inhibited by these PPIs [14, 18]. This does not seem to be the case, at least not to the same extent, with pantoprazole [14, 19] and rabeprazole [14, 20].

Concerns of PPI and clopidogrel interaction were raised when omeprazole was found to inhibit the antiplatelet effect of clopidogrel in an *in vivo* study of 124 patients undergoing elective coronary stent implantation [21]. Several studies have suggested that omeprazole interacts with clopidogrel efficacy by inhibiting the formation of its active metabolite via CYP2C19 inhibition [22, 23]. Whether this occurs with all PPIs or even is of significant amplitude with omeprazole remains, however, a matter of debate [9, 24-29]. However it has

been recently demonstrated that generation of clopidogrel active metabolite and inhibition of platelet function were reduced less by the coadministration of dexlansoprazole or lansoprazole with clopidogrel than by the coadministration of esomeprazole or omeprazole [30].

Since rabeprazole is a less potent CYP2C19 inhibitor than other PPIs [14], we performed a pharmacodynamic antiplatelet activity study of the interaction between standard recommended repeated doses of rabeprazole and clopidogrel in CYP2C19-genotyped healthy male subjects. Omeprazole and placebo were used as controls. Our primary objective was to demonstrate non-inferiority of rabeprazole over placebo using the change in platelet reactivity index (Δ PRI%) in clopidogrel good responders as derived from the VAsodilator-Stimulated Phosphoprotein (VASP) assay as the primary endpoint.

METHODS

Study design

This was a prospective, placebo- and active-controlled, open label, blinded evaluations, randomized 3-way crossover study. The study assessed the influence of rabeprazole (20 mg o.d. for 7 days) and omeprazole (20 mg o.d. for 7 days) on clopidogrel (75 mg o.d. for 7 days) antiplatelet effects and pharmacokinetics in 36 CYP2C19-genotyped non-smoking healthy Caucasian male subjects with normal basal platelet aggregation testing (> 50% aggregation to 1 μ g/mL collagen, 1 to 2 mmol/L arachidonic acid and 10 μ M adenosine diphosphate (ADP)), platelet count, complete blood count and prothrombin time. Subjects gave written informed consent to participate and to have CYP2C19 genotyping (but were not selected on their genotype) and the protocol was approved by the Committee for Protection of Human Subjects Île-de-France II and the French Medicine Agency.

Subjects were randomized based on a Latin square design to receive clopidogrel, 75 mg o.d. in the morning in the fasting state for 7 days during 3 study periods separated by a drug-free period of 2 to 3 weeks together with placebo, 20 mg of rabeprazole or 20 mg of omeprazole given at the same time as clopidogrel. Platelet function evaluation (pharmacodynamics) was performed on day 1 before dosing and on day 7 before and 4 hrs after the last intake of study drugs. Pharmacokinetics of clopidogrel, its inactive carboxylic acid metabolite as well as the active metabolite was determined from blood samples taken before (H0) and at various times after administration of the last dose of clopidogrel with the concomitant drug (either placebo or PPI). Additional blood samples for determination of omeprazole, 5-hydroxy-omeprazole, rabeprazole and rabeprazole-thioether plasma concentrations were taken 3 and 4 hrs post-dose on Day 7 to confirm proper exposure to PPIs.

Pharmacodynamic evaluations

The primary test to assess platelet function was based on the VASP-phosphorylation level measured on whole blood using a flow cytometric assay (Platelet VASP[®], Diagnostica Stago, Biocytex, Asnières, France) and a FACScan flow cytometer (Becton Dickinson, Le Pont de Claix, France). Results were expressed as Platelet Reactivity Index (PRI%) calculated from the mean fluorescence intensity (MFI) of samples incubated with prostaglandin E1 (PGE1) alone or with both PGE1 and ADP simultaneously, using the following formula: $(MFI_{PGE1} - MFI_{PGE1+ADP} / MFI_{PGE1}) \times 100$ as previously described [3]. This test, also referred to as the VASP index, is a test that specifically assesses P2Y12 receptor activity [31], the target of clopidogrel antiplatelet action and it is widely used for monitoring the responsiveness to clopidogrel [32, 33]. The percent change in PRI on study day 7 just before the last administration of study drugs relative to baseline, i.e. prior to drug administrations, percent change in Δ PRI (%) D7H0, was used as the primary study endpoint. Δ PRI (%) relative to Day1 was also calculated for Day 7 H4.

Platelet aggregation was also determined at the same time points as those of VASP-phosphorylation level assessments with ADP-induced platelet optical aggregometry (Biopool, ADP 10 and 20 μ M) using platelet-rich plasma adjusted to 250 x 10⁹/L. Inhibition of Platelet Aggregation (IPA%) induced by ADP was calculated as: $[\text{MPA}(\text{Day } 1) - \text{MPA}(\text{Day } 7) / \text{MPA}(\text{Day } 1) \times 100]$, where MPA is the maximal platelet aggregation induced by ADP. Platelet aggregation tests were performed on a TA-8V optical platelet aggregometer (Soderel Medical, Heillecourt, France) within 3 hours from sampling in all subjects.

Pharmacodynamic evaluations were performed blind to the study period and to CYP2C19 genotype.

Pharmacokinetic evaluations

Blood samples for clopidogrel assay were collected in 6ml EDTA vials stored at +4°C to which 38 μ l of 2-Bromo-3'-methoxyacetophenone (500mM in acetonitrile) were added within 30 sec of sampling to stabilize the active metabolite. Blood samples were centrifuged at +4°C within 30min and stored at -80 °C until assay. Clopidogrel, clopidogrel carboxylic acid, clopidogrel active metabolite, omeprazole, 5-hydroxy-omeprazole, rabeprazole and rabeprazole-thioether, were extracted from plasma on solid phase OASIS HLB cartridge (10mg/1ml, Waters SAS, Milford USA). Chromatographic separation and detection of all compounds was performed on YMC-UltraHT Pro C18 analytical column (YMC, Dinslaken, Germany) using a ultra high performance liquid chromatography coupled to tandem mass spectrometry system (UPLC-Acquity-TQD Waters SAS, Milford, USA). Limits of quantification were 0.1ng/ml for clopidogrel and clopidogrel active metabolite, 5ng/ml for rabeprazole and rabeprazole-thioether, 10ng/ml for clopidogrel carboxylic acid, 50ng/ml for omeprazole and 5-hydroxy-omeprazole.

Pharmacokinetic parameter values were calculated using WinNonlin[®] Professional Version 5.2, or higher, (Pharsight Corp., Mountain View, California). Maximum plasma

concentration (C_{max}) and the time of its occurrence (T_{max}) were obtained from observed values. The area under the concentration-time curve (AUC) in the sampled matrix during a dosing interval was calculated by linear up/log down trapezoidal summation. Apparent terminal rate constant (λ_z) after multiple dosing (1/h), was determined by linear regression of the terminal points of the log linear concentration time curve. Apparent terminal half-life after multiple dosing (h) was determined as $(\ln 2/\lambda_z)$.

CYP2C19 genotyping and activity

The loss-of-function *CYP2C19* variants *2 (rs4244285) and *3 (rs4986893) were tested using PCR-based specific probe hybridization and single base extension. 681G>A and 636G>A comprise the two common reduced functional variants *CYP2C19**2 and *CYP2C19**3, respectively. Subjects with the *CYP2C19**1/*1 genotype were designated as *CYP2C19* extensive metabolizer (EM) subjects.

The molar omeprazole/5-hydroxy-omeprazole metabolic ratio in plasma samples at 3hrs was calculated as an index of *CYP2C19* activity [34-36]. In one EM subject, this ratio was calculated from the blood sample taken at 4 hrs because 5-hydroxy-omeprazole was not detectable at 3 hrs.

Statistical analyses

Sample size was calculated with the assumption that approximately 66% of subjects would be good antiplatelet responders defined as subjects in whom the VASP index on study day 7 relative to study day 1 would decrease by at least 30% with an expected intra-subject standard deviation of differences in Δ PRI of $\leq 14\%$ [37] or a PRI value at day 7 below a cut-off value of 60 %, as recently proposed for clopidogrel 75 mg daily maintenance dose [38]. With these assumptions, 36 subjects were sufficient to conclude for non-inferiority of rabeprazole to

placebo with 10% Δ PRI as the limit of non inferiority with greater than 95% power when true difference in treatment means is equal to 2%. Pharmacodynamic analyses were first performed on good antiplatelet responders as defined above, then on all 36 subjects.

Mixed effect models were fitted to the Δ PRI% data as the dependent variable, and sequence, treatment and period as factors and subject as a random effect. Ninety percent confidence intervals (CI) were calculated for the difference in means between rabeprazole versus placebo. Non-inferiority was concluded if the upper limit of the 90% CI fell below 10%. This non-inferiority limit was chosen because it represents the difference between omeprazole and placebo reported by Gilard et al. [21] (10.7% in absolute value, 13.4% in relative value) which prompted the FDA's warning on PPIs interaction with clopidogrel.

Additional post-hoc analyses were performed to compare the change in VASP index on study day 7 relative to study day 1 with omeprazole and rabeprazole relative to placebo using Wilcoxon signed ranks test in good antiplatelet responders. Post-hoc correlations analyses were performed using Pearson's correlation.

A linear mixed effects model suitable for 3-way crossover design was fit to log-transformed pharmacokinetic parameters and 90% CI for the ratio of the mean pharmacokinetic parameters of clopidogrel were constructed using least-square means and intra-subject variance from the model. The above analysis was performed for clopidogrel active metabolite and clopidogrel major carboxylic acid metabolite. Bioequivalence was considered as demonstrated if the 90% CI of the ratios for AUC_{0-24} and C_{max} between the placebo and PPI study periods fell in the range 80 – 125%.

RESULTS

Thirty six subjects completed the three study periods. Mean age, body weight and body mass index were 33.6 ± 7.9 yrs, 74.1 ± 8.7 kg and 23.6 ± 2.3 kg/m², respectively. Of these 36

subjects, 23 were *CYP2C19*1/*1* EMs, 12 were heterozygous *CYP2C19*1/*2* and one was a poor metabolizer with the *CYP2C19*2/*2* genotype.

Platelet function assays:

Baseline VASP Index before administration of clopidogrel was not significantly different across study periods ($p=0.60$). As expected, there was considerable inter-individual variability in platelet function inhibition as measured by use of the VASP index (VASP Δ PRI%) on day 7 of the clopidogrel plus placebo study period prior to last drug administration (D7H0) (figure 1). The decrease in VASP index was $<30\%$ in 18 subjects while the other 18 subjects were classified as good clopidogrel responders (change of VASP index $>-30\%$). Table 1 shows the results of platelet aggregation studies on day 7 (D7) of each study period before (H0) and 4hrs (H4) after administration of the last dose of clopidogrel together with placebo, omeprazole and rabeprazole.

In good clopidogrel responders as evaluated by VASP assay, the upper limit of the 90% CI non-inferiority threshold of 10% was crossed during co-prescription of omeprazole but not during co-prescription of rabeprazole at D7H0 and D7H4. Therefore, in this pre-defined group of subjects in whom a significant antiplatelet activity was present during administration of clopidogrel with placebo, the antiplatelet effect of clopidogrel during co-administration of rabeprazole was non-inferior to placebo whereas this was not the case during omeprazole co-administration. In this group, the increase of VASP reactivity index relative to placebo did not differ significantly during rabeprazole and during omeprazole ($p=0.067$, figure 2).. However, the change in VASP index from placebo was statistically significant during omeprazole ($p=0.017$) but not during rabeprazole ($p=0.26$) at D7H0 and D7H4 ($p<0.009$ and $p=0.20$, respectively) (Table 1)

When considering the entire population of 36 subjects, VASP index at D7H0 and D7H4 was not significantly altered by co-administration of omeprazole or rabeprazole. The increase of VASP index at D7H4 with omeprazole did not reach statistical significance ($p=0.056$). Between-period differences were less consistent when considering inhibition of platelet aggregation (IPA%) induced by ADP (table 1). The 10% non-inferiority threshold was crossed in all subjects for both omeprazole and rabeprazole only in the presence of ADP $10\mu\text{M}$.

CYP2C19 genotype influenced the antiplatelet effects of clopidogrel. Compared with subjects with the *CYP2C19*1/*2* genotype ($n=12$), EM subjects ($n=23$) had more antiplatelet effect as assessed by the change in VASP index at D7H0 during placebo ($-39.3\pm 0.20\%$ in *CYP2C19*1/*1* vs. $-22\pm 0.15\%$ in *CYP2C19*1/*2*; $p<0.015$). Among the 23 EM subjects, 15 were good antiplatelet responders. One subject became non-responder with omeprazole and none with rabeprazole (figure 3). Among the 12 subjects with the *CYP2C19*1/*2* genotype, only 3 were good antiplatelet responders during administration of clopidogrel with placebo. One subject became non-responder with both rabeprazole and omeprazole (figure 3).

Clopidogrel disposition kinetics:

Table 2 shows the main pharmacokinetic parameters for clopidogrel active metabolite in all subjects. We also analyzed pharmacokinetics parameters in EM subjects homozygous for *CYP2C19*1*1*. Figure 4 shows the plasma concentration vs. time profile of clopidogrel active metabolite in all subjects.

In the entire population, despite a significant fall compared to placebo, the AUC_{0-24} of clopidogrel active metabolite during rabeprazole co-administration remained within the bioequivalence limits relative to the placebo study period. This was not the case during omeprazole co-administration. Bioequivalence was not met for C_{max} during administration of both PPIs. In EM subjects, bioequivalence was not met for any of the measured parameters

during both omeprazole and rabeprazole co-administration. Mean T_{max} was 0.67hr in the 3 study groups.

The AUC₀₋₂₄ and apparent elimination half-life of clopidogrel and its main carboxylic acid metabolite remained within the bioequivalence range during both omeprazole and rabeprazole co-administration (data not shown). Other parameters which were not bioequivalent were: C_{max} of clopidogrel during the rabeprazole study period (ratio of 85.1, 90% CI: 75.1–98.0), C_{max} of carboxylic acid metabolite during rabeprazole (ratio of 82.0, 90% CI: 72.2–93.2) and during omeprazole (ratio of 83.3, 90% CI: 73.3–94.6) co-administration.

In the 23 subjects who were CYP2C19 EMs, AUC₀₋₂₄ of clopidogrel active metabolite decreased significantly during co-administration of omeprazole and rabeprazole (figure 5).

Regression analyses:

Platelet reactivity (VASP PRI) at D7H0 negatively correlated with clopidogrel active metabolite AUC₀₋₂₄ during placebo ($r^2=0.32$; $n=36$; $p<0.001$), rabeprazole ($r^2=0.30$; $n=36$; $p<0.001$) and omeprazole ($r^2=0.18$; $n=36$; $p<0.007$) co-administration. The change of VASP Δ PRI at D7H0 from placebo during each PPI study period positively correlated with the change of clopidogrel active metabolite AUC₀₋₂₄ during co-administration of the corresponding drug, rabeprazole ($r^2=0.11$; $n=36$; $p<0.025$) or omeprazole ($r^2=0.11$; $n=36$; $p<0.027$).

Omeprazole metabolic ratio could not be determined in one EM subject. The change in platelet inhibition (VASP Δ PRI) at D7H0 during the placebo period and the change of platelet aggregation (IPA%) induced by ADP 10 μ M (but not 20 μ M) at D7H0 positively correlated with the omeprazole metabolic ratio ($r^2=0.19$; $n=35$; $p<0.006$ and $r^2=0.17$; $n=35$; $p<0.009$, respectively) a higher metabolic ratio, i.e. less CYP2C19 activity, being associated with less antiplatelet effect. No significant correlation was found between the change of VASP index or

the change in AUC of clopidogrel active metabolite during omeprazole or rabeprazole periods and CYP2C19 activity as assessed by use of the omeprazole metabolic ratio.

There was no correlation between omeprazole plasma concentration at 3hrs (mean, SD: 665±576ng/ml) or rabeprazole (mean, SD: 351±233ng/ml) or rabeprazole-thioether (mean, SD: 129±85ng/ml) plasma concentration at 4hrs and the change in VASP Δ PRI during the corresponding PPI combination therapy.

The fall in clopidogrel active metabolite C_{max} and AUC₀₋₂₄ during rabeprazole and omeprazole were correlated ($r^2=0.56$; $n=36$; $p<0.001$ in both cases) and the slopes of these relationships did not significantly differ from unity.

DISCUSSION

This randomized study, cross over study was designed to analyze the potential interaction between clopidogrel and rabeprazole, omeprazole being used as a putative positive control. It was conducted in healthy male volunteers, thus eliminating potential confounding factors, including smoking, noncompliance and other medications.

Since an inhibitory interaction is not expected to occur in subjects who do not have an adequate response in the absence of inhibitor, the predefined group of VASP antiplatelet good responders was chosen to examine the pharmacodynamic interactions between rabeprazole and clopidogrel. VASP index is considered as a specific test to evaluate P2Y₁₂ inhibition compared to light-transmission aggregometry to predict outcome during dual antiplatelet therapy although both tests have a predictive value [31, 33, 39, 40]. In the group of good VASP antiplatelet responders, clopidogrel antiplatelet effect remained non-inferior to placebo at D7H0 and D7H4 during rabeprazole co-administration whereas it crossed the limit of non-inferiority during omeprazole co-administration. Therefore, from a pharmacodynamic point of view, in subjects in whom clopidogrel elicits a marked antiplatelet effect, inhibition of clopidogrel antiplatelet

action is minimal with rabeprazole whereas a statistically significant reversal of clopidogrel effects is observed with omeprazole.

However, when using aggregometry, a test less specific of P2Y₁₂ but that reflects the global platelet function, inhibition of platelet aggregation induced by ADP 10 μ M significantly decreased with both omeprazole and rabeprazole in the entire population. These results are in line with PK analysis, showing a decreased exposure to clopidogrel active metabolite with both PPIs. AUC₀₋₂₄ and C_{max} of active clopidogrel metabolite significantly decreased with both omeprazole and rabeprazole and conditions of bioequivalence were not met, except for AUC₀₋₂₄ with rabeprazole. This discrepancy between pharmacokinetic and pharmacodynamic parameters (VASP) changes was also found in a drug interaction study which examined the influence of pantoprazole (80mg/day) on clopidogrel antiplatelet effects and exposure to its active metabolite [19]. In this study, no significant change in VASP index (but significant changes in ADP 5 μ M-induced maximum platelet aggregation) was found despite a statistically significant decrease in clopidogrel active metabolite AUC₀₋₂₄ and C_{max} with pantoprazole of the same order of magnitude as what was found in our study. Greater decreases of exposure to clopidogrel active metabolite were found with high dose omeprazole (80mg/day) in the study by Angiollilo et al. [19] and were associated with significant inhibition of VASP and ADP-induced platelet aggregation. In our study the change of VASP index with PPIs was weakly associated ($r^2=0.11$) with the change of clopidogrel active metabolite exposure produced by omeprazole and rabeprazole although the association between VASP index and clopidogrel active metabolite AUC₀₋₂₄ was stronger ($r^2=0.32$) during administration of clopidogrel with placebo. Taken together, these results suggest that a certain extent of pharmacokinetic interaction on clopidogrel active metabolite is necessary to produce a significant pharmacodynamic interaction. This could explain why the amplitude of the pharmacodynamic interaction we found with omeprazole was limited in size.

As expected, [22, 23] CYP2C19 genotype and activity influenced clopidogrel antiplatelet activity in the absence of PPI with greater inhibition of platelet aggregation in homozygous EM subjects compared to subjects with at least one non-functional *CYP2C19* allele. Also, during the placebo study period, clopidogrel-induced change of VASP index and of platelet aggregation induced by 10 μ M (but not 20 μ M) ADP correlated with CYP2C19 activity as assessed by use of the omeprazole metabolic ratio. However, the association was weak with only about 18% of antiplatelet effect explained by the omeprazole metabolic ratio. During PPI administrations, no significant correlation was found between the change of VASP index or the change in AUC of clopidogrel active metabolite and CYP2C19 activity as assessed by the omeprazole metabolic ratio. Such an absence of association by regression analysis raises the question of the role of CYP2C19 inhibition in explaining our findings. Rabeprazole is mainly metabolized by non-enzymatic reduction to rabeprazole-thioether [41] and is a less potent inhibitor of CYP2C19 than omeprazole [14, 42, 43]. This may explain the fact that rabeprazole had less effects than omeprazole on the clopidogrel-induced change of VASP index although the study was not powered to test the statistical significance of this difference. However it does not explain the similarity of the pharmacokinetic interaction on clopidogrel active metabolite with both PPIs. In this respect, pantoprazole [19] and rabeprazole appear to have comparable profiles. Also, rabeprazole-thioether, the main circulating metabolite of rabeprazole, is a CYP2C19 inhibitor [14] and could have contributed to the observed effects. Finally, CYP2C19 is not the only cytochrome P-450 which contributes to the bioactivation of clopidogrel to its active metabolite [4]. CYP2C19 contributes to the first step of clopidogrel metabolism to its 2-oxo unstable metabolite for 45% while CYP1A2 and CYP2B6 contribute for 36% and 19%, respectively. CYP2C19 contributes to the final step of clopidogrel active metabolite formation from 2-oxoclopidogrel for only 20% while CYP3A4, CYP2B6 and CYP2C9 contribute for 40%, 33% and 7%, respectively [4]. It is therefore conceivable that non CYP2C19-mediated mechanisms may contribute to the interaction between PPIs and clopidogrel.

For uniformity, our study included only young male volunteers, a population which does not reflect the diversity of patients with ischemic heart disease who usually receive dual antiplatelet therapy and an initial loading dose of clopidogrel. In the target population, clopidogrel is usually prescribed with aspirin and it has been suggested that inhibition of antiplatelet effect may result from an interaction of PPIs with aspirin absorption [44, 45] independently from the interaction with clopidogrel [46, 47]. Inhibition of clopidogrel absorption by PPIs is unlikely to occur since clopidogrel is a weak base which is not absorbed from the stomach, in contrast to aspirin. To our knowledge only one study compared the effects of omeprazole and rabeprazole on the antiplatelet action of clopidogrel in patients on dual antiplatelet therapy [48]. In this open-labeled study in a limited number of patients, both omeprazole and rabeprazole decreased the effects of clopidogrel on platelet aggregation induced by 10 μ M ADP. However, the authors acknowledged that their study was not placebo-controlled and did not have the power to detect a difference between omeprazole and rabeprazole. Another recent study reported on the interaction between a single 300 mg dose of clopidogrel and rabeprazole (20mg) and did not find an interaction [49]. Our study, despite its limitations indicated above, suggests that the interaction between rabeprazole and clopidogrel is likely to be less pronounced than the interaction between omeprazole and clopidogrel in patients with heart disease. It also shows that the interaction with omeprazole is of small amplitude when the standard therapeutic dose of 20mg/day is used. Under our experimental conditions and PPIs doses, there was no significant pharmacodynamic interaction between rabeprazole or omeprazole and clopidogrel despite a significant decrease in the formation of clopidogrel active metabolite. This is consistent with a previous study with pantoprazole [19] and suggests that there is a threshold of decreased clopidogrel active metabolite formation which is required to produce a pharmacodynamic interaction.

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CONFLICTS OF INTEREST

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Figure Legends

Figure 1: Change of VASP platelet reactivity index (PRI) at trough on day 7 of clopidogrel 75 mg o.d. in the presence of placebo in 36 healthy subjects. Subjects in whom the VASP index on Day 7 relative to Day 1 was decreased by at least 30% were defined as good antiplatelet responders.

Figure 2: Change of VASP platelet reactivity index (PRI) on day 7 at trough of clopidogrel 75 mg o.d. during co-administration of rabeprazole and omeprazole in 18 good antiplatelet responders. Each box plots represents interquartile range with mean (horizontal line in the box) and median (dot in the box) and whiskers represent the 5 - 95 percentiles. $p=0.067$

Figure 3: Change of VASP platelet reactivity index (PRI) at trough on day 7 of clopidogrel 75 mg o.d. in the presence of placebo, rabeprazole and omeprazole in 36 healthy subjects according to CYP2C19 genotypes.

Figure 4: Mean (SD) plasma concentration of clopidogrel active metabolite as a function of time on day 7 of clopidogrel 75 mg o.d. in the presence of placebo, rabeprazole and omeprazole in 36 healthy subjects.

Figure 5: Area under plasma concentration-time curve from 0 to 24 hours of clopidogrel active metabolite on day 7 of clopidogrel 75 mg o.d. in the presence of placebo, rabeprazole and omeprazole in 36 healthy subjects according to CYP2C19 genotypes. * $p<0.001$

Table 1: Antiplatelet effects of clopidogrel 75 mg o.d. for 7 days in the presence of placebo, omeprazole and rabeprazole.

	Treatment	N	Least Square		Pairwise Comparisons			p-value*
			Mean (%)	95% CI	Pair	Difference (%)	90% CI	
VASP ΔPRI (%)								
Good VASP Antiplatelet responders								
Day 7 / Hour 0	RABE	18	-47.3	(-52.5; -42.1)	RABE/OME	-4.1	(-9.2; 1.0)	0.18
					RABE/PLBO	3.4	(-1.7; 8.5)	0.26
	OME	18	-43.2	(-48.4; -38.0)	OME/PLBO	7.5	(2.5; 12.6)	0.017
Day 7 / Hour 4	PBO	18	-50.7	(-55.9; -45.6)				
	RABE	18	-56.2	(-62.5; -49.9)	RABE/OME	-4.9	(-10.5; -0.8)	0.15
					RABE/PLBO	4.4	(-1.2; 9.9)	0.2
Day 7 / Hour 4	OME	18	-51.3	(-57.6; -45.1)	OME/PLBO	9.2	(3.6; 14.8)	0.0087
	PBO	18	-60.3	(-66.8; -54.3)				
All subjects								
Day 7 / Hour 0	RABE	36	-32.1	(-38.8; -25.5)	RABE/OME	-1.6	(-5.1; 1.8)	0.44
					RABE/PLBO	0.4	(-3.1; 3.8)	0.85
	OME	36	-30.5	(-37.5; -23.9)	OME/PLBO	2	(-1.5; 5.5)	0.34
Day 7 / Hour 4	PBO	36	-32.5	(-39.2; -25.9)				
	RABE	36	-39.8	(-47.4; -32.1)	RABE/OME	-3.7	(-7.2; -0.1)	0.089
					RABE/PLBO	0.5	(-3.1; 4.0)	0.82
Day 7 / Hour 4	OME	36	-36.1	(-43.8; -28.5)	OME/PLBO	4.2	(0.6; 7.7)	0.056
	PBO	36	-40.3	(-47.9; -32.6)				
Inhibition of platelet aggregation (IPA) induced by ADP Day 7 / Hour 0								
All subjects								
ADP 20μM	RABE	36	39.4	(32.4; 46.4)	RABE/OME	4.3	(-0.1; 8.8)	0.11
					RABE/PLBO	-0.8	(-5.3; 3.7)	0.77
	OME	36	35.1	(28.1; 42.1)	OME/PLBO	-5.1	(-9.6; -0.6)	0.063
ADP 10μM	PBO	35	40.2	(33.1; 47.2)				
	RABE	36	39.8	(32.7; 46.9)	RABE/OME	0.6	(-4.7; 5.8)	0.86
					RABE/PLBO	-6.3	(-11.6; -1.1)	<0.05
ADP 10μM	OME	36	39.2	(32.1; 46.3)	OME/PLBO	-6.9	(-12.2; -1.6)	0.033
	PBO	35	46.1	(39.0; 53.3)				

VASP: VAsodilator-Stimulated Phosphoprotein; ΔPRI: Change in Platelet Reactivity Index; ADP: adenosine diphosphate; IPA: Inhibition of Platelet Aggregation; CI: Confidence Interval, RABE: rabeprazole; OME: omeprazole; PLBO: placebo; Good Antiplatelet Responders were defined as subjects in whom the VASP index on Day 7 relative to Day 1 was decreased by at least 30%. * p values for equality of means.

Table 2: Pharmacokinetics of Clopidogrel Active Metabolite on day 7 of clopidogrel 75 mg o.d. in the presence of placebo, omeprazole and rabeprazole.

Parameter (unit)	Treatment	N	Geometric Least Square		Pairwise Comparisons			p-value
			Mean	95% CI	Pair	Ratio (%)	90% CI	
All subjects								
AUC ₀₋₂₄ (ng•h/mL)	RABE	36	26.6	(23.1 - 30.6)	RABE/OME	108.1	(100.0 - 116.9)	0.10
					RABE/PLBO	88.4	(81.7 - 95.6)	0.01
	OME	36	24.6	(21.4 - 28.3)	OME/PLBO	81.7	(75.6 - 88.4)	<0.0001
	PLBO	36	30.1	(26.2 - 34.6)				
Cmax (ng/mL)	RABE	36	15.1	(12.5 - 18.1)	RABE/OME	105.8	(90.6 - 123.6)	0.54
					RABE/PLBO	72.1	(61.7 - 84.2)	<0.001
	OME	36	14.3	(11.9 - 17.1)	OME/PLBO	68.1	(58.3 - 79.5)	0.0001
	PLBO	36	20.9	(17.4 - 25.2)				
t1/2 (h)	RABE	19	4.5	(2.7 - 7.7)	RABE/OME	91.5	(56.8 - 147.4)	0.75
					RABE/PLBO	105.3	(65.3 - 169.5)	0.86
	OME	19	5.0	(2.9 - 8.4)	OME/PLBO	115.0	(71.4 - 185.3)	0.62
	PLBO	19	4.3	(2.6 - 7.3)				
CYP2C19 *1/*1								
AUC ₀₋₂₄ (ng•h/mL)	RABE	23	29.8	(25.7 - 34.5)	RABE/OME	107.1	(97.8 - 117.3)	0.21
					RABE/PLBO	82.3	(75.1 - 90.2)	0.0009
	OME	23	27.8	(24.0 - 32.2)	OME/PLBO	76.8	(70.1 - 84.2)	<0.0001
	PLBO	23	36.2	(31.3 - 41.9)				
Cmax (ng/mL)	RABE	23	16.7	(13.3 - 21.1)	RABE/OME	104.1	(86.6 - 125.3)	0.72
					RABE/PLBO	66.8	(55.4 - 80.5)	0.0008
	OME	23	16.1	(12.8 - 20.3)	OME/PLBO	64.1	(53.3 - 77.2)	0.0002
	PLBO	23	25.1	(19.9 - 31.6)				
t1/2 (h)	RABE	10	6.4	(3.3 - 12.6)	RABE/OME	126.5	(62.5 - 256.1)	0.57
					RABE/PLBO	75.8	(37.0 - 155.4)	0.51
	OME	10	5.1	(2.5 - 10.1)	OME/PLBO	59.9	(28.1 - 127.5)	0.25
	PLBO	10	8.5	(4.2 - 17.0)				

AUC₀₋₂₄: area under plasma concentration-time curve from 0 to 24 hours; Cmax: Maximum observed plasma concentration; t1/2: apparent elimination half-life; CI: Confidence Interval, RABE: rabeprazole; OME: omeprazole; PLBO: placebo.

Figure 1: Change of VASP platelet reactivity index (PRI) at trough on day 7 of clopidogrel 75 mg o.d. in the presence of placebo in 36 healthy subjects. Subjects in whom the VASP index on Day 7 relative to Day 1 was decreased by at least 30% were defined as good antiplatelet responders.

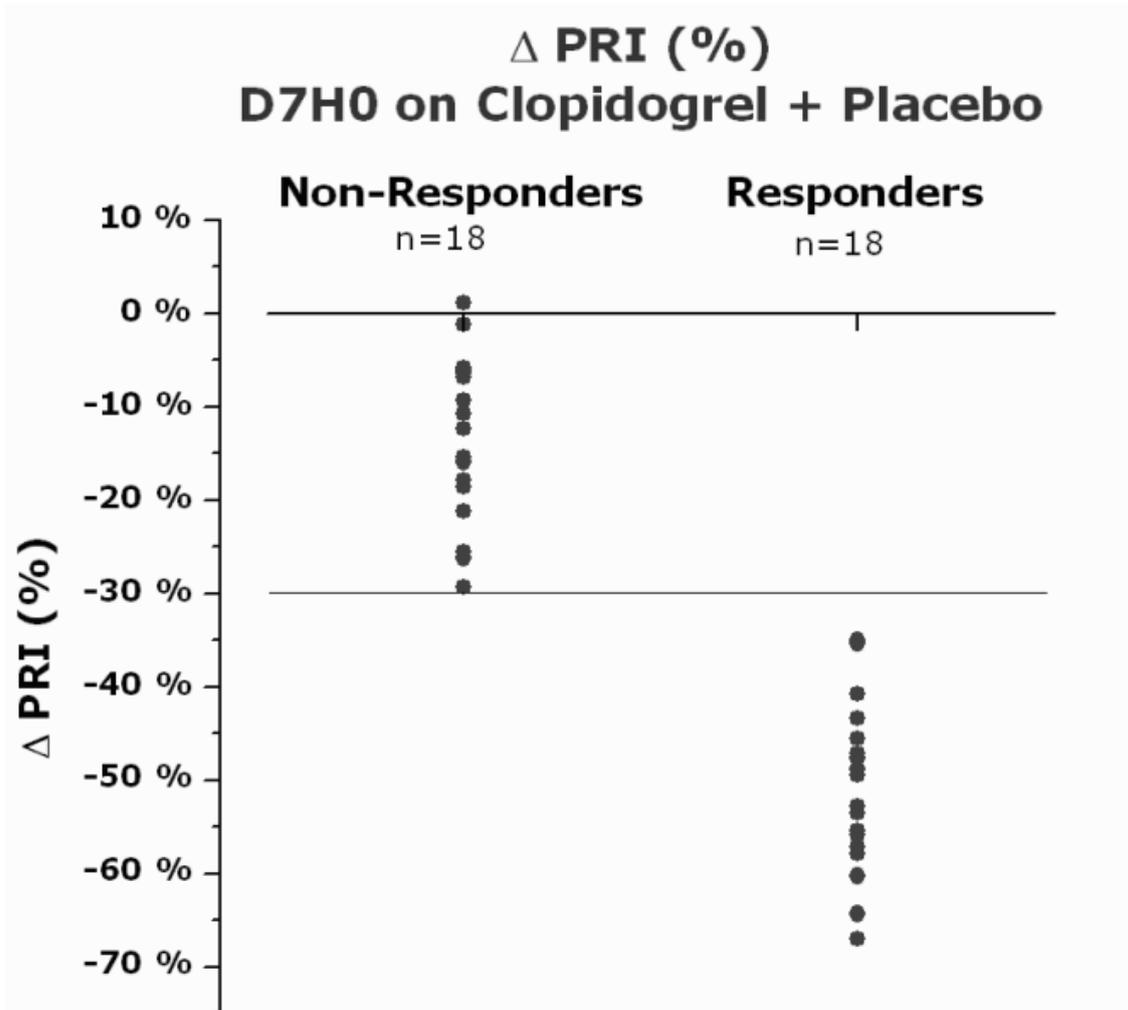


Figure 2: Change of VASP platelet reactivity index (PRI) on day 7 at trough of clopidogrel 75 mg o.d. during co-administration of rabeprazole and omeprazole in 18 good antiplatelet responders. Each box plots represents interquartile range with mean (horizontal line in the box) and median (dot in the box) and whiskers represent the 5 - 95 percentiles. p=0.067

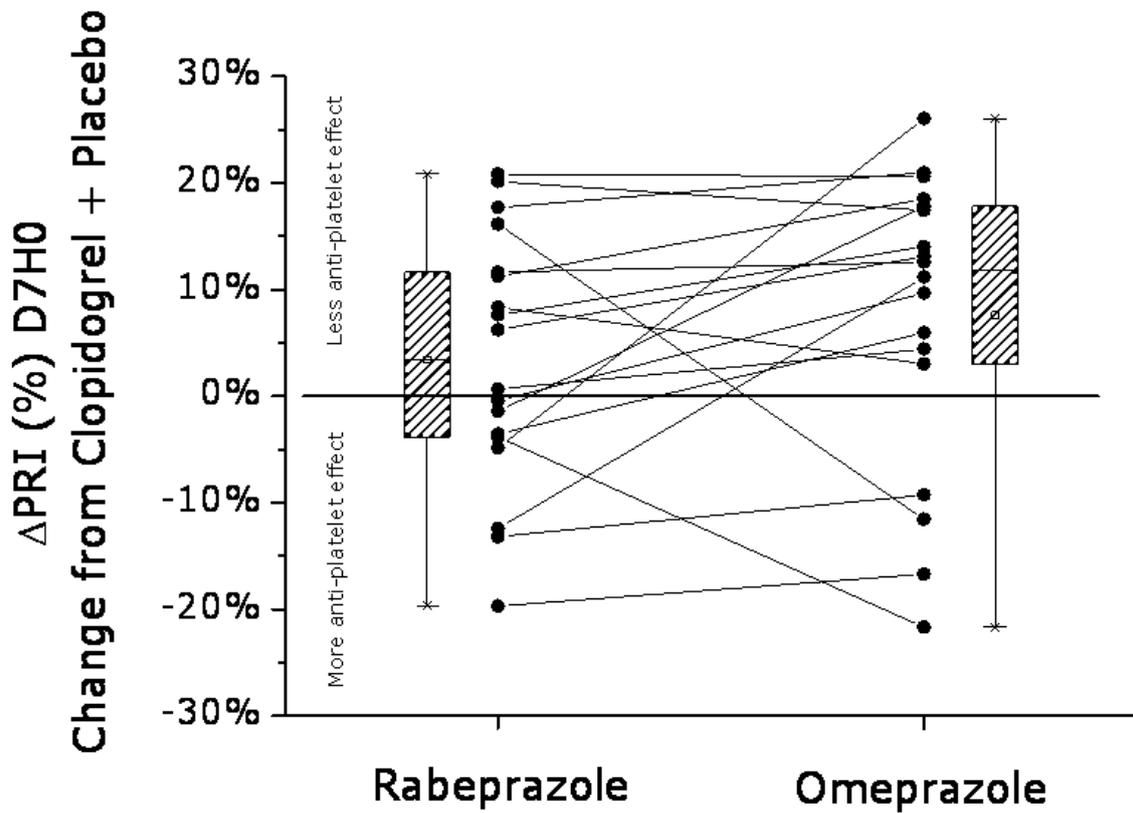


Figure 3: Change of VASP platelet reactivity index (PRI) at trough on day 7 of clopidogrel 75 mg o.d. in the presence of placebo, rabeprazole and omeprazole in 36 healthy subjects according to CYP2C19 genotypes.

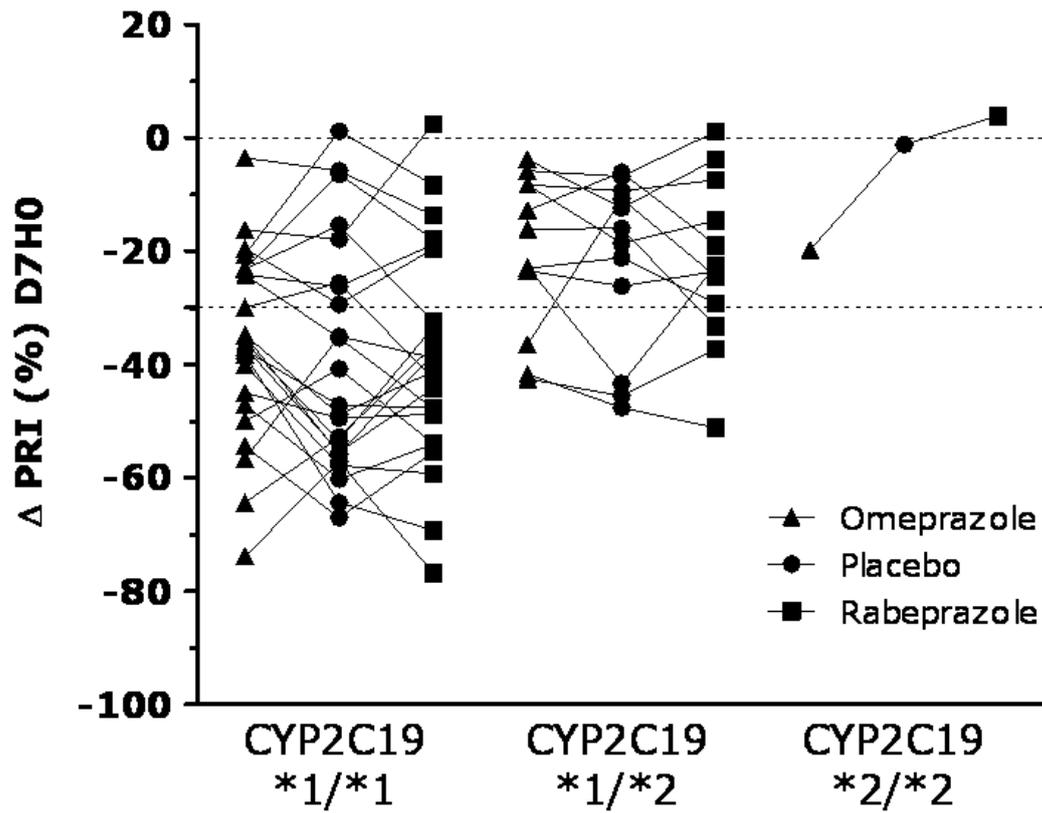


Figure 4: Mean (SD) plasma concentration of clopidogrel active metabolite as a function of time on day 7 of clopidogrel 75 mg o.d. in the presence of placebo, rabeprazole and omeprazole in 36 healthy subjects.

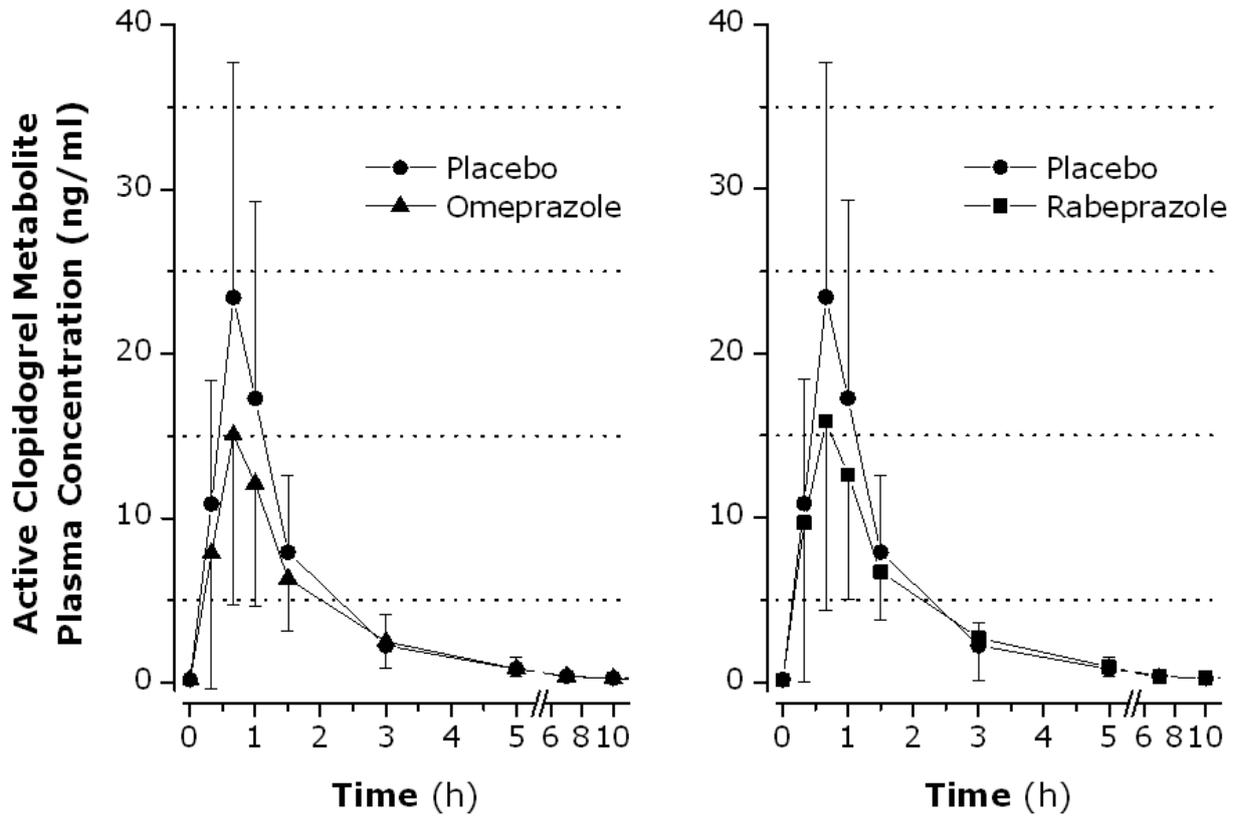


Figure 5: Area under plasma concentration-time curve from 0 to 24 hours of clopidogrel active metabolite on day 7 of clopidogrel 75 mg o.d. in the presence of placebo, rabeprazole and omeprazole in 36 healthy subjects according to CYP2C19 genotypes. * $p < 0.001$

