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The Antiplatelet Effect of Aspirin is Reduced by Proton Pump Inhibitors in Patients With Coronary Artery Disease

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Abbreviations
AU, Aggregation Unit(s); CAD, coronary artery disease;
CYP, cytochrome P450; IQR, interquartile range; PPI(s), proton pump inhibitor(s);
sP-selectin, soluble serum P-selectin; S-TxB₂, serum thromboxane B₂
ABSTRACT

Objective: To evaluate the effect of proton pump inhibitors (PPIs) on the platelet response to aspirin in patients with coronary artery disease (CAD).

Design: Case-control study.

Patients: 418 stable CAD patients, 54 of whom were treated with PPIs. All patients were treated with non-enteric coated aspirin 75 mg/day and received no other antithrombotic drugs.

Main outcome measures: Platelet aggregation was measured by Multiplate® whole blood aggregometry induced by arachidonic acid 1.0 mmol/L and expressed as area under the aggregation curve (Aggregation Units*min). Platelet activation was assessed by soluble serum P-selectin. Compliance was confirmed by serum thromboxane B2 levels.

Results: The distribution of age, sex, body mass index, blood pressure, family history of ischaemic heart disease, smoking, diabetes, and the number of previous ischaemic events did not differ between groups. All patients were compliant with aspirin therapy according to serum thromboxane B2 levels. Platelet aggregation (median 180 (interquartile range 119-312) vs. 152 (84-226) Aggregation Units*min, p = 0.003) and soluble serum P-selectin levels (88.5 (65.2-105.8) vs. 75.4 (60.0-91.5) ng/mL, p = 0.005) were significantly higher in patients treated with PPIs. Furthermore, these patients had significantly higher serum thromboxane B2 levels (geometric mean 1.29 (95% confidence interval 0.96-1.72) vs. 0.92 (0.84-1.01) ng/mL, p = 0.01).

Conclusions: CAD patients treated with PPIs had a reduced platelet response to aspirin in terms of increased residual platelet aggregation and platelet activation compared with CAD patients not taking PPIs. Concomitant use of aspirin and PPIs might reduce the cardiovascular protection by aspirin.

Keywords: Aspirin, coronary artery disease, drug interactions, platelet aggregation, proton pump inhibitors.
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INTRODUCTION

Aspirin is the mainstay of secondary antithrombotic therapy. Accordingly, low-dose aspirin lowers the risk of vascular events by 32% in high-risk patients.[1] However, the platelet response to aspirin is variable and in some patients platelet aggregation is inhibited less than expected.[2] These patients might be at an increased risk of cardiovascular events.[3,4]

Aspirin therapy carries a risk of dyspepsia and upper gastrointestinal bleedings, thus often combined with a proton pump inhibitor (PPI). PPIs protect the gastric mucosal barrier by suppressing the gastric acid production.[5] Under physiological acidic conditions, aspirin is absorbed in its lipid state by passive diffusion across the gastric mucosal membrane according to the pH partition hypothesis.[6] PPIs exert their antacid effect by inhibiting the H⁺/K⁺-exchanging ATPase of the gastric parietal cells, thus raising intragastric pH.[7] In fact, the pH potentially rises above the pKₐ (3.5) of acetylsalicylic acid, causing a pronounced reduction in the lipophilicity of aspirin.[8] According to previous studies, such chemical changes might compromise the bioavailability and therapeutic efficacy of aspirin.[9,10,11]

Previous studies have addressed the effect of PPIs on the antiplatelet effect of clopidogrel.[12,13] Presumably, PPIs attenuate the antiplatelet effect of clopidogrel by competitively inhibiting the cytochrome P450 (CYP) isoenzyme system, in particular CYP2C19, which is responsible for converting clopidogrel to its active metabolite.[14] Less attention has been paid to the potential drug interaction between PPIs and aspirin, although aspirin remains the most frequently used drug worldwide. However, any interaction between PPIs and aspirin remains to be established.

P-selectin is a cell adhesion molecule stored in the α-granules of platelets. If not expressed on the platelet surface, P-selectin might be released into the blood as soluble P-selectin. Soluble serum P-selectin (sP-selectin) is regarded as a marker of platelet activation.[15]

Serum thromboxane B₂ (S-TxB₂) measurements reliably reflect endogenous thromboxane A₂ production, which occurs largely, albeit not exclusively, in platelets.[16] Hence, S-TxB₂ is regarded the most specific test for measuring the inhibitory effect of aspirin on platelets.[17,18]

The main purpose of the present study was to investigate whether patients with coronary artery disease (CAD) treated with PPIs had a reduced platelet response to aspirin in terms of increased residual platelet aggregation and platelet activation compared with CAD patients not taking PPIs.

METHODS

Design and study population

We performed a case-control study including 418 patients angiographically diagnosed with CAD. Among these, 54 patients received PPI treatment.

Patients were identified in the Western Danish Heart Registry and enrolled from November 2007 through April 2009 according to predefined inclusion and exclusion criteria. The Western Danish Heart Registry collects data on patient and procedure characteristics for all interventions performed in interventional centres in the western part of Denmark.[19]

Patients ≥18 years of age with angiographically verified CAD on low-dose (75 mg) non-enteric coated aspirin therapy were included in the study. Exclusion criteria were aspirin intolerance, any acute or chronic disease (apart from CAD), use of anticoagulants or any drugs known to affect platelet function (including clopidogrel and NSAIDs), pregnancy, gastrointestinal bleeding within the last month, platelet count <120 x 10⁹/L, any ischaemic event or
revascularisation procedures (percutaneous coronary intervention or coronary artery bypass grafting) within the previous 12 months, and incapability to give informed consent.

All patients were treated with aspirin 75 mg/day prior to and during study participation. Current medication, including the use of PPIs and aspirin, was registered on the day of blood sampling and subsequently confirmed by reviewing hospital records.

Written informed consent was obtained from all participants. The study was conducted in agreement with the Helsinki-II-declaration, and the study was approved by the Central Denmark Region Committees on Biomedical Research Ethics.

Laboratory measurements
Standardised blood sampling was performed one hour after aspirin intake. Patients rested for 30 minutes prior to sampling in the supine position. Samples were drawn from an antecubital vein into vacuum tubes through a 19G butterfly needle using a minimum of stasis.

Platelet aggregation was measured by the Multiplate® analyser (Dynabyte, Munich, Germany). Multiplate® is a whole blood impedance platelet aggregometer providing simultaneous duplicate measurements.[20] All platelet aggregation analyses were performed within two hours of sampling. Blood was collected in 3.6 mL tubes containing 3.2% sodium citrate and in 3 mL tubes containing hirudin 25 µg/mL. Platelet aggregation was induced by arachidonic acid (1.0 mmol/L). Results are reported as area under the aggregation curve (Aggregation Units(AU)*min).[20] A quality control of a sample from a person with normal coagulation status was performed each day in order to ensure that agonist solutions were appropriately prepared.

sP-selectin was determined by ELISA according to manufacturer’s instructions (R&D systems, MN, USA). Blood was collected in non-anticoagulated glass tubes and allowed to clot at room temperature for 30 minutes prior to centrifugation for 15 minutes at 1000 g. The supernatant serum was recovered and stored at -80°C.

S-TxB2 levels were determined according to Patrono et al.[21] with the modifications that serum was collected after one hour of clotting and that S-TxB2 was measured by ELISA (Cayman Chemical, MI, USA). Blood was collected in non-anticoagulated glass tubes and allowed to clot at 37°C for one hour. Subsequently, it was centrifuged for 10 minutes at 2600 g and the supernatant serum was recovered and stored at -80°C.

Compliance
Compliance was evaluated by face-to-face interviews and pill counting and confirmed by S-TxB2 measurements. In order to optimise compliance, patients received a tablet dosage-box with seven non-enteric coated aspirin tablets for the last seven days prior to blood sampling.

Statistics
Continuous data are presented as mean ± standard deviation if data were normally distributed, as geometric mean with 95% confidence interval if normally distributed when log-transformed, and as medians with interquartile range (IQR) if not. Unpaired data from two groups were compared by the two-sample t test if normally distributed and by the Mann-Whitney test if not. Distributions of categorical variables were compared with the chi-square test and presented as absolute counts and percentages. Multiple linear regression was used to test the effect of PPI treatment on platelet aggregation adjusted for baseline characteristics. A two-tailed probability value of p <0.05 was considered statistically significant. Confidence intervals were calculated at the 95% level. Statistical analyses were performed using GraphPad Prism® version 5.0 (GraphPad Software, CA, USA) and Stata® version 9.0 (StataCorp., TX, USA).

RESULTS
Baseline characteristics of the study population are shown in Table 1.

**Table 1** Baseline characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Patients on PPIs (n = 54)</th>
<th>Control patients (n = 364)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years†</td>
<td>67.0 (10.1)</td>
<td>67.2 (9.4)</td>
<td>0.88</td>
</tr>
<tr>
<td>Males*</td>
<td>41 (75.9)</td>
<td>283 (77.7)</td>
<td>0.77</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking*</td>
<td></td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td>Never</td>
<td>19 (35.2)</td>
<td>134 (36.8)</td>
<td></td>
</tr>
<tr>
<td>Previously</td>
<td>23 (42.6)</td>
<td>148 (40.7)</td>
<td></td>
</tr>
<tr>
<td>Today</td>
<td>12 (22.2)</td>
<td>82 (22.5)</td>
<td></td>
</tr>
<tr>
<td>Family history of IHD*</td>
<td>22 (40.7)</td>
<td>177 (48.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg‡</td>
<td>137.2 (132.6-142.2)</td>
<td>141.8 (139.7-143.8)</td>
<td>0.11</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg‡</td>
<td>81.0 (78.0-84.2)</td>
<td>83.3 (82.1-84.5)</td>
<td>0.17</td>
</tr>
<tr>
<td>Body mass index, kg/m²‡</td>
<td>28.4 (27.2-29.7)</td>
<td>27.4 (27.0-27.8)</td>
<td>0.10</td>
</tr>
<tr>
<td>Diabetes*</td>
<td>21 (38.9)</td>
<td>99 (27.2)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatininum, μmol/L§</td>
<td>87.0 (72.0-106.0)</td>
<td>83.0 (72.0-99.8)</td>
<td>0.40</td>
</tr>
<tr>
<td>Platelets, 10⁹/L‡</td>
<td>238.0 (222.3-254.7)</td>
<td>227.3 (221.9-232.8)</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous MI*</td>
<td>38 (70.4)</td>
<td>244 (67.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Previous PCI*</td>
<td>49 (90.7)</td>
<td>328 (90.1)</td>
<td>0.88</td>
</tr>
<tr>
<td>Previous CABG*</td>
<td>15 (27.8)</td>
<td>71 (19.5)</td>
<td>0.16</td>
</tr>
<tr>
<td>Previous stroke*</td>
<td>7 (13.0)</td>
<td>29 (8.0)</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Medications</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins*</td>
<td>51 (94.4)</td>
<td>325 (89.3)</td>
<td>0.24</td>
</tr>
<tr>
<td>Beta-blocker*</td>
<td>40 (74.1)</td>
<td>270 (74.2)</td>
<td>0.99</td>
</tr>
<tr>
<td>ACE inhibitor*</td>
<td>30 (55.6)</td>
<td>156 (42.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>AT-II receptor antagonist*</td>
<td>9 (16.7)</td>
<td>68 (18.7)</td>
<td>0.72</td>
</tr>
<tr>
<td>Calcium antagonist*</td>
<td>13 (24.1)</td>
<td>94 (25.8)</td>
<td>0.78</td>
</tr>
<tr>
<td>Diuretics*</td>
<td>34 (63.0)</td>
<td>121 (33.2)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* n (%), comparison made using chi-square test. † Mean (standard deviation), comparison made using t test. ‡ Geometric mean (95% confidence interval), comparison made using t test. § Median (interquartile range), comparison made using Mann-Whitney test.

ACE, angiotensin converting enzyme; AT-II, angiotensin 2; CABG, coronary artery bypass grafting; IHD, ischaemic heart disease; MI, myocardial infarction; PCI, percutaneous coronary intervention.

Patients treated with PPIs did not differ with respect to demographic features and risk factors, except for an excess use of diuretics. The distribution of generic PPI variants is given in Table 2.

**Table 2** Distribution of generic PPI variants among patients treated with PPIs (n = 54)

<table>
<thead>
<tr>
<th>Generic drug</th>
<th>Patients, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantoprazole</td>
<td>29 (54)</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>12 (22)</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>8 (15)</td>
</tr>
</tbody>
</table>
Platelet aggregation was significantly higher in patients treated with PPIs (median 180 (IQR 119-312) vs. 152 (84-226) AU*min, p = 0.003) (Figure 1). In a multiple linear regression analysis, the effect of PPIs on platelet aggregation remained significant after adjustment for age, sex, body mass index, smoking, concomitant drug therapy, previous myocardial infarction, and diabetes mellitus (p = 0.013).

Hirudinised blood samples were obtained from a minor part (n = 115, 16 of whom were treated with PPIs) of the study population. These samples confirmed a significantly higher platelet aggregation in patients on PPIs (geometric mean 284 (95% confidence interval 201-401) vs. 158 (135-185) AU*min, p = 0.007).

Platelet activation was assessed by sP-selectin as shown in Figure 2. Patients treated with PPIs had significantly higher sP-selectin levels (median 88.5 (IQR 65.2-105.8) vs. 75.4 (60.0-91.5) ng/mL, p = 0.005) indicating an increased extent of platelet activation. The effect of PPIs on platelet activation remained significant after adjustment for age, sex, body mass index, smoking, concomitant drug therapy, previous myocardial infarction, and diabetes mellitus (p = 0.013).

All patients returned empty pill boxes and claimed to be adherent to aspirin treatment. All patients demonstrated S-TxB2 levels (geometric mean 0.96 (95% confidence interval 0.88-1.05), range 0.04 to 18.18 ng/mL) far below the normal range of 327±123 ng/mL in healthy individuals not on aspirin[17] and well below 30 ng/mL, corresponding to a more than 95% inhibition of platelet COX-1 activity.[22] Patients treated with PPIs had significantly higher S-TxB2 levels than patients not on PPI therapy (geometric mean 1.29 (95% confidence interval 0.96-1.72) vs. 0.92 (0.84-1.01) ng/mL, p = 0.01).

DISCUSSION

The present study is the largest to investigate platelet aggregation in patients on aspirin concomitantly treated with PPIs. We evaluated the platelet aggregation and platelet activation in 418 fully compliant CAD patients treated with non-enteric coated aspirin and no other antithrombotic medications. Our main finding was a significantly higher platelet aggregation in patients treated with PPIs, who also demonstrated an increased extent of platelet activation as compared to CAD patients not treated with PPIs.

Animal studies have shown that omeprazole reduces the analgesic and antipyretic effects of aspirin, likely attributable to the reduction in gastric aspirin absorption.[8,11] Similar findings were reported from a study performed on humans.[23] However, no reduction in the antiplatelet effect of aspirin was observed when administered concomitantly with either omeprazole 20 mg/day[24] or lansoprazole 30 mg/day.[25] We investigated the aspirin-PPI drug interaction in a clinical setting and observed a substantially higher residual platelet aggregation and platelet activation in patients treated with PPIs.

The higher aspirin dose used in the study by Inarrea et al (125 mg/day) might partly explain why their results differ from ours.[24] Hypothetically, dose increments might result in a slightly increased passive diffusion rate across the gastric mucosal membrane despite the elevation in pH caused by PPIs. In that case, the likelihood of detecting a difference using an aspirin dose of 125 mg is obviously reduced. A dose of 75-100 mg is the generally accepted choice for secondary prevention in Europe.[26]

In our study, a non-enteric coated formula of aspirin was used. In contrast, Adamopoulos et al used a coated formula when investigating a potential aspirin-lansoprazole drug interaction.[25] The lower bioavailability of the coated formula might explain why no difference between study groups was observed. The higher bioavailability of the non-enteric coated formula
used in our study might amplify the measurability of the aspirin-PPI drug interaction, thus allowing us to demonstrate the inhibiting effect of PPIs on the antiplatelet effect of aspirin.

At present, a possible drug interaction between clopidogrel and PPIs is the focus of an intense debate. Recent studies suggest that a class effect of PPIs on the antiplatelet effect of clopidogrel does not exist.[13,27] This might be attributable to differences in the inhibitory potency towards CYP2C19. In particular, pantoprazole seems to interfere little, if at all, with the metabolism of clopidogrel.[13] Since all PPIs affect gastric pH to roughly the same extent,[28,29] the aspirin-PPI drug interaction is likely to represent a class effect of PPIs.

Metabolism of another PPI, lansoprazole, depends partly on the CYP3A4 isoenzyme.[29,30] Aspirin is also to some extent metabolised by hepatic Phase I reactions driven by CYP3A4.[31] This prompted Adamopoulos et al to investigate a potential drug interaction between lansoprazole and aspirin.[25] They performed a crossover study that did not confirm any CYP3A4-dependent aspirin-lansoprazole drug interaction.[25] Thus, the aspirin-PPI drug interaction observed in our study might be interpreted as a pH-dependent phenomenon related to changes in the bioavailability and therapeutic activity of aspirin, rather than a question of competitive CYP-inhibition.

Multiplate® aggregometry was performed on citrated blood in all patients as well as on hirudinised blood in a subgroup of patients (n = 115, 16 of whom were treated with PPIs). Hirudin, a selective thrombin inhibitor, has been suggested as an appropriate alternative to citrate due to the undesirable Ca²⁺-chelating properties of the latter.[32] In patients treated with aspirin, we found a significantly higher level of platelet aggregation in patients on concomitant PPI therapy measured both in citrated and in hirudinised blood. In line with previous findings, platelet aggregation was more potently inhibited under citrate preservation, which might result from the acidification and Ca²⁺-chelation caused by citrate.[33] Platelet aggregation was induced by arachidonic acid, which activates platelets specifically through the COX-1 pathway.

In clinical practice, a reduced response to aspirin is often explained by non-adherence to treatment.[34] In our study, all patients were fully compliant, but the patients with increased platelet aggregation had higher S-TxB₂ levels. This finding might suggest that even beyond the 95% level of COX-1 inhibition, platelet aggregation is affected by the actual level of COX-1 inhibition. S-TxB₂ is regarded the most specific test for measuring the inhibitory effect of aspirin on platelets.[17,18]

**Limitations**

The design of our study did not allow any firm conclusions to be drawn on the potential causality between PPI treatment and aspirin response. Furthermore, we did not assess platelet aggregation after withdrawal of aspirin therapy, as this was considered unethical. Thus, we do not know if underlying platelet hyperreactivity *per se* accounts for the higher platelet aggregation in patients treated with PPIs. Whole blood aggregometry might have potential drawbacks such as platelets interacting with other whole blood elements potentially bypassing the platelet inhibition by aspirin.[35] Moreover, platelet aggregometry inherently requires *ex vivo* anticoagulation, which might affect platelet aggregability.[33]

**CONCLUSIONS**

CAD patients treated with PPIs had a reduced response to aspirin in terms of increased residual platelet aggregation and platelet activation compared with CAD patients not taking PPIs. Concomitant use of aspirin and PPIs might leave patients at an increased risk of thrombotic events. These findings may affect the clinical practice of antithrombotic therapy. Having the widespread
use of PPIs in mind, a randomised double blind crossover study (PPI vs. placebo on top of aspirin) is needed to further explore the inhibitory effect of PPIs on aspirin.

**DISCLOSURES**
No conflict of interest is declared.

**STUDY FUNDING**
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FIGURE LEGENDS

Figure 1 Platelet aggregation assessed by Multiplate®
Platelet aggregation (median, IQR, range) in 418 patients with coronary artery disease: 54 patients receiving proton pump inhibitors (PPI) and 364 patients not receiving proton pump inhibitors (no PPI).
AUC, area under the aggregation curve; AU, aggregation unit.

Figure 2 Platelet activation assessed by soluble serum P-selectin
sP-selectin levels (median, IQR, range) in 418 patients with coronary artery disease: 54 patients receiving proton pump inhibitors (PPI) and 364 patients not receiving proton pump inhibitors (no PPI).
sP-selectin = soluble serum P-selectin.
$p = 0.003$

AUC, AU*min

PPI

$n = 54$

No PPI

$n = 364$
The box plot shows the distribution of sP-selectin levels (ng/mL) in two groups: PPI and No PPI. The PPI group has 54 participants, and the No PPI group has 364 participants. The p-value for the comparison is 0.005, indicating a statistically significant difference between the two groups.