Aspirin resistance in off-pump coronary artery bypass grafting

Zanxin Wang, Fei Gao, Jianlong Men, Jing Ren, Paul Modi and Minxin Wei

**OBJECTIVE:** Anti-platelet therapy with aspirin is the cornerstone of treatment after coronary artery bypass grafting (CABG). Aspirin resistance describes the clinical observation of the inability of aspirin to prevent thrombotic complications or the laboratory phenomenon of absence of the effect of aspirin on platelet inhibition tests. Off-pump CABG (OPCAB) is associated with reduced platelet activation and turnover compared to on-pump surgery which may indicate that aspirin is more effective after OPCAB. Our aim was to evaluate the efficacy of aspirin and incidence of aspirin resistance in patients undergoing OPCAB.

**METHODS:** A total of 331 patients was recruited, of which 111 underwent primary OPCAB (group A) and 220 controls with ischaemic heart disease received medical therapy. Arachidonic acid-induced platelet aggregation and urinary 11-dehydro thromboxane B₂ (11-dehydroTxB₂) were measured at baseline and following aspirin administration on days 1, 4 and 10. A 6-month follow-up was completed in patients who developed aspirin resistance.

**RESULTS:** On the first postoperative day, 78 patients (70.3%) were aspirin sensitive (AS) and 33 (29.7%) were aspirin resistant (AR). Of the latter, 18 (16.2%) and five (4.5%) patients remained resistant on days 4 and 10, respectively. AR patients had significantly greater platelet aggregation and urinary 11-dehydroTxB₂ levels at all time points than those in the AS group. All patients in the AR group were AS by 6 months. All controls were sensitive to aspirin with similar platelet aggregation and 11-dehydroTxB₂ to those in the AS group.

**CONCLUSIONS:** Aspirin resistance is a transient phenomenon during the early postoperative period in approximately 30% of patients undergoing OPCAB.

**Keywords:** Aspirin resistance, Coronary artery bypass grafting, Platelet aggregation, Thromboxane

**INTRODUCTION**

The cornerstone of anti-platelet therapy after coronary artery bypass grafting (CABG) is aspirin and its early administration is associated with a reduced risk of death and ischaemic complications [1]. However, not all individuals respond comparably to anti-platelet therapy; some suffer from thromboembolic events despite continuous anti-platelet therapy. This is known as ‘aspirin resistance’ and has been described in more than two-thirds of patients early after CABG [2,3]. The mechanisms of aspirin resistance are uncertain and concepts to explain it are largely hypothetical. It may, in part, be attributable to cardiopulmonary bypass (CPB), but a significantly diminished anti-platelet effect of aspirin has also been reported after carotid surgery [4]. Off-pump CABG (OPCAB) reduces platelet activation and turnover compared to on-pump CABG which may indicate that aspirin should be more effective after OPCAB than conventional CABG [5,6]. However, little is known about the incidence of aspirin resistance after OPCAB compared to on-pump CABG. If indeed there is a difference, then this would indicate that mechanisms independent of CPB are also important.

Aspirin inhibits platelet aggregation through irreversible acetylation of platelet cyclooxygenase (COX), blocking the transformation of arachidonic acid (ARA) into thromboxane A₂ (TxA₂) [7]. There are two isoforms of cyclooxygenase (COX-1 and COX-2), of which COX-1 is ubiquitously expressed and is the only isoform relevant for platelet aggregation. Due to the instability of TxA₂, it is rapidly converted to stable and inactive thromboxane B₂ (TxB₂), the metabolite of which can be measured in urine, 11-dehydro thromboxane B₂ (11-dehydroTxB₂). A myriad of tests are currently available to assess the anti-platelet effect of aspirin, but these tests are not equally effective and correlate poorly amongst themselves [8,9]. ARA-induced platelet aggregation and measurement of TxB₂ are two of the most sensitive methods [10]. Our aim was to evaluate the efficacy of aspirin and the incidence of aspirin resistance in patients undergoing OPCAB.

**PATIENTS AND METHODS**

A total of 331 patients consented to participate in the study of which 111 patients underwent primary OPCAB (group A);
another 220 control patients with ischaemic heart disease (only stable angina pectoris) were treated with optimal medical therapy (group B). The study was approved by the local ethics committee. Exclusion criteria were: (1) acute myocardial infarction, (2) percutaneous coronary intervention within 30 days before surgery, (3) stroke within 6 months, (4) concomitant surgery (valvular or MAZE procedure), (5) renal insufficiency, (6) liver disorders, (7) platelet count <150 x 10^9 l^-1; INR (international normalised ratio) >1.2; activated partial thromboplastin time ratio >1.2, (8) those receiving platelet transfusions and (9) those receiving anti-platelet therapy within 5–7 days before surgery.

**Study protocol**

All patients who received OPCAB were admitted to hospital 1 week before surgery to finish preoperative preparation. No anti-fibrinolytics (aprotinin and tranexamic acid) were used perioperatively. Aspirin and clopidogrel were discontinued 5–7 days prior to surgery and replaced by low-molecular-weight heparin until the day before surgery. OPCAB was undertaken via median sternotomy with internal thoracic artery, radial artery and/or saphenous vein as conduits. Heparin (1.5 mg kg^-1) was administered to achieve an activated clotting time (ACT) >300 s. After the end of the grafting procedure, the effect of heparin was reversed by protamine administration (1:1 ratio) to achieve an ACT to around 150–170 s. Postoperative treatment in the intensive care unit (ICU) was standardised. Low-molecular-weight heparin therapy was begun at night of the surgery day. Patients in the present research received mechanical ventilation not longer than 2 days. Aspirin (100 mg) was administered after weaning the ventilator. Low-molecular-weight heparin therapy was begun from the night of the surgery day to 10 days after OPCAB. Some of the patients who needed longer mechanical ventilation were excluded. Control patients received aspirin 100 mg day^-1 and did not receive medical therapy before admission. To patients who developed aspirin resistance, clopidogrel 75 mg day^-1 was also prescribed. Samples were collected at the following time points between 06:00 and 07:00 prior to daily aspirin administration:

- **group A** – blood and urine samples preoperatively and 1, 4 and 10 days after aspirin administration.
- **group B** – blood samples prior to aspirin administration and after administration on day 1. Repeated measurement was performed if platelet aggregation was ≥20% on day 1 after aspirin treatment. Urine samples were collected before and 1, 4 and 10 days after aspirin administration.

**Platelet aggregation and urinary 11-dehydroTxB2 assessment**

Platelet-rich plasma was prepared immediately after blood collection by centrifugation of 5 ml of anti-coagulated blood at 150 g for 10 min at room temperature. Platelet-rich plasma was adjusted to a platelet count of 200 000–300 000 μl^-1. Platelet-poor plasma was obtained from the remaining sample by centrifugation at 2500 x g for 20 min. Samples were assayed within 2 h of collection by light transmission aggregometry (Platelet Aggregation Profiler, Chrono-Log, USA). Platelet aggregability was calculated as total aggregation (% at 5 min) and aspirin resistance was defined as a mean aggregation of ≥20% with 0.5 mg dl^-1 ARA [11,12]. Urine samples were stored at ~80 °C for batch measurement of 11-dehydroTxB2 using an enzyme immunoassay kit (11-dehydro Thromboxane B2 EIA Kit, Cayman Chemical, MI, USA).

**Statistical analysis**

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows (version 13.0). Continuous data are presented as mean ± standard deviation and analysed using either a t-test, repeated measures analysis of variance or one-way analysis of variance (ANOVA), as appropriate. Categorical variables were expressed as frequencies and analysed with χ²-test. p-Values <0.05 were considered significant.

**RESULTS**

There were no significant differences in baseline characteristics between patients in groups A and B, or between groups AS and AR (Tables 1 and 2). In group A, aspirin was withdrawn 7 ± 2 days prior to surgery whereas no patients in group B had received aspirin previously. In group A, there were no perioperative deaths, myocardial infarctions (MIs) or major complications (cerebral, renal or hepatic).

Preoperative platelet aggregation was ≥20% in all patients in group A. Postoperative aspirin resistance was identified in 33 (29.7%) patients on day 1 after aspirin treatment was commenced (AR group), which decreased by days 4 and 10 of treatment (18 (16.2%) and 5 (4.5%) patients, respectively). As many as 78 patients (70.3%) were defined as aspirin sensitive (AS group) on day 1 after aspirin treatment was commenced and all remained so by the fourth and tenth day (Fig. 1a).

There was no significant difference in preoperative urinary 11-dehydroTxB2 between AS and AR groups (Fig. 1b). In both groups, urinary 11-dehydroTxB2 decreased with time following aspirin administration, but at all time points levels were higher in the AR group (p = 0.049). There was no significant difference in preoperative platelet counts between AR and AS groups (198.8 ± 87.6 x 10^9 l^-1 vs 217.8 ± 62.8 x 10^9 l^-1, respectively) (Fig. 1c). In both groups, postoperative platelet counts increased more in AS than AR patients (P = 0.003). There was no significant difference in platelet counts between AR and AS groups at any time point.

**Table 1:** Patient characteristics stratified into surgical (A) and control (B) patients

<table>
<thead>
<tr>
<th></th>
<th>Surgical (A)</th>
<th>Control (B)</th>
<th>P</th>
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<tbody>
<tr>
<td>n</td>
<td>111</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>80/31</td>
<td>134/86</td>
<td>0.087</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.3 ± 9.0</td>
<td>64.5 ± 12.7</td>
<td>0.905</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>57 (51%)</td>
<td>92 (42%)</td>
<td>0.079</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>71 (64%)</td>
<td>121 (55%)</td>
<td>0.171</td>
</tr>
<tr>
<td>Diabetes n (%)</td>
<td>34 (31%)</td>
<td>53 (24%)</td>
<td>0.180</td>
</tr>
<tr>
<td>Hyperlipidaemia, n (%)</td>
<td>41 (37%)</td>
<td>75 (34%)</td>
<td>0.571</td>
</tr>
<tr>
<td>Creatinine</td>
<td>84 ± 21</td>
<td>82 ± 22</td>
<td>0.740</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>55 ± 13</td>
<td>56 ± 13</td>
<td>0.498</td>
</tr>
</tbody>
</table>

Data are presented as either mean ± SD or counts. LVEF: left ventricular ejection fraction.
but no significant difference was found between groups ($p = 0.053$). A 6-month follow-up was 100% complete in aspirin resistant (AR) patients and all patients were now AS (mean platelet aggregation, $11.5 \pm 3.4\%$, and mean urinary 11-dehydroTxB$_2$, $50.3 \pm 15.4$ ng l$^{-1}$) with no deaths, MIs or thromboembolic events.

In control patients, platelet aggregation was $8.7 \pm 2.8\%$ on day 1 after aspirin was given with no patients being AR. The change in urinary 11-dehydroTxB$_2$ was similar to those in the AS group. After taking aspirin, the trend of urinary 11-dehydroTxB$_2$ decreased in all patients. Those who developed aspirin resistance had significantly higher levels than the AS group (Fig. 2).

**DISCUSSION**

This study demonstrates that nearly 30% of patients are AR on day 1 after OPCAB but that this is a transient phenomenon with only 4.5% of patients remaining so by postoperative day 10. This has important implications for graft patency. Aspirin resistance can be defined clinically as an ischaemic event while on aspirin therapy. Laboratory assays of aspirin resistance are surrogate measures as inhibition of platelet aggregation in vitro does not necessarily translate into prevention of thrombosis in vivo. However, recent data have shown that aspirin resistance is an independent predictor of vein graft thrombosis [13]. One of the best-evaluated in vitro methods is light transmission aggregometry that measures the increase in light transmission through a platelet suspension when platelets aggregate using ARA as an agonist. Aspirin’s inhibition of COX-1 is best assessed directly by measuring its metabolite, 11-dehydroTxB$_2$, in urine [14].

Each assay, however, measures a distinct aspect of platelet function and definitions of aspirin resistance are not consistent, which may explain the variation in the reported prevalence of aspirin resistance after CABG from 10% up to >90% [15-17]. Aspirin resistance acquired after CABG would appear to be a transient phenomenon, especially within the first month after surgery. In 24 on-pump CABG patients, there were 11 weak responders and four non-responders on day 10 (total 15/24, 62.5%) versus only one weak responder and no non-responders by 1 month after surgery (total 1/24, 4%) [18]. Similarly, in 25 patients undergoing on-pump CABG, the prevalence of aspirin resistance on days 5, 10 and 6 months after surgery was 93%, 86% and 0%, respectively, using a thromboxane assay, and 100%, 75% and 33% using a platelet function analyser (PFA-100) method [19]. In comparison, our data demonstrate that aspirin resistance was seen in fewer patients early after OPCAB but that it too had disappeared by 6 months. Poston et al. reported a remarkably similar incidence of aspirin resistance in 225 OPCAB patients (30% on day 3) [13]. This raises the question about the

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**Table 2**: Characteristics of surgical patients stratified as aspirin resistant or sensitive

<table>
<thead>
<tr>
<th></th>
<th>Aspirin resistant</th>
<th>Aspirin sensitive</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>33</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>23/10</td>
<td>57/21</td>
<td>0.823</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63 ± 9</td>
<td>65 ± 9</td>
<td>0.248</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>22 (67)</td>
<td>49 (63)</td>
<td>0.907</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>8 (24)</td>
<td>26 (33)</td>
<td>0.226</td>
</tr>
<tr>
<td>Hyperlipidaemia, n (%)</td>
<td>10 (30)</td>
<td>31 (40)</td>
<td>0.769</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>54 ± 12</td>
<td>56 ± 12</td>
<td>0.582</td>
</tr>
<tr>
<td>Preoperative creatinine</td>
<td>84 ± 21</td>
<td>82 ± 22</td>
<td>0.740</td>
</tr>
<tr>
<td>(μmol l$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin withdrawal preop (days)</td>
<td>7 ± 2</td>
<td>6 ± 1</td>
<td>0.588</td>
</tr>
<tr>
<td>Distal anastomoses</td>
<td>3.2 ± 0.9</td>
<td>3.1 ± 0.9</td>
<td>0.734</td>
</tr>
<tr>
<td>Blood salvage (ml)</td>
<td>420 ± 246</td>
<td>375 ± 153</td>
<td>0.137</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>200 ± 41</td>
<td>213 ± 43</td>
<td>0.141</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD or counts. LVEF: left ventricular ejection fraction.
underlying mechanisms of the (procedurally) ‘acquired’ transient aspirin resistance seen after CABG and indicates that it is multifactorial and not just attributable to the effects of cardiopulmonary bypass.

CPB leads to increased platelet turnover due to mechanical disruption, adhesion to the bypass circuit, activation and haemodilution, with an increased amount of newly generated platelets capable of forming thromboxane despite aspirin treatment [11,20]. The platelet count typically falls after CPB followed by an increase during the early postoperative period in approximately 30% of patients undergoing OPCAB which has disappeared by 6 months after surgery.

Platelet turnover is believed to be less after OPCAB compared to on-pump CABG. This may explain why platelet aggregation was inhibited significantly to 55.7 ± 16.3% of control by postoperative day 5 in 14 OPCAB patients treated with aspirin 100 mg day\(^{-1}\), whereas aggregation remained unchanged after CPB in 15 on-pump patients (105.8 ± 26.9% of control) [16]. Though these data may indicate that aspirin is more effective after OPCAB than after conventional CABG, our data clearly demonstrate that aspirin resistance still occurs albeit in fewer patients. The clue to the common pathway may be in the platelet count, which gradually increased postoperatively, peaking on day 4 and remaining above preoperative levels until the tenth postoperative day. Thus, generation of new platelets in OPCAB clearly occurs and this would therefore be a plausible common mechanism for aspirin resistance.

It may be that competing forces of reduced coagulopathy versus reduced aspirin resistance are at play after OPCAB compared to on-pump CABG. It would be reasonable to expect that aspirin resistance in a less coagulopathic setting (i.e., OPCAB) may have effects on graft patency but appropriate clinical trials of modified anti-platelet strategies are lacking. In an observational study of 591 patients undergoing elective OPCAB, 266 patients were treated with postoperative aspirin monotherapy and 325 patients received dual anti-platelet therapy with aspirin and clopidogrel [22]. In the multivariate analysis, postoperative clopidogrel independently decreased adverse cardiac events (odds ratio 0.2, 95% confidence interval 0.1–0.45, \(p < 0.0001\)) and symptom recurrence (OR 0.3, 95% CI 0.15–0.99, \(p < 0.0001\)). There was no significant difference in the incidence of bleeding complications between groups. However, other studies reported that the patency rates in patients treated with clopidogrel plus aspirin compared to those in the aspirin monotherapy group did not reach statistical significance and the process of saphenous vein graft (SVG) intimal hyperplasia did not reduce [23,24]. These may be explained by the fact that different populations have different sensitivities to anti-platelet medicine.

In control patients, platelet aggregation was 8.7 ± 2.8% on the first day after aspirin administration with no resistance detected. By contrast, the incidence of aspirin resistance is 20–30% in the US and Germany [12] which suggests that the Chinese population is more sensitive to aspirin than that in Western countries.

Our study has several potential limitations. First, the dose of aspirin is important as the incidence of aspirin resistance is less in patients who receive 325 mg compared to 100 mg early after CABG [25]. Second, measurement of urinary 11-dehydro-TxB\(_2\) levels can be confounded by thromboxane derived from non-platelet sources, for example, nucleated cells such as monocytes or vascular endothelial cells which are endowed with substantial amounts of thromboxane synthase. However, because this is likely to have had a similar effect for all patients, we believe that higher levels of 11-dehydro-TxB\(_2\) truly represent aspirin resistance.

In summary, aspirin resistance is a transient phenomenon during the early postoperative period in approximately 30% of patients undergoing OPCAB which has disappeared by 6 months after surgery.

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**Conflict of interest:** none declared.

**REFERENCES**


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Figure 2: (a) Arachidonic acid-induced platelet aggregation in controls (group B) and (b) urinary 11-dehydro-TxB\(_2\) in aspirin resistant (AR) and sensitive (AS) patients and controls. Data are presented as mean ± SD. * \(p < 0.05\).


