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Pantoprazole significantly interferes with antiplatelet effect of clopidogrel: Results of a pilot randomized trial

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ABSTRACT

Background: The CYP2C19*2 polymorphism is significantly associated with residual platelet reactivity (RPR) and maybe a major confounding factor in studies evaluating pharmacological interactions with clopidogrel.
Objectives: We sought to evaluate the influence of a proton pump inhibitor (PPI), pantoprazole, indicated as relatively less influent than other PPIs, on the antiplatelet effect of clopidogrel, considering a stratification of the population for the presence of cytochrome 2 C19*2 polymorphism.
Methods: 105 patients with ST elevation myocardial infarction (STEMI), treated with percutaneous coronary angioplasty (PCI) and who received dual antiplatelet therapy, were randomized between pantoprazole (n = 54) or ranitidine (n = 51). RPR was evaluated by Platelet Function Analyzer-100 (PFA-100) with collagen-epinephrine (CEPI) and collagene-ADP (CADP) cartridges and by light transmitted aggregometry with 10 μM adenosin diphosphate (ADP) and 1 mM arachidonic acid (AA), on 5 (T0) and 30 (T1) days after PCI.
Results: Demographic, clinical and procedural data and the prevalence of CYP2C19*2 polymorphism were similar between the two groups. Not statistically differences were observed for CEPI-CT and for the maximal aggregation (MA) values with AA stimulus at both times. We observed a significant increase in MA values with ADP in PPI group at T0 ($p = 0.01$) and T1 ($p = 0.03$). At the multiple regression analysis PPI use remained significantly associated with ADP-MA both at T0 ($p = 0.05$) and T1 ($p = 0.03$).
Conclusions: This is the first documentation in a randomized trial, after correction for the bias of CYP2C19*2 polymorphism, that pantoprazole increases the ADP-MA in patients treated with dual antiplatelet therapy.

1. Introduction

The use of clopidogrel on top of aspirin after percutaneous coronary angioplasty (PCI) is the rule in clinical practice for inhibition of platelet activation and aggregation [1–3]. However, major adverse cardiovascular events (MACE) occur in patients taking clopidogrel and aspirin. A growing body of evidence demonstrates that residual platelet reactivity (RPR) is associated with increased risk of adverse clinical events [4–6].

In particular, the inter-individual variability in clopidogrel response is mostly due to the two steps of pharmacokynetic activation of the prodrug clopidogrel, mediated by several isoforms of the cytochrome P450 (CYP450), to generate the active metabolite [7–9]. The hepatic CYP450 is involved in the metabolism of several drugs, such as statins [10,11], and proton pump inhibitors (PPI) [12–14].

Several functional polymorphisms have been found in genes encoding isoforms of CYP450, including the isoenzyme CYP2C19 that seems to be involved in both the biotransformation of clopidogrel and metabolism of proton pump inhibitors (PPI).

Recently, the loss-of-function CYP2C19*2 allele has been associated with decreased metabolization of clopidogrel, poor anti-platelet effect, and increased cardiovascular events. In high risk vascular patients, the CYP2C19*2 polymorphism is a strong predictor of adverse cardiovascular events and particularly of stent thrombosis [15,16]. Since the different PPIs are metabolized by CYP2C19 at varying degree, their metabolic interaction with clopidogrel is variable [12–14].

Although the current evidence remain controversial about the potential interaction between proton pump inhibitors and anti-platelet effect of clopidogrel [17,18], the FDA and the EMEA have issued a notice of caution concerning the coadministration of clopidogrel and PPIs [19,20].

However, the investigators of PRINCIPLE-TIMI 44 and TRITON-TIMI-38 have noted that PPIs exert a modest reduction on the inhibitory effect of clopidogrel on platelet function and this effect was not reflected in a significant increase in clinical events [21].

The lack of a full gastric protection by PPIs due to an excess of caution for clopidogrel interference may have serious clinical implications with an excess of major gastric bleedings [22].

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The purpose of the present study was to define the implication of pantoprazole as compared to a H2-receptor antagonist (ranitidine) in RPR, as determined by specific or global tests of platelet function, and the role of 2 C19*2 polymorphism as a major confounding factor.

2. Methods

2.1. Study design

We conducted a prospective randomized open trial. A total of 105 consecutive patients with ST elevation myocardial infarction (STEMI) undergoing primary PCI were considered for inclusion. They received aspirin (100 mg/day) and clopidogrel (loading dose 300 mg, followed by 75 mg/day). Periprocedural glycoprotein IIbIIIa antagonists were used at the discretion of the operators. After written informed consent was obtained, patients were randomized to 2 treatment groups: 54 patients were treated with 40 mg pantoprazole (PPI), whereas 51 patients received 150 mg ranitidine (H2-RA). The time of follow-up was 30 days.

The use of an H2-RA was considered on the basis of recent evidence of the literature. Taha et al. [23] have shown that H2-RA versus placebo are very effective in preventing gastric and duodenal ulcers in patients on low dose aspirin and can be considered as an alternative to PPIs without significant interaction with clopidogrel.

Exclusion criteria were: in-hospital death for other causes than STEMI, previous history of thrombocytopenia (<150,000 platelets/ml) or bleeding disorders, liver disease, gastrointestinal ulcer or pregnancy and noncompliance to dual drug antiplatelet treatment.

The institutional review board approved the study protocol.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.2. Blood samples

Peripheral venous blood samples anticoagulated with sodium citrate (0.109 M) were drawn at 5 days (T0) (patients discharge from the department) and 30 days (T1) after the beginning of the maintenance therapy (100 mg/day aspirin and 75 mg/day clopidogrel). The first tube was not used for the platelet function testing. The timing of the blood samples was chosen to rule out the temporary effect of gpIIbIIIa inhibition.

2.3. Platelet function tests

2.3.1. PFA-100

The Platelet Function Analyzer (PFA-100) system (Siemens, Germany) is a point of care assay for the assessment of a quantitative measure of primary hemostasis at high shear stress [24,25].

The anticoagulated blood is drawn through an aperture in a nitrocellulose membrane coated with collagen and epinephrine (CEPI) or collagen and ADP (CADP). Platelet aggregation is measured as the closure time (CT) that platelets take to occlude this forum until a maximum of 300 s.

In this study we performed the test with collagene/epinephrine (CEPI) and collagen ADP (CADP) coated cartridge.

PFA-100 presents several advantages in clinical practice because of the easy and speedy implementation (about 5 minutes) and the small volume of blood (0.8 mL) necessary to perform the test.

Platelet counts and hematocrit may affect results, for this reason we excluded all patients with a platelet count <50×10^9 or hematocrit <25%.

2.3.2. Aggregometry

Light transmittance aggregometry is the most common method of assessing platelet function [26].

The platelet aggregation was measured in a platelet rich plasma (PRP) obtained by centrifugation of sodium-citrate anticoagulated blood for 15 min at 100 g.

The platelet count in PRP was standardized from 180×10^9/L to 320×10^9/L by dilution with platelet poor plasma (PPP). The PPP, obtained by centrifugation at 2400 g for 20 min, was used for setting the 100% line of aggregation.

PRP was stimulated with 10 μM adenosin diphosphate (ADP) and 1 mM arachidonic acid (AA).

ADP-induced and AA-induced platelet aggregation were performed to measure the responsiveness to clopidogrel and aspirin respectively.

Whole blood aggregometry was performed using a light transmittance aggregometer (Mascia Brunelli).

2.3.3. Residual platelet reactivity (RPR)

As previously described, we consider patients with RPR those with CEPI-CT<190 s and CADP-CT<82 s [27]. RPR by ADP was defined in the presence of a maximal platelet aggregation ≥70%, while for AA, RPR was defined as a maximal aggregation ≥20% [5,28].

2.3.4. Genetic polymorphism analysis

Genotyping of the subjects for the CYP2C19*2 polymorphism was performed by allelic discrimination assay and the detection system - ABI prism 7900HT Sequence Detection System (Applied Biosystems); Allele definition follow the nomenclature of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (www.cypalleles.ki.se).

2.4. Statistics

The number to treat was estimated on the basis of previous observational studies, by our group and others [27]. We estimated that a study sample size of 100 would enable a one-half standard deviation (SD) difference (i.e., a 10% difference in ADP-aggregometry and CEPI-CT between groups) to be detected, with an 80% statistical power and a 5% alpha risk.

The characteristics of the 2 groups were compared using chi-square tests for qualitative variables, *t*-test for continuous normally distributed variables and Mann-Withney test for continuous not-normally distributed variables. Stepwise multivariable logistic regression analysis was used to estimate possible associations between RPR and the use of pantoprazole. The logistic model included sex, smoking habit, use of statins, calcium channel blockers, diabetes mellitus, hypercholesterolemia, high sensitivity CRP levels, glycoprotein IIbIIIa inhibitors premedication and CYP 450 2 C19 polymorphism.

The main end point compared the mean values variation of ADP-MA and CEPI-CT during the 1 month treatment period in the 2 groups.

The secondary end points were the RPR with ADP-aggregometry (≥70%), with PFA-100 (< 190 sec) and with AA-aggregometry (≥20%). A value of $p<0.05$ was considered statistically significant.

3. Results

Between July 2009 and February 2010, following predetermined exclusion criteria, 105 patients were included in the study and randomized into two treatments arms and followed for a period of 1 month.

No patients experienced major adverse cardiovascular events (MACE), defined as death, myocardial infarction or reinfarction, stent thrombosis, stroke, major bleeding and hospitalization for cardiovascular reasons.

Clinical, procedural and laboratory data of patients in the PPI and H2-RA groups were comparable, including the percentage of periprocedural glycoprotein IIbIIIa antagonists use and concomitant use of cytochrome P450 metabolized drugs (Table 1).

To evaluate a possible interference of the CYP2C19*2 polymorphism on platelet function tests, we assessed the distribution of this polymorphism in the two groups of pantoprazole and ranitidine. According to literature data [7–9], the *2 allele is associated with a lower response to clopidogrel, which translates into a residual platelet reactivity measured by platelet function tests and an increase in adverse cardiovascular events.

Although the prevalence of the *2 carriers (heterozygous *1*2 + homozygous *2*2) was lower in patients receiving PPI treatment (27%) than the group treated with H2-RA (40%), the difference did not reach statistical significance ($p=0.16$), as shown in Fig. 1.

Data of platelet function assessed by the PFA-100 test done with the cartridge collagen/epinephrine are illustrated in Fig. 2. The median of the closure times (CEPI-CT) is shown at five days (left) and one month (right) after the revascularization procedure. CEPI-CT values at T0 and T1 were not significantly different between the H2-RA group and PPI group: median 300 sec (range 90–300, 25–75 percentiles: 156–300) for H2-RA group and median 292 sec (range 79–300, 25–75 percentiles: 138–300) for PPI group at T0 ($p=0.23$); median 281 sec (range 99–300, 25–75 percentiles: 132–300) for H2-RA group and median 287 sec (range 107–300, 25–75 percentiles: 137–300) for PPI group at T1 ($p=0.83$).

We did not collect a sufficient number of data for statistical analysis with regard the platelet function measured by PFA-100 using the collagen/ADP cartridge.

Light transmittance aggregometry using arachidonic acid (AA) as stimulus was performed to evaluate residual platelet reactivity related to aspirin therapy.

Values of AA maximal amplitude (MA) were not significantly different between the two groups at both times: median 4% (range 0–23, 25–75 percentiles: 2–6) in the H2-RA group and median 4% (range 0–85, 25–75 percentiles: 2–7) in the PPI group at T0 ($p=0.52$), median 3% (range 0–35, 25–75 percentiles: 1–7) in the H2-RA group and 3%