The effect of methylprednisolone treatment on fibrinolysis, the coagulation system, and blood loss in cardiac surgery

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Abstract

The purpose of this study was to examine steroid pretreatment in order to decrease postoperative coagulopathy disorders and bleeding. In this randomized double-blinded study, the efficacy of low versus high doses of methylprednisolone on the coagulation system and postoperative bleeding was compared in patients who were undergoing cardiac surgery with cardiopulmonary bypass (CPB). The platelet response to agonists, D-dimer concentration, tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI-1) antigens, and platelet receptors CD42b, CD62P, and CD41a were evaluated.

Results: The platelet response to agonists was reduced. The mean concentrations of D-dimer and tPA antigen increased although PAI-1 concentration did not show any significant changes following heparin neutralization. Postoperative expression of CD42b showed no changes in comparison with preoperation values in both groups. There was a significant increase in the expression of CD62P with a methylprednisolone dose of 15 mg/kg, while there was just a slight increase with a dose of 5 mg/kg. CD41a, as a fibrinogen receptor, was increased significantly after CPB in both groups. Significant data were shown in decreasing blood loss with a high dose of methylprednisolone.

Conclusion: Methylprednisolone at a dose of 15 mg/kg reduced bleeding, probably by increasing CD62P after heparin neutralization, which can activate platelet activation in favor of better hemostasis.

Keywords: Cardiac surgery, cardiopulmonary bypass, coagulopathy, methylprednisolone, fibrinolysis

1. Introduction

Coronary surgery with cardiopulmonary bypass (CPB) is the most common heart surgery in the world and it is almost always associated with acquired hemostatic disturbance that induces postoperative bleeding (1–5). Excessive bleeding and high usage of blood products after CPB are among the most important complications of cardiac surgery (6–8) in association with economic consequences. Reoperation for significant bleeding is required in 3%–11% of patients after cardiac operation with increased morbidity and hospital mortality (9,10), and this is a result of acquired hemostatic defect with no identifiable surgical bleeding sources in 30%–50% of these cases (3,9). CPB activates a variety of systems associated with inflammatory triggers following blood contact with nonbiological surfaces and trauma, consumption of coagulation factors because of hemodilution and increased fibrinolytic system activity, and reduced platelet function (11,12).

Prophylactically, agents that have antifibrinolytic properties are used to restrict these complications (13). Corticosteroids have been recommended to attenuate inflammatory response during cardiac surgery in order to decrease the proinflammatory to antiinflammatory interleukin ratio (14–19). In this way, some clinical benefits of methylprednisolone as an inflammatory agent during CPB have been reported previously (20), although there is always concern about the potential adverse effects, such as vein graft occlusion and ischemic events with methylprednisolone (4). There is some evidence in favor of interaction between inflammatory and coagulation systems, supported by the hemostatic derangement observed in severe inflammation (2,12).

The main purpose of this study was to assess this bidirectional relationship by examining the effect of two administered doses of methylprednisolone (5 and 15 mg/kg) on blood losses and blood product requirements,
coagulation, fibrinolysis, and expression of platelet receptors before operation and 5 min after heparin neutralization with protamine. The platelet response to different agonists, the expression of platelet receptors (CD41a, CD42b, CD62P), tissue plasminogen activator, plasminogen activator inhibitor, and D-dimer were evaluated both before operation and 5 min after protamine administration.

2. Materials and methods

2.1. Study design

The study was reviewed and approved by the Shahid Beheshti University ethics committee and signed informed consent was obtained from every participant. Due to the presence of extensive inflammation processes during CPB, the ethics committee had not permitted a group without steroid administration. The study was also registered in the Iranian Registry of Clinical Trials (IRCT201010233438N2). There was a significant difference in postoperative bleeding between two groups in the first 28 patients, so we terminated the study at that stage. A total of 28 nondiabetic patients (20 males, 8 females) undergoing elective CABG with CPB in our institution between September 2010 and May 2011 were enrolled in a prospective, randomized, double-blinded study. The exclusion criteria were ages under 50 or over 80, previous cardiac surgery, emergency or combined procedures, renal or hepatic dysfunction, history of recent peptic ulcer disease (<6 months), hematologic or coagulation disorders, and history of antiinflammatory or anticoagulant treatment in the 5 days prior to the operation.

2.2. Methylprednisolone administration and CPB

2.2.1. Technique

Patients were randomly assigned to receive a single intravenous bolus (either 5 or 15 mg/kg) of methylprednisolone (Urbason, Sanofi-Aventis, Frankfurt, Germany) during induction of anesthesia.

2.2.2. Anesthesia and CPB

Anesthesia was administered in each group in an identical fashion according to the standard protocol of the institution. Weight-related doses of morphine and midazolam were given for preoperative medication. Anesthesia was induced using a weight-adjusted combination of sufentanil, thiopental, and pancuronium and it was maintained with isoflurane, a supplemental infusion of fentanyl, atracurium, and midazolam.

A standard surgical technique was used for all patients. Coronary surgery was performed with a left internal mammary graft to the left anterior descending coronary artery in all patients and vein grafts to other territories. Nonpulsatile roller pump flows were adjusted to maintain a cardiac index greater than 2.4 L min⁻¹ m⁻² with moderate hypothermia (30–32 °C). Cardiac protection was achieved by intermittent antegrade cold blood cardioplegia. An initial dose of heparin (300 IU/kg) was given to reach an activated clotting time (ACT) greater than 480 s with additional bolus doses given as required to maintain the ACT values above 400 s. After CPB, the heparin was reversed by the administration of protamine (1 mg per 100 U heparin) with ACT of <130 s. Intravenous epinephrine as incremental or infusion doses was administered to maintain mean arterial pressure between 50 and 80 mmHg. No steroids were added to the prime and neither aprotinin nor tranexamic acid was used.

Cross-matched packed red blood cells were added whenever the hematocrit level fell below 20%. The whole process of patient care during the preoperative period was performed by surgeons and nurses who were not involved in the study.

After completion of surgery, patients were transferred to the ICU. Patients were weaned from mechanical ventilation after becoming hemodynamically stable, responding to verbal stimulation and when the blood loss was below 100 mL/h. Cardiovascular and respiratory values were recorded every 15 min before extubation and then hourly until discharge from the ICU. Prophylactic measures were started on the first postoperative day with orally given aspirin (325 mg once per day) and subcutaneous injection of heparin twice daily in both study groups. Blood glucose concentrations were monitored regularly to maintain the plasma glucose between 100 and 200 mg/dL.

Patients were discharged from the ICU after becoming hemodynamically stable and having normal blood gases during spontaneous breathing with satisfactory renal function.

2.2.3. Blood sampling

Blood samples were taken twice: before induction of anesthesia (T1) and 5 min after heparin neutralization (T2). The samples were transferred immediately to the lab. The platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained from the blood mixed with sodium citrate by centrifuging of the sample at 600 and 2500 × g for 10 min, respectively. Platelet aggregation was assessed by PRP immediately. PPP was stored and frozen until analysis. Flow cytometry analysis was performed on blood mixed with EDTA.

2.2.4. Laboratory assessments

Platelet counts and hematocrit measurements were performed using an automated cell counter (Sysmex K800, Japan). Prothrombin time (PT) and partial thromboplastin time (PTT) (Biolabo, France) were determined by a semiautomatic coagulometer (Option 4 Plus, UK). The platelet function was evaluated by aggregometer (PACKS-4, France). Adenosine 5I-diphosphate (ADP; 0.2 μM), ristocetin (Risto; 7.5 mg), collagen (Coll; 100 μg),
arachidonic acid (AA; 5 mg), ristocetin cofactor (1.0 mg) (all from Helena, France) were used as stimulants. Samples were transferred to the laboratory within less than 2 h. The PRP counts were adjusted to a platelet count of 250 × 10^9/L and the results were recorded as percentages at 3 min.

An enzyme-linked immunosorbent assay (ELISA) was used for measuring tissue plasminogen activator (tPA; ARK011A) and plasminogen activator inhibitor (PAI-1; ARK012A) antigens (Hyphen Biomed, France) in accordance with the manufacturer’s instructions (normal reference values are less than 10 ng/mL and 25 ng/mL, respectively). Briefly, standards and samples were diluted, then added to a microplate precoated with tPA and PAI-1 antigens. After incubation and washing, the immunoconjugate, which is a monoclonal antibody coupled to horse radish peroxidase, was introduced. Peroxidase substrate tetramethylbenzidine (TMB) was added following the new incubation and washing step. The color development was stopped by adding stop solution. Results were read by microplate reader (Lab System Multiscan, USA).

D-dimer concentration was measured to assess the fibrinolysis system (normal reference value is less than 0.5 µg/mL). The test was done by the immunoturbidimetric method (Stago, France). This assay is based on the change in turbidity of a microparticle suspension that is measured by photometry (STACOMPACT, France).

Samples were centrifuged at 1,500 × g for 15 min for flow cytometry analysis with a platelet count of more than 100 × 10^9/L. Briefly, an aliquot of the cells (100 µL) was incubated with 20 µL of monoclonal antibodies CD41a-FITC, CD42b-FITC, and CD62P-PE (all from Becton Dickinson Pharmingen, USA) at 4 °C in the dark for 30 min. An isotype control as a negative control also was used for each sample. Samples were diluted and fixed with 700 µL of PBS/BSA/paraformaldehyde and were evaluated up to 24 h using flow cytometry (Paratec, PASIII) and FLOWMAX version 2.4e software.

### 2.3. Statistical analysis

All the data were presented as mean ± SD. The preoperative and postoperative characteristics of the groups were compared by Mann–Whitney analysis. Intragroup differences of variables in each test were assessed by Wilcoxon’s ranked sum test. P ≤ 0.05 was considered statistically significant.

### 3. Results

Demographic data and pre- and intraoperative characteristics, as well as hemostatic variables, were not significantly different before and after the surgery between the two groups (5 and 15 mg/kg of methylprednisolone) (Tables 1 and 2). There was no mortality or wound infection in either group during the 30 days after operation (data not shown).

#### 3.1. Effect of methylprednisolone on platelet aggregation

The platelet response to different agonists (ADP, AA, Coll, Risto) decreased significantly after heparin neutralization in comparison with preoperation values (P < 0.05). There was no significant difference between the two groups of methylprednisolone doses. However, the ristocetin cofactor activity increased significantly in both groups after heparin neutralization (P < 0.05). There was no difference in ristocetin cofactor activity between the two groups (P > 0.05) (Figure 1).

#### 3.2. Effect of methylprednisolone on expression of platelet membrane glycoproteins

CD42b expression as an adhesion receptor to endothelial cells showed no significant changes (P > 0.05) after heparin neutralization in comparison with preoperation

### Table 1. Demographic data and data from CPB.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>5 mg/kg methylprednisolone</th>
<th>15 mg/kg methylprednisolone</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>14</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Euro score</td>
<td>2.0 ± 1.6</td>
<td>2.5 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>EF</td>
<td>49.0 ± 10</td>
<td>43.0 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of CPB (min)</td>
<td>112 ± 26</td>
<td>110 ± 29</td>
<td>NS</td>
</tr>
<tr>
<td>Aortic time (min)</td>
<td>65.5 ± 14.6</td>
<td>66.5 ± 15.1</td>
<td>NS</td>
</tr>
<tr>
<td>Intubation time (min)</td>
<td>480 ± 315</td>
<td>537 ± 209</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation. NS: Not significant. EF: Ejection fraction. Euro score: European System for Cardiac Operative Risk Evaluation was used to assess the severity of the patients’ clinical conditions.
values in both groups. However, CD62P increased significantly after heparin neutralization with the 15 mg/kg methylprednisolone dose (P < 0.05) compared with preoperation values. This effect was not seen significant statistically for the methylprednisolone dose of 5 mg/kg. The level of CD62P expression did not show any significant changes between the two doses (P > 0.05). CD41a, as a fibrinogen receptor, increased significantly after CPB in both groups (P < 0.05). However, this increase was not significantly different between the two groups (P > 0.05) (Figure 2).

3.3. Effect of methylprednisolone on fibrinolysis markers
D-dimer concentration showed significant differences after heparin neutralization in both groups (P < 0.05). In contrast, the levels of tPA antigen increased significantly in both groups after heparin neutralization (P < 0.05). PAI-1 antigen had no significant changes after CPB (P > 0.05) (Figure 3).

3.4. Effect of methylprednisolone on postoperative bleeding and blood product requirements
A significant decrease in blood loss was observed at a dose of 15 mg/kg versus 5 mg/kg (736 ± 479.36 versus 1153 ± 531.01 mL, P < 0.05) of methylprednisolone. The need for fresh frozen plasma and packed red cells was decreased at the 15 mg/kg dose in comparison with 5 mg/kg, though this was not statistically significant (P > 0.05) (Figure 4).

4. Discussion
The problem of hemorrhage following CPB is one of the most significant complications contributing to increasing mortality and morbidity (21,22). Bleeding causes the consumption of required blood products that it is neither healthy nor cost-effective (23). The main finding of the present study was that a single high dose of methylprednisolone administration led to a significant reduction of postoperative bleeding in patients undergoing cardiac surgery with CPB. We also attempted to determine the responsible mechanism(s) that might have occurred with each dose by analyzing platelet functions.

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Different studies have shown that platelet aggregation in response to different agonists is impaired (24,25). In our study, platelet aggregation to agonists ADP, collagen, arachidonic acid, and ristocetin was diminished significantly after heparin neutralization in both methylprednisolone dosage groups in comparison with preoperation values (P < 0.05). There was no difference between the two groups statistically (P > 0.05). Similar to a previous study (26), ristocetin cofactor activity increased significantly in both groups after heparin neutralization (P < 0.05), and there was no significant difference between the two groups (P > 0.05).

The D-dimer concentration and tPA and PAI-1 antigens as fibrinolysis markers were investigated both before the operation and after heparin neutralization.

Table 2. Hemostatic variables before and after surgery in both groups of methylprednisolone (5 and 15 mg/kg).

<table>
<thead>
<tr>
<th></th>
<th>5 mg/kg</th>
<th>15 mg/kg</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Platelet count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperation</td>
<td>212 ± 10^3 ± 62</td>
<td>243 ± 10^3 ± 82</td>
<td>NS</td>
</tr>
<tr>
<td>6 h after surgery</td>
<td>182 ± 10^3 ± 36</td>
<td>205 ± 10^3 ± 63</td>
<td>NS</td>
</tr>
<tr>
<td>12 h after surgery</td>
<td>172 ± 10^3 ± 43</td>
<td>186 ± 10^3 ± 54</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperation</td>
<td>40 ± 3.0</td>
<td>40 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>6 h after surgery</td>
<td>32 ± 4.0</td>
<td>32 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>12 h after surgery</td>
<td>29 ± 2.0</td>
<td>30 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>PT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperation</td>
<td>13 ± 0.8</td>
<td>13 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>6 h after surgery</td>
<td>15 ± 2.0</td>
<td>16 ± 5.0</td>
<td>NS</td>
</tr>
<tr>
<td>12 h after surgery</td>
<td>14 ± 1.0</td>
<td>14 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>PTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperation</td>
<td>29 ± 2.0</td>
<td>28 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>6 h after surgery</td>
<td>46 ± 25</td>
<td>41 ± 25</td>
<td>NS</td>
</tr>
<tr>
<td>12 h after surgery</td>
<td>44 ± 34</td>
<td>54 ± 26</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation. NS: Not significant.
Figure 1. Comparison of platelet response to four different agonists, ADP (adenosine 5I-diphosphate), AA (arachidonic acid), collagen, and ristocetin, preoperation and postoperation (heparin neutralization) with doses of 5 and 15 mg/kg methylprednisolone. A) Platelet response to ADP. B) Platelet response to AA. C) Platelet response to collagen. D) Platelet response to ristocetin. E) Ristocetin cofactor activity. There was a significant statistical difference between pre- and postoperative responses in both groups and in all parts (P < 0.05). Results are shown as mean ± standard deviation.
Figure 2. Comparison of expression of platelet receptors (CD42b, CD62P, and CD41a) preoperation and postoperation (heparin neutralization) with doses of 5 and 15 mg/kg methylprednisolone. A) Platelets of CD42b+. B) Platelets of CD62P+. C) Platelets of CD41a+. D) Platelets of CD41a+·CD62P+. E) Platelets of CD42b+·CD62P+. There was a significant statistical difference between pre- and postoperative expression in CD62P+, CD41a+·CD62P+, and CD42b+·CD62P+ with a dose of 15 mg/kg and CD41a+ in both groups (P < 0.05). Results are shown as mean ± standard deviation.
The D-dimer concentration increased significantly (P < 0.05) in both dosage groups after heparin neutralization. The tPA levels also increased significantly (P < 0.05) after heparin neutralization in both groups, while PAI-1 as a tPA inhibitor showed no significant changes (P > 0.05). Fibrin can induce tPA synthesis by endothelial cells, which can convert plasminogen to plasmin (26). Thrombolytic agents such as tPA can affect platelet function by degrading the platelet surface receptors such as vWF and fibrinogen receptors (27). Increasing of tPA antigen is likely because of surgery and release from endothelial cells (28). Results related to the fibrinolysis system have been controversial. Different studies have shown different results in different settings (11,27). However, analysis of other fibrinolysis factors such as α₂ antiplasmin complex tPA-PAI-1 that could play a role in reducing bleeding is recommended.

While some studies have argued that CPB would affect expression of platelet surface receptors such as GP Ib (CD42b), GP IIb-IIIa (CD41a-CD61), or GMP140 (CD62-P) (8,25), others have postulated that extrinsic factors such as the oxygenator, hypothermia, hemodilution, and heparin are involved in platelet dysfunction (29–31). In our study, vWF receptors (CD42b) showed no changes (P > 0.05) after heparin neutralization in both groups of methylprednisolone. In contrast, fibrinogen receptor (CD41a) increased significantly (P < 0.05) in both groups following heparin neutralization. In our study, interesting data were shown for the expression of CD62P.

Figure 3. Comparison of A) tPA (tissue plasminogen activator) antigen, B) D-dimer concentration, and C) PAI-1 (plasminogen activator inhibitor) antigen preoperation and postoperation (heparin neutralization) with doses of 5 and 15 mg/kg methylprednisolone. There was a significant statistical difference between pre- and postoperative values in both groups (P < 0.05). Results are shown as mean ± standard deviation.
CD62-P or GMP140 as α-granules that are intracellular and expressed during platelet activation showed significant increase with methylprednisolone at a dose of 15 mg/kg (P < 0.05). An increase was also observed at a dose of 5 mg/kg, although it was not statistically significant (P > 0.05).

It has been reported that treatment with steroids can reduce postoperative bleeding (20). According to our data, we postulate that at a high dose (15 mg/kg) methylprednisolone preserves vWF as an adhesion factor to endothelial cells and helps start the coagulation cascade. The significant increase of CD62P as a platelet activation marker at a dose of 15 mg/kg methylprednisolone following increasing CD41a, as a fibrinogen receptor that contributes in aggregation of platelets, shows that platelets can activate and aggregate better at a high dose of methylprednisolone. It seems that platelets at the 15 mg/kg dose might play a role in less blood loss associated with blood product requirements, but this was not statistically significant (P > 0.05). A possible explanation might be the relative low threshold of transfusion in our center. There was no significant difference observed between staying in the ICU and regular hospital stays.

Various studies have shown different results in the expression of the platelet receptors (CD41a, CD42b, and CD62P) with relation to different clinical settings during CPB (8,25,31–33). Literature reviews report that various doses of methylprednisolone (1–30 mg/kg) have been used during CPB for the past 30 years. However, an optimal dose for obtaining the desirable effects and avoiding potential side effects has not been identified yet (17). Based on our observations, however, a single dose of methylprednisolone, 15 mg/kg, seems to be sufficient for both reduction of bleeding and control of hyperglycemia and infection.

Our study has not considered the effect of methylprednisolone on other coagulation factors and neither fibrinolytic or kallikrein cascades nor platelet function. Larger randomized studies are necessary to shed light on other factors involved in the bleeding and blood product requirements.

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Figure 4. Comparison of blood loss with doses of 5 and 15 mg/kg methylprednisolone 24 h after operation with significant statistical differences (P < 0.05) (A). Need for blood products was not statistically different between groups (P > 0.05) (B).
References


