Platelet Function during Cardiopulmonary Bypass using Multiple Electrode Aggregometry: Comparison of Centrifugal and Roller Pumps

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Abstract

Blood trauma may be lower with centrifugal pumps (CP) than with roller pumps (RP) during cardiopulmonary bypass (CPB), because, unlike RPs, CPs do not compress the tubing and shear stress is considered lower in CP than in RP. However, relative platelet function remains unclear. Using multiple electrode aggregometry (MEA), we compared platelet function with CP and RP. Ten swine underwent CPB for 3 h, with five each weaned off using CP and RP. Platelet function was measured using MEA, as were hemoglobin concentration and platelet count, before sternotomy, after heparin infusion, 30 min and 3 h after starting CPB, after protamine infusion, and 60 min after stopping CPB. Platelet activation was initiated with adenosine diphosphate (ADP), arachidonic acid (AA) and thrombin receptor activating protein 6 (TRAP). Fibrinogen, platelet factor 4 (PF4), and beta-thromboglobin (beta-TG) concentrations were measured before sternotomy and 60 min after stopping CPB. In the CP group and using ADP, aggregation was significantly reduced 30 min ($p=0.019$) and 3 h ($p=0.027$) after starting CPB, recovering to baseline 60 min after stopping CPB. In the RP group, aggregation was significantly decreased 30 min ($p=0.007$) and 3 h ($p=0.003$) after starting CPB and after protamine administration ($p=0.028$). With AA, aggregation significantly decreased 30 min after starting CPB in both the CP ($p=0.012$) and RP ($p=0.016$) groups, slightly

increasing 3 h after starting CPB and after protamine infusion and recovering to baseline 60 min after CPB cessation. With TRAP, aggregation in the CP and RP groups decreased 30 min after starting the pump, although changes were not significant; aggregation gradually recovered after 3 h and returned to baseline 60 min after the pumps were stopped. There were no significant differences at all sampling points of MEA. In both groups, fibrinogen, PF4 and beta-TG concentrations were similar 60 min after pump cessation and before sternotomy. Platelet function, evaluated with MEA, was lowest 30 min after CPB was started, but did not decrease over time in either group. Using MEA, platelet function using CP and RP did not differ significantly. Platelet dysfunction was caused mainly by initial contact with foreign materials and may not be modified by type of pump.

Key Words: Centrifugal pump, Roller pump, Platelet function, Multiple electrode aggregometry, Contact with foreign materials

Cardiopulmonary bypass (CPB) during open heart surgery is associated with hemorrhagic defect, likely due to platelet dysfunction (1). Blood trauma may be lower with centrifugal pumps (CP) than with roller pumps (RP), since CPs work on a constrained-force principle, and, unlike RPs, do not compress the tubing. The maximum estimated shear stress has been reported higher inside RPs (994 N/m^2) than CPs (20-100 N/m^2) (2, 3). Use of CPs resulted in higher platelet counts, reduced platelet activation, lower free hemoglobin concentrations and fewer inflammatory responses than RP during CPB (4-7). Other studies, however, have found that hemolysis and blood loss using CPs and RPs did not differ (8, 9). The benefits to platelet function of CPs, relative to RPs, are unclear, although one study reported that platelet aggregation with RPs was significantly greater, which may result in a higher rate of postoperative thrombotic complications (10).

Multiple electrode aggregometry (MEA) is a whole blood impedance aggregometry method that allows platelet function to be measured at a patient's bedside. MEA is highly sensitive to drug induced platelet inhibition and to the effects of CPB (11-18). We therefore used MEA to compare platelet function in swine undergoing CPB with CP or RP.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of Shinshu University. The study included two month-old crossbred ([White-Landrace] ×Duroc) swine, of mean weights 47.4 ± 0.8 kg in the CP group (n=5) and 47.8 ± 1.3 kg in the RP group (n=5). The swine were fasted overnight and pre-medicated with 15 mg/kg ketamine and 0.5 mg atropine sulfate intramuscularly. Anesthesia was induced with 4–5% halothane and maintained with 1 mg/kg/h ketamine and 0.8–1.0 mg/kg/h vecuronium intravenously. After orotracheal intubation, ventilation was maintained with a volume control ventilator (ADV1000, Mera, Tokyo, Japan) at 10 ml/kg per min for 12 breaths per minute. Electrocardiography and heart rate were monitored throughout (Life Scope12, Nihon Kohden, Tokyo, Japan). The animals were maintained on 10 ml/kg/h lactated Ringer's via the ear vein. The internal carotid artery and the jugular vein were exposed. A 16 G single lumen catheter (Anthron, Toray, Tokyo, Japan) and seven French triple lumen catheters (CS17703-E, Arrow International, Inc, Reading, PA, USA) were inserted into the ascending aorta and superior vena cava for blood sampling and for continuous monitoring of arterial blood and central venous pressure (Life Scope12, Nihon Kohden). Median sternotomy was performed. Each animal was injected with 300 units/kg of heparin, and activated clotting time was maintained at over 500 sec

(Hemochron 400; Technidyne, Minnesota, United States) by additional heparin, if necessary. CPB was initiated by cannulation of the ascending aorta and right atrium. Each CPB circuit consisted of either polycarbonate CP (Gyro Pump, Medtronic Japan, Tokyo, Japan) or RP (Heart Assist System; HAS-150, Senko Medical Cooperation, Tokyo, Japan) and a membrane oxygenator (Affinity, Medtronic Japan, Tokyo, Japan) with 3/8-inch polyvinyl chloride (PVC) tubing. The tubing, pumps and oxygenator were not coated with heparin. The priming volume of the circuit was 500 ml, and the circuit was filled with lactated Ringer's solution. To avoid air-blood contact, no blood reservoir, suction or vent was used. Pump flow was maintained at 2 L/min, and the oxygenator was connected to a heat exchanger to maintain rectal temperature between 37 and 38°C, which is normal for swine. After 3 h, CPB was discontinued, all the cannulas were withdrawn, and heparin was neutralized with 3 mg/kg protamine sulfate.

MEA and laboratory analysis

MEA, hemoglobin concentration and platelet count were measured before sternotomy, after heparin infusion, 30 min and 3 h after starting CPB, after protamine infusion, and 60 min after stopping CPB. Fibrinogen, platelet factor 4 (PF4), and beta-thromboglobin (beta-TG) concentrations were measured before sternotomy and 60 min after stopping

CPB using porcine ELISA kits (Cusabio Biotech, Hubei, China). MEA was performed using Multiplate® (Dynabyte GmbH, Munich, Germany). Blood samples were drawn into PICO samplers (Radiometer, Copenhagen, Denmark) containing heparin; 300 µl of whole blood were mixed with 300 µl of pre-warmed isotonic saline in a test cell and stirred for 3 min at 37 °C. Platelet activation was initiated with three different agonists, adenosine diphosphate (ADP; final concentration 6.5 µM; Instrumentation Laboratory, Munich, Germany), arachidonic acid (AA; final concentration 0.5 mM; Instrumentation Laboratory) and thrombin receptor activating protein 6 (TRAP; final concentration 32 µM; Instrumentation Laboratory). Changes in electrical impedance were recorded for 6 min after activation. The area under the aggregation curve indicates overall platelet activity, expressed as aggregation units (U).

Statistical analysis

All statistical analyses were performed using SPSS PASW Statistics 18 (SPSS, Chicago, IL, USA). Data were expressed as mean ± standard deviation. Statistical comparisons between CP and RP were assessed by Student's t-tests and Mann-Whitney test and by Student's t-tests and repeated ANOVA between time points. Correlation coefficients were used to examine relationships between data. A probability less than

0.05 was regarded as statistically significant.

RESULTS

MEA

Mean ± SD baseline aggregation responses to ADP were 73.8 ± 14.0 U in the CP group and 73.4 ± 16.5 U in the RP, with neither changing after heparin infusion (p=0.567 for CP, p=1.000 for RP). In the CP group, aggregation was significantly reduced, to 55.4 ± 9.6 U (p=0.019) 30 min after CPB was started, was maintained for 3 h after starting CPB, and recovered to 64.3 ± 13.1 U 60 min after CPB was stopped. In the RP group, aggregation was significantly decreased, to 53.8 ± 17.8 U (p=0.007) 30 min after CPB started, to 62.8 ± 14.9 U (p=0.003) after 3 h of CPB and to 59.8 ± 22.8 U (p=0.028) after protamine administration, increasing to 68.5 ± 13.5 U 60 min after the pump was stopped. No significant difference between the pumps was observed at any sampling point (Fig 1).

Using AA, mean aggregation at baseline was 77.4 ± 16.7 U in the CP group and 73.4 ± 13.7 U in the RP group. In the CP group, aggregation dropped significantly, to 53.6 ± 10.8 U (p=0.012), 30 min after CPB started, increasing to 65.5 ± 16.8 U 3 h after starting CPB, to 62.3 ± 16.6 U after protamine infusion and to 76.5 ± 24.4 U 60 min

after CPB cessation, with the latter not differing significantly from baseline. In the RP group, aggregation decreased significantly from baseline, to 56.8 ± 20.9 U (p=0.016), 30 min after starting CPB, and then increased to 63.8 ± 20.0 U 3 h after starting CPB and to 62.3 ± 25.1 U after protamine infusion, with neither differing significantly from baseline. There were no significant differences between the two pumps (Fig 2).

Using TRAP, baseline aggregation in the CP and RP groups was 6.4 ± 7.6 U and 2.6 ± 1.9 U, respectively, decreasing to 1.6 ± 3.0 U and 1.6 ± 2.6 U, respectively, 30 min after starting the pump, although neither differed significantly from baseline. Aggregation began to recover in both groups after 3 h and reached baseline 60 min after both pumps were stopped. There were no significant differences between CP and RP at all the sampling points (Fig 3).

Laboratory analysis

Fibrinogen, PF4 and beta-TG concentrations were lower than baseline 60 min after pump cessation in both groups, although neither change was significant. In addition, there were no significant differences between the CP and RP groups (Fig 4).

At baseline, mean hemoglobin concentration was 12.6 ± 0.7 g/dl in the CP group and 11.2 ± 0.8 g/dl in the RP group, decreasing in both groups after 3 h pumping, after

protamine infusion and 60 min after pump cessation. The baseline platelet counts were $29.6 \pm 5.9 \times 10^4/\mu l$ in the CP group and $27.9 \pm 7.3 \times 10^4/\mu l$ in the RP group. Platelet counts did not significantly change in the CP group, but decreased significantly after heparin infusion in the RP group.

Aggregation and hemoglobin concentration

The correlation between aggregation and hemoglobin concentration is shown in Fig 5. Aggregation initiated with ADP ($r=0.84$; $p=0.532$), AA ($r=0.101$; $p=0.451$) and TRAP ($r=0.247$; $p=0.062$) did not correlate significantly with hemoglobin concentration.

DISCUSSION

We utilized ADP, AA and TRAP as platelet agonists. ADP binds to platelet ADP receptors and is blocked by clopidogrel. AA is converted to thromboxane A2 by platelet cyclooxygenase and is blocked by aspirin. TRAP is a potent agonist that mimics the platelet-activating action of thrombin. ADP and AA induce platelet activation via protease-independent receptors (19), whereas thrombin activates protease-activated receptors (20). Using both ADP and AA, we found that platelet aggregation was significantly lower than baseline 30 min after starting CPB in both the CP and RP

groups, was maintained at this level until 3 h after starting CPB and subsequently returned to baseline. A similar pattern was observed for TRAP, except that the decrease from baseline 30 min after starting CPB was not significant. These results suggest that a protease-independent receptor plays a major role in decreased platelet aggregation during CPB. We found that, at baseline, TRAP-induced aggregation was much reduced compared with previous studies in humans (12, 14, 17, 18, 21, 22), suggesting that swine platelets may not react with TRAP.

Minimum platelet aggregation was observed 30 min after starting CPB with both pumps but did not decrease thereafter. Prolongation of CPB has been associated with platelet dysfunction (23). Following initial platelet activation, their morphology begins to recover, despite CPB continuing (24). We found that the decrease in platelet aggregation occurred immediately after contact with fluid and foreign materials, but that platelet aggregation did not decrease further over time. Hemodilution has also been reported to reduce platelet aggregation (25), and coagulation time measured by light transmission aggregometry decreased as hematocrit increased (26). In our experiment, the priming volume in the circuit was only 500 ml, and no change in hemoglobin concentration was observed 30 min after starting CPB, despite platelet aggregation decreasing maximally at this time. In addition, platelet aggregation did not

correlate with hemoglobin concentration. It is difficult to conclude that platelet dysfunction observed during CPB was caused by hemodilution. Rather, platelet dysfunction was more likely caused by the initial contact with foreign materials.

This study was conducted with CP, which is made of polycarbonate and PVC tubing. Platelet activation has been reported at platelet-polycarbonate interfaces, with transmission electron microscopy showing changes in the internal structures and adhesion interfaces of activated platelets (27). Scanning electron microscopy showed that platelets attached to PVC surfaces, and chandler loop analysis showed that PVC increased the numbers of circulating platelets positive for CD62P, a marker of platelet activation (28). Circulation of citrated whole blood through polycarbonate and PVC circuits resulted in the loss of 58% and 2%, respectively, of platelets within the first few minutes (29). However, to our knowledge, no studies have compared platelet function following contact with polycarbonate and PVC. Further studies are needed to assess the effect on platelet function of contact with foreign materials during CPB.

Aggregometry has shown that administration of heparin before starting CPB significantly reduced responses to collagen (30). Moreover, aggregation in response to AA was found to decrease significantly after heparin administration without CPB (31). Others have reported, however, that heparinization had no effect on ADP- or

collagen-induced aggregation (32). Differences between these studies may have been due to differences in aggregometers. The first two studies (30, 31) used Chrono-Log®, an impedance aggregometer with re-usable electrodes (21). Although these electrodes should have been cleaned between analyses, cleaning was impractical and a possible source of measurement error. Our finding, of no reduction in aggregation after infusion of 300 units/kg heparin, supports results showing that heparinization had no effect on platelet aggregation (32). We used disposable test cells in a Multiplate®, thus eliminating a source of measurement errors, finding that heparin did not alter platelet aggregation initiated with ADP, AA and TRAP during CPB.

Using MEA, we observed no significant differences in platelet function between CPs and RPs. Although studies have assessed the effects of these two types of pump on platelet count and platelet activation, their conclusions were conflicting (4-10). For example, one study reported that CP was superior in preventing blood cell damage (4), whereas others reported that CPs had no advantages over RPs in platelet count, bleeding and transfusion requirement (8, 9). These studies were performed on patients undergoing coronary artery bypass grafting, with the circuits including cardiotomy reservoirs and suction. In this study, however, the circuit consisted only of a pump, oxygenator and tubing, with no blood reservoir, suction or vent. Thus, the only variable

that could have affected platelet function was pump type. We found, however, that aggregation responses to ADP, AA and TRAP did not differ by pump type. Heparin coating may have a greater influence on platelet activation than pump type (7), and shorter pump runs may overshadow the effect of pump head design on hemostasis (9). We therefore utilized non heparin coated circuits and ran CPB for as long as 3 h. The lack of clear clinical benefits has reduced the use of the more expensive CPs; in Europe, for example, only 10% of CBPs are performed using CPs (33), which is a trend supported by our results.

PF4 is a cytokine and beta-TG a platelet-specific protein released from alpha-granules of activated platelets. Studies have reported that PF4 and beta-TG concentrations were significantly higher at the end of than before CPB (4, 6, 24, 34). These studies were performed on patients undergoing open heart surgery, with CPBs including suction and blood reservoirs. In contrast, we found that PF4 and beta-TG concentrations did not change significantly from baseline after CPB in both pumps. Our circuits, which consisted of a pump, an oxygenator and tubing, did not include a blood reservoir or suction. These findings therefore indicate that PF4 and beta-TG may be more affected by a reservoir and suction than by a pump and circuit.

Platelet function is multifactorial in clinical situations, and cannot be determined only

by coagulation tests during surgery. Animal models are essential for in vivo evaluation of coagulation, and swine are increasingly used in cardiovascular and platelet research. However, data from swine cannot be directly transposed to human beings. Flow cytometry showed that antibodies to platelet receptors bound to human, but not to swine, platelets (35). These species specific differences indicate that care should be taken in adapting our results to clinical situations.

CONCLUSION

Platelet aggregation was lowest 30 min after starting CPB with both pumps, but did not decrease thereafter. There were no significant differences between CPs and RPs in platelet function, as measured by MEA. Platelet dysfunction may be caused primarily by initial contact with foreign materials and is likely not modified by use of CPs.

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Legends

Fig 1: Changes in platelet aggregation in response to ADP in the CP and RP groups. In the CP group, aggregation was significantly reduced from baseline 30 min and 3 h after starting CPB, recovering 60 min after CPB was stopped. In the RP group, aggregation was significantly lower than baseline 30 min and 3 h after CPB was started and after protamine administration. PRE: before sternotomy, HEP: after heparin infusion, 30MIN: 30 min after starting CPB, 3H: 3 h after starting CPB, PRO: after protamine infusion, POST: 60 min after CPB stopped. #: significant difference from before sternotomy.

Fig 2: Changes in platelet aggregation in response to ASPI in the CP and RP groups. In both groups, aggregation was significantly lower 30 min after starting the pump than at baseline, but recovered thereafter. PRE: before sternotomy, HEP: after heparin infusion, 30MIN: 30 min after starting CPB, 3H: 3 h after starting CPB, PRO: after protamine infusion, POST: 60 min after CPB stopped. #: significant difference from before sternotomy.

Fig 3: Changes in platelet aggregation in response to TRAP in the CP and RP groups. In both groups, aggregation was reduced from baseline 30 min after the pump was started, although these changes were not significant. PRE: before sternotomy, HEP: after heparin infusion, 30MIN: 30 min after starting CPB, 3H: 3 h after starting CPB, PRO: after protamine infusion, POST: 60 min after CPB stopped.

Fig 4: Changes in fibrinogen, PF4 and beta-TG concentrations during CPB. All were lower than baseline 60 min after both the CP and RP were stopped, although these changes were not significant, and the differences between these two groups were not significant. PRE: before sternotomy, POST: 60 min after CPB stopped.

Fig 5: Correlation between aggregation and hemoglobin concentration. Aggregation initiated with ADP (Panel A), AA (Panel B) and TRAP (panel C) did not correlate significantly with hemoglobin concentrations.