A Point-of-Care Assessment of the Effects of Desmopressin on Impaired Platelet Function Using Multiple Electrode Whole-Blood Aggregometry in Patients After Cardiac Surgery

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BACKGROUND: Blood loss after cardiac surgery can be caused by acquired platelet dysfunction after cardiopulmonary bypass. Monitoring of platelet function is clinically important for the identification of patients experiencing such platelet dysfunction. 1-Deamino-8-D-arginine vasopressin (desmopressin acetate, DDAVP) has been shown to augment platelet function and to reduce blood loss in patients with platelet dysfunction. In this study, we examined the feasibility of whole blood multiple electrode aggregometry (MEA) for the detection of cardiopulmonary bypass–induced platelet dysfunction and investigated its ability to monitor DDAVP treatment.

METHODS: Fifty-eight consecutive patients with blood loss exceeding 150 mL/h in the first 2 consecutive hours after cardiac surgery were screened for suspected isolated platelet dysfunction. Twenty-two patients had suspected isolated platelet dysfunction and were enrolled in the study. Platelet dysfunction was assumed if conventional coagulation analyses (platelet count, activated partial thromboplastin time, international normalized ratio, and fibrinogen) did not show abnormal values as defined for transfusion of allogenic blood products, and no surgical cause of bleeding was suspected. Eleven patients received 0.3 μg/kg DDAVP, and 11 patients received no therapy in a nonrandomized manner. MEA was performed after stimulation with thrombin receptor–activating peptide (TRAPtest, 32 μM), adenosine diphosphate (ADPtest, 6.4 μM), and arachidonic acid (ASPItest, 0.5 mM) before and 2 hours after intervention. Conventional laboratory variables were recorded. The Mann-Whitney test was used to detect differences between the groups, and the Wilcoxon test was used to detect differences before and after intervention.

RESULTS: All enrolled patients showed platelet dysfunction that manifested as impaired platelet aggregation in MEA before intervention. After the intervention, platelet function improved in the DDAVP group (49 U [30/72 U], median [25th/75th percentile] postintervention vs 15 U [8/21 U] preintervention for the ADPtest [P < 0.001]; 35 U [24/54 U] vs 14 U [7/28 U] for the ADPtest [P = 0.002]; and 85 U [66/115 U] vs 64 U [26/88 U] for the TRAPtest [P = 0.007]). In contrast, MEA remained unchanged in the control group (22 U [10/50 U] postintervention vs 33 U [14/57 U] preintervention for the Animaltest [P = 0.175]; 17 U [12/20 U] vs 14 U [10/28 U] for the ADPtest [P = 0.147]; and 65 U [41/89 U] vs 57 U [30/91 U] for the TRAPtest [P = 0.123]).

CONCLUSIONS: Impaired platelet function after cardiac surgery can be assessed at the bedside using MEA. The effect of DDAVP on impaired platelet function can also be detected as significant improvement in platelet aggregation to all activators. This device might be helpful for the identification of patients who may benefit from DDAVP therapy. (Anesth Analg 2010;110:702–7)

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quired platelet dysfunction caused by mechanical trauma and cardiopulmonary bypass (CPB)-induced platelet activation contributes to coagulopathy after cardiac surgery. Hypothermia and heparin treatment during cardiac surgery may also cause impaired platelet function. Administration of the vasopressin analog 1-deamino-8-D-arginine vasopressin (desmopressin acetate, DDAVP) is a potential therapeutic option for improving platelet function after cardiac surgery.2 DDAVP treatment can lead to a 3-fold increase in plasma concentrations of von Willebrand factor and factor VIII3 and hence to enhanced platelet-platelet and platelet-subendothelium interactions5 via the glycoprotein (Gp)IIb/IIIa platelet receptors and the GpIb platelet receptors, respectively. Randomized administration of DDAVP to patients with diagnosed CPB-related platelet dysfunction after cardiac surgery decreased postoperative blood loss and transfusion requirements.6 However, the effectiveness of DDAVP treatment in reducing hemorrhage in unselected cardiac surgery patients without confirmed platelet function impairment after CPB could not be demonstrated7; thus, this treatment is not recommended for routine use after cardiac surgery by other studies.8 Nonetheless, at our institution, DDAVP infusion is an accepted therapeutic option that is sometimes used in patients with excessive postoperative bleeding after CPB.

In this context, a simple and quick method for the detection of clinically relevant CPB-induced platelet dysfunction is of particular interest. Such a method would...
allow reliable point-of-care identification of patients who could potentially benefit from DDAVP treatment. We therefore selected multiple electrode aggregometry (MEA) to determine platelet function after cardiac surgery. MEA is a newly developed technique for platelet function testing in whole blood and has been shown to be applicable at the point-of-care. MEA is a modification of classic whole blood impedance aggregometry, which has been shown to be appropriate for the assessment of CPB-induced platelet dysfunction. MEA is increasingly being used for the assessment of platelet function in various fields, including cardiac surgery.

We hypothesized that (1) clinically relevant CPB-induced platelet dysfunction could be detected by MEA, and (2) the effect of DDAVP on platelet dysfunction could be monitored with this device in patients after CPB.

METHODS

Study Population and Intervention

After IRB approval, adult patients after elective cardiac surgery with CPB who had discontinued antiplatelet therapy 5 or more days before the operation were consecutively selected between May and October 2008. The local ethics committee waived the requirement for written informed consent for 2 reasons: (1) no randomized intervention (except common treatment) was performed, and (2) MEA analysis is part of the routine bedside coagulation monitoring at our institution.

Cardiac surgery patients with CPB were screened for study eligibility on admission to the intensive care unit (ICU). Eligibility criteria were as follows: (1) postoperative blood loss through chest tubes of >150 mL/h in the first 2 hours after admission to the ICU, and (2) lack of surgical reintervention based on the decision of the surgeon. Eligible patients were enrolled in the study if CPB-related platelet dysfunction was suspected as the cause of the increased postoperative bleeding. CPB-related platelet dysfunction was assumed if the results of postoperatively performed routine coagulation testing were abnormal as defined by hematocrit <25%, international normalized ratio ≥1.7, activated partial thromboplastin time ≥50 seconds, platelet count <80/mL, or fibrinogen <150 mg/dL, requiring transfusion of allogenic blood products in bleeding patients. All patients were tracheally intubated, and their lungs were ventilated. Sedation was performed with a continuous infusion of 1 to 3 mg·kg⁻¹·h⁻¹ propofol.

Blood Sampling and Intervention

Two hours after admission to the ICU (T1), blood was drawn from a central venous line placed before the operation. The first 10 mL of blood was discarded. Blood was then collected for MEA analysis into 4.5-mL tubes containing 25 μg/mL hirudin as an anticoagulant (Dynabyte, Munich, Germany) following the recommendations of the manufacturer. After drawing blood at T1, 0.3 μg/kg DDAVP (CSL Behring, Marburg, Germany) was administered IV (DDAVP group) over a 30-minute period, or no intervention was performed (control group) in a nonrandomized setting. The group assignment of the patients was not blinded. Instead, it was based on the judgment of the attending anesthesiologist, with no input from any person involved in the study. Two hours after intervention or at a similar time in the control group (T2), blood was drawn again for MEA analysis and a routine coagulation profile (international normalized ratio, activated partial thromboplastin time, platelet count, and fibrinogen) was performed.

Platelet Function Assays

Platelet function analysis was performed using the Multiplate analyzer, a novel whole blood impedance aggregometer (Dynabyte) with 5 channels for parallel MEA analyses. The method has been described in detail elsewhere. Briefly, 300 μL preheated saline (37°C) and 300 μL hirudin-anticoagulated whole blood were placed into the test cell. Platelet aggregation was initiated using arachidonic acid (ASPItest, 0.5 mM), adenosine diphosphate (ADPtest, 6.4 μM), or thrombin receptor-activating peptide (TRAP-6, TRAPtest, 32 μM) using commercially available test reagents (Dynabyte, Munich, Germany). Increased impedance caused by attachment of platelets to the test cell electrodes was continuously measured over 6 minutes. Platelet aggregation was quantified as the area under the aggregation curve (AUC [U]). Reference ranges for healthy subjects obtained from the manufacturer were 75 to 136 U for the ASPItest, 53 to 122 U for the ADPtest, and 94 to 156 U for the TRAPtest.

Statistical Analysis

Statistical analysis was performed using SigmaStat software (Version 3.1, Jandel, San Rafael, CA). Because “reference ranges” for MEA analysis in patients without excessive bleeding after CPB have not been determined until now, the sample size calculation was based on the analysis of our previous pilot experience collected in 15 nonbleeding subjects after CPB. Sample size analysis (expected difference of means for ASPItest 30 U, expected std 20 U, desired power 0.8, and P < 0.05) revealed a required sample size of at least n = 9 to detect statistically significant differences in platelet aggregation before and after treatment. The Mann-Whitney rank sum test was used to detect differences between groups. The Wilcoxon signed rank test was used to detect differences before and after intervention. We used the Fisher exact test to compare the number of patients in the 2 groups who ceased taking clopidogrel for 5 or more days before operation.

RESULTS

The enrollment of patients during the 6-month study period is shown in Figure 1. Fifty-eight patients without prior antiplatelet therapy fulfilled the eligibility criteria with increased bleeding and no suspected surgical bleeding site. Twenty-two patients (38%) with suggested isolated platelet dysfunction were prospectively included in the study. Eleven patients received an infusion of 0.3 μg/kg DDAVP (DDAVP group), whereas 11 patients did not (control group). The baseline characteristics of the 2 groups and analyses of conventional coagulation laboratory, calcium, pH, and temperature at study inclusion (T1) were not significantly different between the groups (Tables 1 and 2).
Platelet aggregation results obtained by MEA at inclusion (T1) were less than the lower reference range for all assays in both groups, indicating platelet dysfunction in patients of both groups. There were no significant differences between the groups (Fig. 2, Table 3).

Platelet aggregation after intervention (T2) in the DDAVP group was significantly improved in all assays when compared with the measurement at the beginning of the study (T1). In contrast, platelet aggregation at T2 and T1 were not different in the control group (Fig. 2, Table 3). Platelet concentrates were not transfused in the DDAVP group or in the control group.

The number of patients who ceased taking clopidogrel for 5 or more days before operation was very low and not different in the 2 groups (1 of 11 in the control group and 2 of 11 in the DDAVP group, \( P_{\text{H11005}} 1.0 \)).

**DISCUSSION**

The main finding of this study was that we were able to reliably detect improved platelet function by MEA after DDAVP administration in the study population. Platelet aggregation in response to all investigated agonists was significantly improved in the DDAVP group (Fig. 2). In contrast, aggregation remained unchanged for all assays performed on the control group. Furthermore, clinically relevant acquired platelet dysfunction after CPB could be detected by MEA at the bedside. This dysfunction was detected as impaired platelet aggregation in all studied assays.

Despotis et al.\(^6\) previously used hemoSTATUS analyses to select patients with platelet dysfunction after CPB. One hundred one patients with CPB-related platelet dysfunction after cardiac surgery were identified using this device, which assessed platelet function by analyzing the effect of platelet-activating factors on kaolin-activated clotting time.\(^{17}\) However, this point-of-care assay has not been shown to be superior to routine coagulation tests for predicting blood loss after cardiac surgery,\(^{18,19}\) and it is no longer clinically available.
To evaluate MEA in a comparable context, we selected bleeding patients with suspected isolated CPB-induced platelet dysfunction after cardiac surgery, because patients with antiplatelet therapy before surgery and those with severe plasmatic coagulation disorders were excluded. In addition, in these patients, DDAVP was expected to have the strongest effect on platelet aggregation. The selection of patients with clinically relevant suspected platelet dysfunction was based on clinical variables (blood loss >150 mL/h for at least 2 consecutive hours after admission and no need for surgical reintervention) and routine laboratory analyses (values below/above institutional cutoffs for transfusion of blood products in bleeding patients) to exclude patients with severe plasmatic coagulation abnormalities. This selection method was effective. In both the control and DDAVP groups, platelet aggregation at the start of the study (T1) was far less than the lower boundary of the reference range for healthy subjects (75 U for ASPItest, 53 U for ADPtest, and 94 U for TRAPtest) (Fig. 2).

We detected the largest increase in platelet aggregation when using the ASPItest; there was a 227% improvement after intervention compared with the preintervention values. Smaller increases were detected for the ADPtest (150%) and TRAPtest (33%). Even if MEA changes only improved to the lower limit of normal values in the DDAVP group, this finding is in agreement with a very recent study that determined CPB-induced changes in platelet function using MEA.20 Platelet aggregation in MEA in patients without prior antiplatelet therapy who showed no excessive bleeding after cardiac surgery was considerably less than the normal reference values.20 This might reflect a clinically nonrelevant CPB-induced platelet function impairment. However, reference ranges for MEA analysis obtained by patients who do not show excessive diffuse bleeding after CPB have not been determined until now. Therefore, a simple comparison of normal reference values (determined on healthy subjects having no CPB-induced platelet dysfunction) to MEA values obtained by patients after CPB might be misleading.

The duration of DDAVP effect has been shown to be between 4 and 6 hours.21 Therefore, it can be assumed that the measuring point T2, which was 2 hours after DDAVP infusion, adequately detected the influence of DDAVP on platelet aggregation. The general improvement in platelet function was presumably attributable to DDAVP-induced release of factor VIII and von Willebrand factor3 and the expression of platelet Gp receptor Ib.22 However, there might also be other mechanisms that could explain the

Figure 2. Platelet aggregation assessed by multiple electrode aggregometry (area under the aggregation curve [AUC] [U]) using the arachidonic acid–induced platelet aggregation test, adenosine diphosphate test, and thrombin receptor–activating peptide test in the control and 1-deamino-8-D-arginine vasopressin groups at measuring points T1 and T2. Horizontal lines show the lower reference ranges of normal values. *P < 0.05 between T1 and T2 using Wilcoxon signed rank test (n = 11 for both groups).

Table 3. Platelet Aggregation in Multiple Electrode Aggregometry (MEA) (AUC, [U]) at Study Inclusion (T1) and 2 h After Intervention in the 1-Deamino-8-D-Arginine Vasopressin (DDAVP) Group or at a Similar Time in the Control Group (T2)

<table>
<thead>
<tr>
<th>Measuring point</th>
<th>Control</th>
<th>DDAVP</th>
<th>P</th>
<th>Control</th>
<th>DDAVP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPItest</td>
<td>33 (14/57)</td>
<td>15 (8/21)</td>
<td>0.07</td>
<td>22 (10/50)</td>
<td>49 (30/72)</td>
<td>0.03</td>
</tr>
<tr>
<td>ADPtest</td>
<td>14 (10/28)</td>
<td>14 (7/28)</td>
<td>1.00</td>
<td>17 (12/20)</td>
<td>35 (24/54)</td>
<td>0.01</td>
</tr>
<tr>
<td>TRAPtest</td>
<td>57 (30/91)</td>
<td>64 (26/88)</td>
<td>0.79</td>
<td>65 (41/89)</td>
<td>83 (66/115)</td>
<td>0.29</td>
</tr>
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</table>

For comparison the Mann-Whitney rank sum test was used. Data are depicted as the median (25th/75th percentile). ASPI = arachidonic acid; ADP = adenosine diphosphate; TRAP = thrombin receptor activating peptide.
distinctive increase in arachidonic acid–induced platelet aggregation (ASPTest). There is most likely an additional direct effect of DDAVP on platelets, because DDAVP treatment leads to increased platelet serotonin concentration. Serotonin leads to increased thromboxane generation and may enhance cyclooxygenase-mediated platelet aggregation in the ASPTest. However, investigation of the direct effect of DDAVP on platelets was not an objective of our study.

Several methods are used for the detection of CPB-induced platelet dysfunction. Whole blood impedance aggregometry (Chrono-log, Havertown, PA) has been shown to be the most appropriate. However, the time-consuming cleaning of the electrodes and the unacceptably large variation in assay results caused by electrode contamination prevent quick and simple assessment of platelet function at the bedside. The development of single-use test cells and half-automatic pipetting in MEA eliminates these drawbacks. Therefore, MEA might be an applicable method for the perioperative assessment of platelet function. The reliability of this test in low platelet counts remains questionable because no data are available.

There were some limitations in this study. First, the study population was small. However, the expected differences in the means and sds used for sample size analysis were based on our previous pilot experience studying the effect of DDAVP on platelet aggregation using MEA. Despite the small sample size, the study results were highly significant and consistent; therefore, conclusions can still be drawn.

Second, the study was designed as a nonrandomized, prospective, controlled trial. Administration of DDAVP was based on the judgment of the physician in the ward. Because of the controversial data on administering DDAVP in cases of CPB-induced platelet function impairment, no standardized treatment protocol for the use of DDAVP has been established in our institution. These physicians’ biases toward DDAVP are based, therefore, not on the patient, but rather on the physicians’ own judgments, which were not involved in the study. Despite these limitations, this study has clinical implications. MEA detects DDAVP-associated improvement in platelet function. Furthermore, this easy-to-use platelet function assay could be helpful as a point-of-care test to identify patients with clinically relevant platelet dysfunction after CPB. In addition, it could be helpful for the identification of patients who may benefit from DDAVP administration.

DISCLOSURE
Christian F. Weber, Michael Spannagl, and Csilla Jámbor have received speaking honoraria and/or research support from CSL Behring AG, Hattersheim, Germany, and Dynabyte, Munich, Germany.

REFERENCES


