Platelet hyperfunction is decreased by additional aspirin loading in patients presenting with myocardial infarction on daily aspirin therapy

Ingrid Fuchs, MD; Alexander O. Spiel, MD; Martin Frossard, MD; Ulla Derhaschnig, MD; Eva Riedmüller, MD; Bernd Jilma, MD

LEARNING OBJECTIVES

After participating in this activity, the participant should be better able to:
1. Interpret laboratory measures of platelet function.
2. Evaluate effect of aspirin infusion on platelet function.
3. Relate the usefulness of aspirin infusion to individuals with acute coronary syndromes.

Objective: Currently 162–325 mg aspirin is recommended for the treatment of acute coronary syndrome. We tested the effect of an additional loading dose of 250 mg aspirin at the onset of acute coronary syndrome in patients who were already on chronic therapy with 100 mg aspirin.

Design: This was a prospective trial in patients presenting with symptoms suggestive of acute coronary syndrome that included a randomized, double-blind, placebo-controlled trial subgroup.

Setting: An emergency department in a tertiary care center.

Patients: Consecutive patients with symptoms suggestive of acute coronary syndrome were enrolled, including a cohort already on chronic aspirin therapy.

Interventions: Patients received an intravenous infusion of 250 mg aspirin.

Measurements and Main Results: Platelet function was measured with a platelet function analyzer in 234 patients before and after aspirin infusion. Aspirin infusion prolonged collagen epinephrine closure times in almost all patients. Aspirin infusion further lowered thromboxane B2 levels in patients with acute coronary syndrome who were on chronic aspirin therapy before admission. Concomitantly, collagen epinephrine closure times increased by 22% from 223 secs (95% confidence interval, 192–255 secs) before to 273 secs (95% confidence interval, 252–294 secs) after aspirin infusion ($p < .01$). Eleven patients with ST-elevation myocardial infarction on daily aspirin therapy (53%) displayed platelet hyperfunction (collagen epinephrine closure times $<193$ secs). Additional aspirin infusion further decreased platelet function in these patients with ST-elevation myocardial infarction (30% prolongation of collagen epinephrine closure times; $p < .01$), and only two patients with ST-elevation myocardial infarction still displayed platelet hyperfunction ($p = .02$).

Conclusion: Aspirin loading in the emergency room further reduced thromboxane B2 levels and further inhibited platelet function in many patients with acute coronary syndrome already on 100 mg aspirin. (Crit Care Med 2010; 38:1423–1429)

KEY WORDS: randomized controlled trial; aspirin; platelet reactivity; acute coronary syndrome
INTRODUCTION

After completing this CME activity, readers should be able to explain how to measure platelet function and the effect of aspirin infusions on platelet function. In addition, readers should be able to relate the usefulness of aspirin infusion in patients with acute coronary syndromes (ACS).

Myocardial infarction is a medical emergency that has a number of life-threatening complications (1–4), leads to a substantial number of admissions to intensive care units (5), and is still associated with considerable mortality (6, 7). Antiplatelet therapy with aspirin is not only a mainstay treatment for ACS (8) but may also be beneficial in critically ill patients preventing organ injury (9).

Current guidelines recommend that aspirin should be administered to patients with myocardial infarction as soon as possible after hospital presentation or as soon as possible after the diagnosis is deemed probable (10–12). Most patients with ACS receive aspirin doses between 75 and 325 mg/day (13) with significant regional differences (14). Currently doses of 162–325 mg chewable aspirin are recommended for unstable angina or non-ST-elevation myocardial infarction (STEMI) (12). Recently, the recommended aspirin dose for patients with STEMI has been increased from 75–162 mg to 162–325 mg (11). Although it is not entirely clear what evidence motivated that shift, two recent trials (nonrandomized for aspirin) have shown that slightly higher aspirin doses were associated with lower rates of myocardial infarction at 6 months (13) or all-cause mortality (14) in patients with ACS.

There is increasing evidence that a significant proportion of patients with coronary artery disease taking aspirin have normal platelet function (15). However, it is unknown whether a limited increase from 100 mg aspirin within the recommended dose range may further inhibit platelet function and decrease the number of poor responders in patients with ACS.

We hypothesized that platelet hyperreactivity is common among patients with ACS and that additional aspirin infusion may further reduce platelet function in patients with ACS who have already been taking 100 mg aspirin. This effect was quantified by measurement of shear dependent platelet plug formation with the platelet function analyzer and thromboxane B₂ levels.

MATERIALS AND METHODS

The study protocol and the informed consent form were approved by the Ethics Committee of the Medical University of Vienna and followed the Helsinki Declaration. Written informed consent was obtained from all patients. This was a prospective and interventional study. Patients with chest pain suggestive of an ACS were eligible. All patients enrolled were admitted to and monitored at the coronary care unit of the emergency department of the University Hospital from 2002 to 2006. Aspirin was infused at a dose of 250 mg, which maximally inhibits platelet function (16). In patients presenting with unstable angina pectoris or non-STEMI, the study was conducted in a randomized, double-blind, placebo-controlled, crossover design. Randomization was concealed until treatment by individually sealed opaque envelopes, the randomization code was generated by www.randomization.com by staff members of the secretariat, participants were enrolled by the treating physicians, nurses opened the envelopes after the patient had signed the informed consent form, prepared the syringes with the study drugs, and numbered them with the sequence number. The treating physician, participat-
ing patients, and technician doing the assessments were blinded to the group assignment. After an initial blood collection, patients received one study drug (aspirin or placebo); this was followed by blood collection for further platelet function testing after 5 mins; then the alternative study drug was infused, again followed by blood collection after an additional 5 mins so that platelet function testing relative to aspirin infusion was determined at three consecutive time points (0, 5, and 10 mins). This was done to guarantee effective treatment and to demonstrate that acute effects of aspirin infusion on platelet function tests were indeed the result of aspirin infusion and not any other intervention. In patients with STEMI, the study was conducted in an open fashion so that all patients immediately received aspirin to avoid a delay in the administration of active treatment with proven efficacy. Because GPIIb/IIIa inhibitors are well known to have an impact on platelet function tests (17), these agents were infused after the effects of aspirin infusion had been quantified. The patient, the attending physician, and the medical assistant who analyzed the blood samples were blinded to the treatment algorithm. The diagnosis of the various subtypes of ACS was according to the World Health Organization criteria and the Consensus Document of the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction (18). Other reasons for troponin T elevations (19) had to be excluded in patients with non-STEMI. Patient management and therapy followed current guidelines, including percutaneous coronary interventions or thrombolysis.

Blood Collection and Laboratory Analysis. Methods have been described in detail in previous publications (20, 21). Briefly, serum thromboxane B2 (TXB2) levels were measured by a commercial enzyme immunoassay (22). Plasma levels of von Willebrand factor ristocetin cofactor were assayed by turbidimetry using a commercial kit, which consists of lyophilized platelets and ristocetin, using the α coagulation timer (23). Blood for platelet testing was collected in 3.8% citrate. The platelet function analyzer was used for measuring platelet function, because high shear rates prevail at sites of stenosis in the coronary circulation. The PFA-100 measures the time that is needed until a platelet plug forms after activation of platelets by pathophysiologically relevant stimuli (e.g., collagen and adenosine diphosphate or collagen and epinephrine [CEPI]) and was crossvalidated against other devices (24, 25). Aspirin only affects CEPI closure times (CT) but has minimal effect on collagen and adenosine diphosphate CT (16, 26).

Daily aspirin (100 mg/day) prolongs CEPI-CT to 225–250 secs measured 24 hrs after the last dose (16). This prolongation is similar to the CEPI-CT of 250 secs and 275 secs (in 3.8% citrate) 5 mins after infusion of 100 mg and 300 mg aspirin, respectively (16).

Table 2. Demographics and laboratory values

<table>
<thead>
<tr>
<th></th>
<th>No ACS (n = 68)</th>
<th>ACS (n = 166)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>55 (52–59)</td>
<td>58 (56–59)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.1 (26.0–28.1)</td>
<td>27.6 (26.9–28.4)</td>
</tr>
<tr>
<td>Creatinine, µM/L</td>
<td>1.1 (1.0–1.1)</td>
<td>1.1 (1.1–1.1)</td>
</tr>
<tr>
<td>Glycated hemoglobin, %</td>
<td>6.4 (5.8–7.1)</td>
<td>6.1 (5.9–6.2)</td>
</tr>
<tr>
<td>Triglycerides on admission, mg/dL</td>
<td>177 (139–216)</td>
<td>208 (186–229)</td>
</tr>
<tr>
<td>Cholesterol on admission, mg/dL</td>
<td>208 (196–220)</td>
<td>224 (218–231)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>49 (42–56)</td>
<td>41 (39–43)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>137 (117–157)</td>
<td>128 (123–133)</td>
</tr>
<tr>
<td>Fibrinogen, µM/L</td>
<td>374 (347–402)</td>
<td>417 (398–436)</td>
</tr>
<tr>
<td>Platelet count, ×10³/µL</td>
<td>220 (208–233)</td>
<td>243 (233–253)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42.3 (41.5–43.2)</td>
<td>44.0 (42.7–45.2)</td>
</tr>
<tr>
<td>von Willebrand Factor RCO activity, %</td>
<td>169 (143–195)</td>
<td>204 (187–220)</td>
</tr>
<tr>
<td>Leukocyte count, ×10⁹/L</td>
<td>11.9 (7.9–16.0)</td>
<td>12.0 (10.2–13.9)</td>
</tr>
<tr>
<td>Creatine kinase myocardial fraction, U/dL*</td>
<td>&lt;6</td>
<td>66 (52–80)</td>
</tr>
<tr>
<td>Troponin T, ng/mL*</td>
<td>&lt;0.01</td>
<td>3.46 (2.61–4.32)</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*P < .05 Mann-Whitney U test for descriptive purposes only. Mean ± 95% confidence intervals.

Table 3. Response to aspirin infusion in patients with and without acute coronary syndrome

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Controls' CEPI-CT,* secs</th>
<th>ACS CEPI-CT, secs</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>179 (161–198)</td>
<td>175 (162–189)</td>
<td>.03</td>
</tr>
<tr>
<td></td>
<td>284 (272–297)</td>
<td>266 (255–277)</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>156 (138–174)</td>
<td>157 (141–172)</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>282 (267–298)</td>
<td>259 (244–275)</td>
<td></td>
</tr>
<tr>
<td>Recent aspirin</td>
<td>229 (164–295)</td>
<td>189 (152–225)</td>
<td>.97</td>
</tr>
<tr>
<td></td>
<td>268 (206–300)</td>
<td>285 (268–300)</td>
<td></td>
</tr>
<tr>
<td>Chronic aspirin</td>
<td>238 (193–283)</td>
<td>223 (192–255)</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td>300 (300–300)</td>
<td>273 (252–294)</td>
<td></td>
</tr>
</tbody>
</table>

CEPI-CT, collagen epinephrine closure time; ACS, acute coronary syndrome.

*CEPI-CT was measured with the platelet function analyzer (PFA-100); *p < .01 Wilcoxon pairs test; *Mann-Whitney U test for descriptive purposes only. Data are given as mean ± 95% confidence intervals.

RESULTS

Patient Characteristics

We enrolled a total of 302 consecutive patients who presented to the emergency department with chest pain (21). Paired platelet function tests before and after aspirin infusion were available in 234 patients of whom 166 presented with ACS (Fig. 1). Forty-four of those patients with ACS were on chronic pretreatment with 100 mg aspirin. Compliance checks were done by questioning the patients regarding the reg-
ularity of their medication intake, and compliance >90% was reported. Demographic variables and selected laboratory values of these 234 patients are presented in Tables 1 and 2. As expected, there were no immediate adverse effects of placebo or aspirin infusion.

Serum TXB₂ Levels

Serum for measurement of TXB₂ levels was available from 50 aspirin-naïve patients and 22 patients on chronic aspirin pretreatment. Median TXB₂ serum levels were 6.1 ng/mL (interquartile range, 1.6−20.3) in aspirin-naïve patients and 1/60 of that value in the aspirin-pretreated patients (0.09 ng/mL [interquartile range, 0.03−0.43]; p < .001 between groups). Four of the aspirin-pretreated patients had TXB₂ levels >0.6 ng/mL (2.2, 2.5, and 3.5 ng/mL, including one outlier with 51.6 ng/mL). This indicates approximately 90% compliance in our population with chronic aspirin pretreatment. Aspirin infusion further decreased TXB₂ levels to 0.04 ng/mL (interquartile range, 0.01−0.07) in aspirin-naïve patients and to 0.04 ng/mL (interquartile range, 0.01−0.04) in patients already on aspirin, (p < .001 vs. baseline for both groups), and the maximum in any patient was 0.5 ng/mL. At baseline, TXB₂ levels correlated inversely with CEPI-CT in the aspirin-naïve patients (r = −.53, p < .001), just as well as in the aspirin-pretreated patients (r = −.62, p = .01).

Immediate Effect of Aspirin Infusion on Platelet Function

In a subgroup of patients (n = 52), we conducted a randomized, placebo-controlled, double-blind, crossover trial on the acute effects of aspirin infusion on CEPI-CT. This substudy was performed to dem-
onstrate that any change in CEPI-CT within 5–10 mins after aspirin infusion is indeed a result of aspirin and not confounded by any concomitant intervention within this timeframe. Based on a previous publication, we considered that five lung passages (5 mins) would guarantee sufficient distribution of aspirin in the vascular system (16). Therefore, the short interval of 5 mins between aspirin infusion and measurement was validated. Indeed, as depicted in Figure 4, aspirin prolonged CEPI-CT (mean CEPI-CT, 278 secs; 95% CI, 259–296 secs) within 5 mins, whereas placebo did not alter CEPI-CT (mean CEPI-CT, 158 secs; 95% CI, 140–174 secs; p < .0001 between groups; Figure 4).

**Table 4. Response to aspirin infusion in relation to underlying atherothrombotic disease**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CEPI-CT, secs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstable angina</td>
<td>289 (278–300)</td>
</tr>
<tr>
<td>Non-ST-segment elevation myocardial infarction</td>
<td>282 (269–295)</td>
</tr>
<tr>
<td>ST-segment elevation myocardial infarction</td>
<td>259 (245–273)*</td>
</tr>
</tbody>
</table>

CEPI-CT, collagen epinephrine closure time.

*p < .05 vs. non-ST segment elevation myocardial infarction or unstable angina. Data are presented as mean ± 95% confidence intervals.

**Risk Factors for High Residual Platelet Reactivity After Aspirin Infusion**

Among patients with STEMI, the proportion of patients with platelet hyperfunction after aspirin infusion was significantly higher than in other patients with ACS (20% vs. 4% in non-STEMI unstable angina pectoris, p = .01).

The following variables did not correlate with platelet hyperfunction after aspirin infusion in a univariate correlation analysis: plasma levels of creatinine, uric acid, triglycerides, cholesterol, C-reactive protein, fibrinogen, hematocrit, platelet count, or body mass index. Furthermore, there was no influence of any drug intake, blood group, sex, or comorbidity such as history of hypertension, diabetes, nicotine abuse, previous myocardial infarction, or known coronary, cerebral, or peripheral artery disease on the responsiveness to aspirin. Significant predictors of poor response to aspirin were baseline collagen and adenosine diphosphate CT (r = .5, p < .01) (16, 27), leukocyte count on admission (r = —.27, p < .01), peak troponin T level (r = —.33, p < .01), and, as expected (28), von Willebrand factor ristocetin cofactor activity (r = —.47, p < .01).

Primary percutaneous coronary intervention was the primary treatment in the majority of patients with STEMI (77%).

As a result of less frequent aspirin premedication, the baseline CEPI-CT was somewhat lower in the patients with STEMI undergoing percutaneous coronary intervention (162 secs) as compared with thrombolysis (198 secs; p = .135), but the CEPI-CT values after aspirin infusion were identical (258 secs).

**DISCUSSION**

Platelet hyperfunction is commonly seen among patients with coronary artery disease who are on aspirin therapy (29, 30). Our study confirms and extends these observations to a large number of patients with ACS, in particular to patients with STEMI. Among aspirin-pretreated patients with ACS, 37% responded poorly to chronic therapy with 100 mg aspirin (Fig. 3). This falls within the CI of a systematic review on aspirin nonresponders in patients with acute vascular events (95% CI, 37% to 47%) (29) and demonstrates the external validity of our study.

Our results provide evidence that chronic intake of 100 mg aspirin is not maximally effective in more than half of the patients with ACS (Fig. 3) presenting to an emergency department. For the first time, we demonstrate that aspirin infusion (250 mg) decreases platelet hyperfunction in these patients to a similar degree as that observed for aspirin infusion in aspirin-naïve patients (Figs. 2 and 3; Table 3).

The decreased platelet reactivity after aspirin infusion is consistent with the further reduction of TXB2 levels in these patients. The reduction in TXB agrees with the ex vivo finding of a previous study (31), and by itself could be beneficial because it reduces levels of this potent vasoconstrictor. Although noncompliance may be a possible cause of apparent resistance, only few of the chronically pretreated patients seemed noncompliant or had incomplete

---

Figure 3. Response to aspirin infusion in patients with acute coronary syndrome (ACS) chronically pretreated with 100 mg aspirin. Collagen epinephrine closure time (CEPI-CT) values were stratified to high, moderate, or low platelet reactivity before (open bars) and after (shaded bars) an intravenous loading with aspirin (250 mg). The number of patients with platelet hyperreactivity (CEPI-CT <193 secs) decreased in all patients with ACS (p = .02 McNemar chi-square), importantly even in the subgroup of patients with ST-elevation myocardial infarction (STEMI). Columns depict number of patients.
bioavailability of low-dose aspirin, because baseline TXB₂ levels indicated compliance of approximately 90%. One possible explanation for the suboptimal inhibition of platelets by chronic aspirin intake is that after the last dose, the newly produced platelets generate sufficient amounts of thromboxane to synergistically act with other agonists. In line with that notion is the observation that TXB₂ levels also decreased in patients already on aspirin.

Our data provide first evidence that an acute increase in the dose of aspirin from 100 mg to a total of 350 mg aspirin has a beneficial antiplatelet effect in patients with ACS as measured by platelet function testing under high shear conditions. This is in good agreement with the more pronounced effects of 325 mg as compared with 100 mg aspirin on thromboxane levels and CEPI-CT values in patients undergoing coronary surgery (32) as well as another study in patients after coronary artery bypass grafting (33). However, ultimate proof for additional aspirin loading in ACS would require a multicentered randomized placebo controlled trial designed to detect a clinically relevant outcome in patients with ACS already on aspirin. This is a major undertaking in terms of recruitment, because only approximately 20% of our patients with ACS were chronically pretreated with aspirin. In addition, the concomitant administration of thienopyridines or GPIIb/IIIa antagonists may mask any beneficial effects of additional aspirin therapy and may inflate the sample size well beyond feasible limits. Notably, additional aspirin administration should not delay appropriate therapy with P₂Y₁₂ receptor or GPIIb/IIIa antagonists, both of which are important drug classes for patients with ACS (34, 35).

As depicted in Figure 4, the effect of infused aspirin is very rapid and hence a suitable alternative to “chewable” aspirin, for which pharmacokinetic data in patients with ACS are very scarce. Apart from the immediate onset of action, and 100% bioavailability, infused aspirin was preferred to “chewable” aspirin for feasibility reasons in this trial. Different centers may not wish to change their current treatment from “chewable” aspirin to the somewhat more expensive infused aspirin, because there is no proven clinical benefit. However, it is conceivable that our findings will be reproducible with “chewable” aspirin, although the onset of action may be delayed.

**Limitations.** For reasons of feasibility in an emergency department, other platelet function tests such as light transmission aggregometry could not be performed. The PFA-100 provides an integral measure of platelet function in whole blood rather than a measure of biochemical aspirin resistance such as TXB₂ levels. However, there is ample evidence that platelet hyperfunction and/or short CEPI-CT correlate with the degree of myocardial necrosis and predict worse outcome and mortality in patients with coronary artery disease or ACS (20, 21, 36–40). In eight studies comprising 847 subjects, aspirin nonresponders were more likely to have vascular events than responders (relative risk: 1.6; 95% CI, 1.2–2.3) (29). This estimate is in good agreement with the risk estimate of 2.3 (95% CI, 1.2–4.3) for recurrent ACS when CEPI-CT was <300 secs in our patients (20). Furthermore, platelets are even more hyperfunctional in patients whose myocardial infarction leads to cardiac arrest (41). These data indicate that the CEPI-CT is a biologically and clinically relevant surrogate endpoint. Yet our study design, in which every patient eventually received an additional aspirin infusion, cannot provide any information on beneficial effects in terms of altering outcome. Finally, our study has not determined the optimal aspirin dose for ACS, but 250 mg aspirin was given to all patients irrespective of previous intake of 100 mg aspirin. Thus, we cannot exclude that a lower loading dose of aspirin may have produced similar effects so that the recommended aspirin doses should not be exceeded. However, our functional data support the recent increase in the recommended aspirin dose from 75–162 mg to 162–325 mg for the treatment of STEMI (11).

In conclusion, aspirin infusion in the emergency room further reduced thromboxane B₂ levels and further inhibited platelet function in many patients with ACS already on 100 mg aspirin.

**CONCLUSIONS**

After completing this CME activity, readers should be able to explain how to measure platelet function and the effect of aspirin infusions on platelet function. In addition, readers should be able to relate the usefulness of aspirin infusion in patients with ACS.
REFERENCES