A prospective randomized trial comparing the clinical effectiveness and biocompatibility of heparin coated circuits and PMEA-coated circuits in pediatric cardiopulmonary bypass

ABSTRACT
We compared the clinical effectiveness and biocompatibility of poly-2-methoxyethyl acrylate (PMEA)-coated and heparin-coated cardiopulmonary bypass (CPB) circuits in a prospective pediatric trial.

Infants randomly received heparin-coated (n=7) or PMEA-coated (n=7) circuits in elective pediatric cardiac surgery with CPB for ventricular septum defects. Clinical and hematologic variables, respiratory indices, and hemodynamic changes were analyzed perioperatively. Demographic and clinical variables were similar in both groups. Leukocyte counts were significantly lower 5 minutes after CPB in the PMEA group than the heparine group. Hemodynamic data showed that PMEA caused hypotension within 5 minutes of CPB. The respiratory index was significantly higher immediately after CPB and 1 hour after transfer to the intensive care unit (ICU) in the PMEA group, as were levels of C-reactive protein 24 hours after transfer to the ICU.

Our study shows that PMEA-coated circuits, unlike heparin-coated circuits, cause transient leukopenia during pediatric CPB and perhaps systemic inflammatory respiratory syndrome after pediatric CPB. (153 words)
Introduction
Cardiopulmonary bypass (CPB) induces inflammatory responses and increases postoperative morbidity. Contact between the blood and the artificial surface of the CPB circuit produces post-perfusion syndrome, which in severe cases causes systemic inflammatory response syndrome (SIRS), acute respiratory district syndrome and other acute lung injuries, sepsis, and even multiple organ failure.\textsuperscript{1-5} To improve the biocompatibility of non-physiological surfaces and thus reduce the incidence of systemic inflammatory responses, the medical device industry has developed different coating materials for CPB circuits.\textsuperscript{2, 6-10}

Since the first description of heparin bonding on colloidal graphite surfaces in 1963,\textsuperscript{11} preparing CPB circuits with biocompatible materials has increased in importance. Heparin-coated CPB circuits can potentially decrease postoperative blood loss as well as the need to transfuse blood and blood products.\textsuperscript{6} They can also reduce complement activation and subsequent cytokine release.\textsuperscript{12, 13}

It has been reported that heparin-coated circuits reduce CPB-induced inflammation.\textsuperscript{7} Poly-2-methoxyethyl acrylate (PMEA)-coated circuits have good biocompatibility,\textsuperscript{8} and PMEA is one of the best blood-compatible polymers, as determined via various approaches.\textsuperscript{8, 12} Although it is currently being used for practical applications such as oxygenators and the tubes in CPB circuits, how it produces blood compatibility is still not fully understood.\textsuperscript{14}

Modification of the blood-contacting surfaces in CPB circuits has been shown to increase platelet number, improve platelet function, and decrease fibrinolysis, and inflammation after cardiac surgery in adults.\textsuperscript{15} However, there have been only a few studies of the influence of hematologic changes on clinical results in pediatric patients. When larger CPB circuits and higher flow rates are used, better outcomes in congenital heart surgery might be expected.\textsuperscript{3, 15-17}

We compared the biological effectiveness and biocompatibility of PMEA-coated and heparine-coated circuits in pediatric cardiac surgery with CPB.
Material and Methods

Studies on human subjects were performed according to the principles of the Declaration of Helsinki. The patients’ parents received a detailed description of the operative procedures and provided informed consent. The Okayama University Institutional Investigational Review Board approved the project.

Fourteen infants aged 3 to 5 months who required elective pediatric cardiac surgery with CPB for ventricular septal defects were enrolled in this study. The patients were randomly divided into two groups by drawing cards from sealed envelopes; the heparin group received heparin-coated circuits (n=7), and PMEA group received PMEA-coated circuits (n=7).

Surgical procedure

For CPB, we used a hollow-fiber membrane oxygenator (RX-05; Terumo, Tokyo), an open hard-shell reservoir (Baby-RX; Terumo), an arterial filter (CX-AF02; Terumo), and a roller pump for perfusion (HAS; MERA, Tokyo). All blood-containing surfaces except the cannulae in the CPB circuit were heparin-coated or PMEA-coated; both types of circuits were obtained from TERUMO. The bypass tubing set consisted of a 3/16 inch × 1/4 inch A-V loop coated with heparin or PMEA and a 1/4 inch pump boot. The only difference between the CPB circuits was the coating material of the tubing; i.e., either heparin or PMEA.

The extracorporeal circuit and the oxygenator were primed with acetate Ringer’s solution, mannitol, riboflavin sodium phosphate, and sodium bicarbonate. We used a CDI 500 arterial gas and venous saturation hematocrit monitor (Terumo) and maintained the hematocrit level at more than 25% during CPB by adding packed red blood cells. Systemic anticoagulation was achieved via intravenous heparin (4 mg/kg) administration. After administration of an initial pre-CPB dose of heparin (300 IU/kg), activated clotting time (ACT) was maintained at more than 400 seconds during CPB.

Intra-operative management was the same in both groups. The initial cardiopulmonary perfusion flow rate was 150–180 mL/min per kg to maintain venous saturation above 80%. Perfusion pressures were maintained at 40–60 mmHg. Moderate hypothermia (30–32°C) was applied to all patients. Normal systemic vascular resistance was maintained via administration of methoxamine hydrochloride (1 mg/kg) and...
chlorpromazine hydrochloride (0.5–1.0 mg/kg). Blood gasses were regulated according to the alpha-stat regimen, and sodium bicarbonate was administered when the base excess dropped below -3.0 mmol/L. We used dilutional ultrafiltration with a polyethersulfone membrane (Aquastream AS-04; JMS, Tokyo) and bispectral index monitoring (40–70; ASPECT; Aspect Medical Systems, Boston, MA) during CPB. Modified ultra filtration (MUF) was performed in all cases.

Anesthesia
Anesthesia was induced via intravenous injection of high-dose fentanyl (total, 50–150 µg/kg), midazolam (0.2-0.4 mg/kg), and pancuronium (0.1 mg/kg). All patients were weaned from CPB via intravenous infusion of dopamine (5 µg/kg per min), dobutamine (5 µg/kg per min), and/or nitroglycerine (1 µg/kg per min). Some patients required intravenous infusion of these inotropes and vasodilators at higher doses for successful weaning from CPB. When necessary, chlorpromazine hydrochloride (1.0 mg/kg) was used during the rewarming phase. Anticoagulation was reversed using intravenous protamine sulfate (3 mg/kg).

Blood sampling and biochemical analysis
Blood was drawn through a syringe into a cuvette within an ABL800 FLEX (Radiometer Medical, Copenhagen, Denmark) gas analyzer set at 37°C. One microliter of the specimen was hemolyzed via ultrasound (30kHz), and hemoglobin content was assessed spectrophotometrically at 128 different wave lengths (478–672 nm). The hemoglobin content of the blood sample was determined using the Lambert-Beer hemoglobin equation; hematocrit content was based on hemoglobin content and determined using an internal algorithm. Serum hemoglobin, erythrocyte, thrombocyte and leukocyte levels were measured using an ADVIA 2120 hematology system (Siemens AG, Eschborn, Germany) and the following methods: a novel cyanide-free colorimetric method (serum hemoglobin), cytograms (erythrocytes and thrombocytes) and flow cytometry and cytochemical peroxidase staining (leukocyte). Amount of total protein, C-reactive protein (CRP), and albumin in serum, were measured using a JCA-BM 8040 automated analyzer (JEOL, Tokyo, Japan) and the CRP-Latex (II) X2 assay (CRP) and the 2-reagent biuret method, modified bromocresol purple method
Arterial blood (for measurement of hemoglobin, erythrocyte, leukocyte, thrombocytes, D-dimer fibrin, and total protein levels) was sampled at 7 time points: (1) after induction of anesthesia, (2) just before CPB (5 minutes after heparin administration), (3) 5 minutes after CPB, (4) 30 minutes after CPB, (5) after CPB (5 minutes after protamine sulfate administration), (6) 24 hours after admittance to the intensive care unit (ICU), and (7) just before discharge from the ICU. Albumin and serum globulin were sampled at 3 time points: (1) after induction of anesthesia, (2) 24-hours after admittance to the ICU, and (3) just before discharge from the ICU. C-reactive protein (CRP) was sampled at 4 time points: (1) just before CPB, (2) after 1 hour after admittance to the ICU, (3) 24 hours after admittance to the ICU, (4) just before discharge from the ICU. At each time point, 5 mL of blood were withdrawn. Two milliliters of the blood sample were used for ACT measurement, 2 mL for biochemical examination of the blood, including CRP and D-dimer levels, and 0.5 mL for blood gas analysis and electrolyte measurement. The medical laboratory at the Okayama University Hospital performed all assays.

**Respiratory index (RI)**

The RI is an indicator of oxygenation due to various pulmonary complications. To standardize alveolar-arterial oxygen gradients to the inspired fraction of oxygen during ventilation, the RI was calculated as follows: alveolar-arterial oxygen tension gradient/arterial oxygen tension. Calculations were made immediately before and after CPB, 1 hour after admittance to the ICU, and immediately before extubation.

**Hemodynamic monitoring**

Hemodynamic data were compared between the two groups intra-operatively and post-operatively. The data included systolic arterial pressure (SAP), mean arterial pressure (MAP), and diastolic arterial pressure (DAP). Hemodynamic monitoring data were recorded at 5 time points: (1) just before CPB (5 minutes after heparin administration), (2) 5 minutes after CPB, (3) just after aortic clamp-off, (4) just after CPB, and (5) 1 hour after admittance to the ICU.
Clinical outcome
The following items were recorded: the time of mechanical ventilator support; postoperative blood loss; amounts of transfused packed red blood cells, fresh frozen plasma (FFP), and platelets; the length of hospitalization, and morbidity and mortality rates. Ventilation was measured from the end of surgery to the time of tracheal extubation. Patients were discharged from hospital when they were apyrexial, in an overall satisfactory stable condition, and able to perform basic routine tasks. Mortality was defined as all-cause, 30-day.

Statistical analysis
Data were analyzed using SPSS software for windows version 20 (SPSS Inc, Chicago, IL). All data were expressed as median and range. The $\chi^2$ and Kruskal-Wallis tests were used to evaluate differences between groups for statistical significance. A p-value of $<0.05$ was considered to have statistical significance. A sample power of 3.0 was used for all data to determine the number of samples necessary for detecting statistically significant differences between the two groups.\textsuperscript{18,19} Power analyses were conducted using MANOVA comparisons, a significance level of 0.05, and a power of 0.6.
Results
There were no complications or mortalities. Patient demographic data and clinical variables are summarized in Table 1. There were no differences between the heparin and PMEA groups in sex, age, weight, or body surface area. Operative and CPB parameters (CPB time, aortic cross-clamp time, minimum temperature during CPB, filtration volume during CPB and MUF, and urine output and bleeding during surgery were also similar as were postoperative parameters (urine, and chest tube output in the first 24 hours after surgery; amounts of packed red blood cells, FFP, and platelets transfused in the first 24 hours after surgery; time to extubation; and hospital stay). In contrast, the amount of packed red blood cells transfused during surgery was significantly higher in the PMEA group than the heparin group (P = 0.03).

Leukocyte counts were significantly lower at after 5 minutes after CPB in the PMEA group than the heparin group (P = 0.002) (Table 2).

Amounts of D-dimer fibrin, a marker of fibrin degradation, were not significantly different between the two groups, whereas amounts of CRP were significantly higher in the PMEA group 24 hours after the patients was admitted into the intensive care unit (ICU) (P = 0.003).

Respiratory index were significantly higher just after CPB (P = 0.01) and after 1 hour after admittance to the ICU in PMEA group (P = 0.01) (Table 3).

Hemodynamic data showed that PMEA circuits caused hypotension within 5 minutes after CPB initiation (SAP, P = 0.01, MAP, P = 0.008, DAP, P = 0.002) (Table 4).
Discussion

Interaction between blood components and the non-physiological surfaces of CPB components (e.g., tubes, oxygenator, reservoir, arterial filter and cannulae) during CPB induces several pathophysiologic responses including fibrinolysis, complement activation, inflammatory cytokine release, coagulation and bradykinin release, and leukocyte, platelet, and endothelial cell activation. The consequent whole body inflammatory response can ultimately lead to post-perfusion syndrome. Manufactures of CPB equipment have modified or are currently modifying the surfaces of CPB circuits and components. It is well known that coated circuits and components reduce CPB-induced inflammation.

Unlike traditional non-coated circuits, heparin-coated circuits have the potential to improve resource utilization in patients undergoing cardiac surgery. However, new technology and accompanying changes in clinical and surgical practice must be validated before implementation to prevent harm to patients. Future innovations include modification of the polymer resin during manufacture, and coating the inner surface of the tubing and oxygenators with additives after they have been manufactured.

PMEA is a synthetic polymer, that is quickly and easily applied, thus eliminating the need for potentially hazardous linkers or organic animal components, (e.g., heparin) that may cause, at least to some extent, allergic reactions, thrombocytopenia, or bovine spongiform encephalopathy via viral transfer. Previous clinical and experimental studies have shown that PMEA surfaces are more compatible with platelets, white blood cells, and complement system components and less likely to induce process such as coagulation and protein adsorption than uncoated surfaces. As described by Gunaydin et al., PMEA coating has a hydrophobic polyethylene backbone and a chemically inactive outer surface, and therefore little reactivity with blood components.

Surface structure affects protein adsorption at the molecular level. Both the amount and conformation of absorbed proteins play a major role in platelet adhesion. Previous studies have shown that coating circuit surfaces with PMEA inhibits protein adsorption and the denaturation of adsorbed proteins. Significantly less protein is adsorbed onto PMEA-coated circuits than uncoated circuits.
Despite the advantages of PMEA-coated circuits, our results clearly show that they cause transient leukopenia during pediatric CPB. Transient leukopenia, mainly granulocytopenia and monocytopenia, occurs when circulating cells are trapped within the pulmonary vasculature owing to complement activation via a non-traditional pathway. Exposure of blood to a CPB circuit activates the complement system, mainly through this alternative pathway. CPB circuits do not contain endothelial cells, which normally regulate cofactor C3 activity, on their interior wall. Therefore when blood contacts extracorporeal circuits, it along with kallikrein stimulates the formation of C3a and C5a, which have anaphylactic and chemotactic activity. Contact of blood with negatively charged surfaces cleaves, factor XII, which is normally present in an inactive complex with prekallikrein, factor XI, and high molecular weight kininogen (HMWK). The cleavage products (alpha and beta factor XIIa) subsequently transduce all contact-initiated response. Beta-factor XIIa converts inactive prekallikrein into active kallikrein, which detaches the vasodilator bradykinin from HMWK.

Ikuta et al. found that the PMEA-coated circuits better prevented platelet activation than heparin-coated and non-coated circuits and perioperatively inhibited the activity of inflammatory cytokines to a similar extent as heparin-coated surfaces, but were slightly inferior in reducing complement activation. Complement activation leads to neutrophil activation, which explains why our results show significantly lower leukocyte counts for PMEA-coated circuits than heparin-coated circuits at the beginning of CPB. From this, it can be inferred that PMEA-coated circuits activates complement via the alternative pathway and entraps leukocytes within the pulmonary vasculature. Accordingly, the transient leucopenia caused by PMEA-coated circuits reduces perfusion pressure during pediatric CPB and may leads lower hemodynamic data during surgery.

Our results also clearly show that PMEA-coated circuits have a higher RI than heparin-coated circuits just after CPB and 1 hour after transfer of the patient to the ICU. They were also associated with significantly higher CRP levels 24 hours after transfer to the ICU. Hazama et al. showed that neutrophil elastase levels were significantly lower for heparin-coated circuits than PMEA-coated circuits immediately and 4 hours after CPB. Transient leukopenia may account for post-CPB increases in the RI and CRP levels in patients receiving PMEA-coated circuits. Increasing in the after CPB predict
development of SIRS as a result of post-perfusion syndrome. However, the differences of between Heparin and PMEA groups; amount of red blood cells transfused during surgery may influence the RI, CRP level and hemodynamic change during surgery.

CPB circuit coating may system be of greater importance in pediatric patients than adult patients owing to the relatively large interface between the artificial surface interface and the blood. Pediatric patients are at particular risk for post-operative coagulation because of increased hemodilution in pediatric CPB, and a higher ratio of the internal surface area of the circuit to blood volume in children than adults. Additional patient-related factors include chronic cyanosis and polycythemia, which are more common in patients with congenital heart disease. On the basis of the results of our study, we recommend the use of heparin-coated circuits than PMEA-coated circuits for pediatric CPB.

The major limitation of the present study was small sample size. In summary, our findings show that PMEA-coated circuits unlike heparine-coated circuits, cause transient leukopenia during pediatric CPB. They also tended to cause systemic inflammatory respiratory syndrome after CPB. Therefore, we concluded that heparin-coated circuits are better than PMEA-coated circuits in pediatric CPB.
References


Figure Legend

Table 1: Patient Demographic and Clinical Variables
PMEA: poly-2-mthoxyethyl acrylate, CPB: cardiopulmonary bypass, MUF: modified ultrafiltration
Data are reported as median and range.
†P<0.05, heparin versus PMEA

Table 2: Perioperative Clinical Variables
Data are reported as median and range. Power analysis was higher than 0.6 for all data.
†P<0.05, heparin versus PMEA
*P<0.05, after induction of anesthesia versus 5 min after CPB
PMEA: poly-2-mthoxyethyl acrylate, CPB: cardiopulmonary bypass, ICU: intensive care unit, CRP: C-reactive protein

Table 3: Changes in the Respiratory Index
Data are reported as median and range. Power analysis was higher than 0.6 for all data.
†P<0.05, heparin versus PMEA
PMEA: poly-2-mthoxyethyl acrylate, CPB: cardiopulmonary bypass, ICU: intensive care unit

Table 4: Hemodynamic change during CPB
Data are reported as median and range. Power analysis were higher than 0.6 for all data.
†P<0.05, heparine versus PMEA
PMEA: poly-2-mthoxyethyl acrylate, CPB: cardiopulmonary bypass, ICU: intensive care unit