

## Experimental Studies on Artificial Blood Usage for Hemodilution during Cardiopulmonary Bypass

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**Background:** Although hemodilution is usually utilized during cardiopulmonary bypass (CPB), hemodilution can cause adverse effects such as hypotension and hypoxia. The purpose of this study was to evaluate a novel perfluoro-octyl bromide (PFOB) emulsion, one of perfluorochemicals (PFCs) emulsions, administered during hemodilution CPB.

**Methods:** Fifteen dogs were subjected to CPB for 2 hours under mild hypothermia. Animals were divided into three groups; control group, hemodilution group and PFOB group. During the experiment, hemodynamics, complete blood count and blood chemistry were monitored. In addition, serum complement titer (CH50), bradykinin and histamine concentrations were also measured. **Results:** Heart rate (HR) was markedly elevated in the hemodilution groups ( $p < 0.05$ ). Mean arterial pressure (MAP) did not change in the three groups. White blood cell (WBC) and platelet (PLT) count did not significantly differ among the three groups. Plasma lactate concentration was markedly elevated only in hemodilution group during late phase of CPB ( $p < 0.05$ ). In the hemodilution group, CH50, bradykinin and histamine, were markedly elevated during the CPB and just after CPB ( $p < 0.05$ ).

**Conclusions:** The present study demonstrated possible benefits of the new PFC emulsion during cardiac surgery by counteracting the adverse effects of hemodilution during CPB. (*Ann Thorac Cardiovasc Surg* 2005; 11: 238–44)

**Key words:** cardiopulmonary bypass, perfluoro-octyl bromide, hemodilution

### Introduction

Patients undergoing cardiac surgery frequently require transfusion because of bleeding and hemodilution during

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cardiopulmonary bypass (CPB). There are two main complications of autologous transfusion, infection and immune reactions. Despite extensive testing of the bank blood, the possibility of these complications remains a concern. Some patients may refuse transfusion on religious grounds. Currently, to avoid autologous transfusion, many institutions are utilizing various modalities such as autologous transfusion,<sup>1-3)</sup> administration of aprotinin, reduction of the priming volume of the CPB circuit, and acceptance of the lower hematocrit (Ht) levels during CPB.<sup>4)</sup> However, certain groups of patients such as the elderly, neonates and infants still remain at high risk from CPB. In addition, CPB generally induces systemic inflammatory reaction by activating complement and various vasoactive substances including histamine and bradykinin.<sup>5,6)</sup> It has been known that this inflammatory reaction

causes capillary leak syndrome with high morbidity in children.<sup>7,8)</sup> Thus it is also important to prevent from inflammatory reaction caused by CPB.

Perfluorochemicals (PFCs) have been used clinically as artificial blood substitutes in the past.<sup>9)</sup> The first generation artificial bloods were not clinically useful because they were unstable at normal temperature and had a low ability for oxygen transportation. Recently new base and emulsification technology has led to the development of second generation PFCs.<sup>10)</sup> We introduced new emulsifying technology and developed a novel PFC emulsion with an improved stability and oxygen carrying capacity.<sup>11)</sup> During cardiac surgery avoidance of a low Ht is most necessary during the time the patient is on CPB. Therefore, the short blood retention time of PFCs should not be problematic during this brief period of support.

The purpose of this study was to evaluate the properties of the novel PFC emulsion during hemodilution CPB from the main view of hemodynamic parameters and inflammatory reaction including complement and vasoactive substance activation.

**Material and Methods**

**Animal**

Female beagle dogs weighing 10-12 kg (n=15) were randomly divided into three groups, control (n=5), hemodilution (n=5) and perfluoro-octyl bromide (PFOB, n=5) group. Animal care complied with the Principle of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH), publication No. 86-23 revised 1985.

**Artificial blood**

The composition of PFC emulsion is shown in Table 1. This PFC solution constituted 28% of PFOB as a base, 12% of a perfluoroalcohol esters with oleic acid (FO-9982), 2.4% of yolk lecithin and 0.12% of one type of polyethylene glycol (PEG), 1, 2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-PEG, (DSPE-50H). This artificial blood is thought to possess properties such as resisting phagocytosis by the reticuloendothelial system because the molecule is surrounded by PEG, and decreased platelet (PLT) activation by the same mechanism. The emulsion is stabilized by bonds with fatty acid fluoride and its average particle size was about 170 nm.

**Table 1. Composition of artificial blood**

Perfluoro-octyl bromide	28%
FO-9982	12%
Yolk lecithin	2.4%
DSPE-50H	0.12%
Distilled water	57.48%

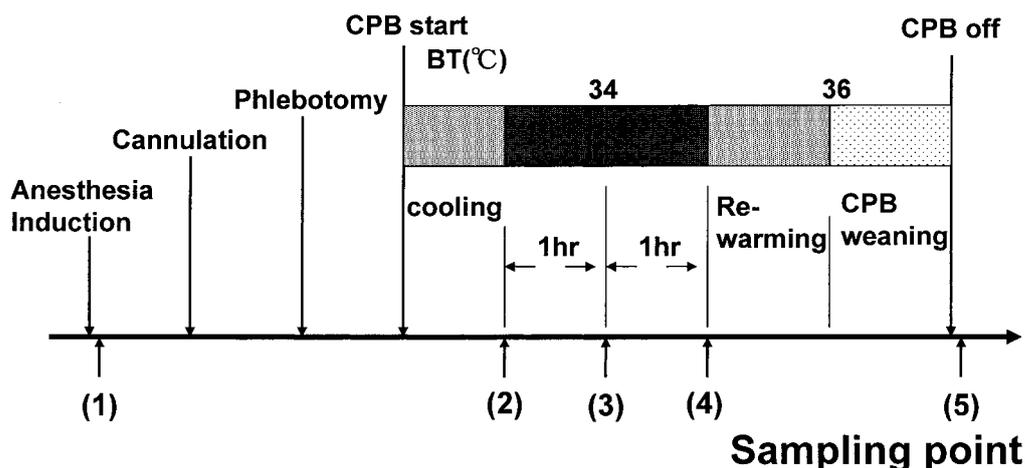
DSPE-50H = 1, 2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-polyethylene glycol (PEG); FO-9982 = perfluoroalcohol esters with oleic acid.

**Surgical procedure**

The dogs were anesthetized with ketamine hydrochloride (5 mg/kg) and thiamylal sodium (10 mg/kg), and maintained by additional injection of 10 mg/kg thiamylal sodium if needed. All animals were placed in a supine position and ventilated through a cuffed endotracheal tube by a volume controlled ventilator (Servo 900, Siemens Elema, Stockholm, Sweden). The ventilator was set at a tidal volume of 10-15 ml/kg and respiratory rate of 15-20 times/min to maintain normocapnia. The administration of heparin (50 IU/kg) was performed 5 min before cannulation. The CPB circuit consisted of SAFE MINI (POLYSTAN, Vaerlose, Denmark) as an oxygenator and a reservoir with a roller pump. The priming volume was 600 ml, and the circuit was primed with 540 ml acetate Ringer's solution and 60 ml bovine serum albumin (BSA). Ten Fr. Bio-Medicus arterial cannula and 14 Fr. Bio-Medicus venous cannula (Medtronic, Inc., Minneapolis, MN, USA) were inserted into the left femoral artery and the left jugular vein, respectively. Electrocardiogram and arterial blood pressure were continuously monitored. A catheter with thermometer was placed in the bladder through the urethra, and the urine volume and bladder temperature (BT) as a central temperature indicator were monitored.

**Protocol**

The experimental protocol is shown in Fig 1. After anesthesia, all monitoring lines were placed. Following CPB 900 ml of blood was saved in hemodilution and PFOB groups to adjust the Ht between 14 and 15%. In hemodilution groups, 900 ml phlebotomy was performed before CPB induction, and it was replaced with 900ml acetate Ringer's solution and 60 ml BSA. In PFOB group, 600 ml PFOB, 300 ml acetate Ringer's solution and 60 ml BSA were administered during phlebotomy. BSA was added for maintaining an osmotic pressure. The pump was started and the flow was kept between 70 and 80 ml/



**Fig. 1.** Experimental protocol.

Numbers are point of taking blood samples.

CPB = cardiopulmonary bypass; BT = bladder temperature.

kg/min. Animals were cooled to 34°C at the BT by the heat exchanger placed in the CPB and maintained for 2 hours, then re-warmed to 36°C. After the animals were re-warmed, CPB was weaned and stopped. Blood in the circuit and phlebotomized blood was returned to the animals. Drugs to support the blood pressure were not required except for sodium bicarbonate. Seven percent bicarbonate was administered as follows: 7% sodium bicarbonated (ml) = body weight × base deficit /3. Blood samples were taken at the point of pre-CPB (1), temperature 34°C (2), 1 hour after 34°C (3), 2 hours after 34°C (4) and after-CPB (5), and analyzed for blood cell counts (red blood cell (RBC), white blood cell (WBC), PLT and Ht, lactate, complement titer (CH50) and vasoactive substances (histamine and bradykinin).

#### Measurement of vasoactive substances (bradykinin and histamine)

The concentrations of bradykinin and histamine were measured using commercial ELISA Kits (Pennisula Laboratories, Inc., Belmont, CA, USA).

#### Statistical analyses

Data are presented as means ± the standard error (SEM). Statistical analysis was performed using Stat View version 5.0 (SAS institute Inc., Cary, NC, USA). Corrections of the hemodilution effect during CPB were made according to a formula described by Roth-Isigkeit A and his associates: corrected concentration = (measurement concentration × preoperative Ht)/Ht at time of sampling.<sup>12</sup> Comparisons were done using repeated measurement

analysis of variance (ANOVA), using Bonferroni *t* test for post hoc analysis. *P* value less than 0.05 was deemed significant in the experimental data.

## Results

### Hemodynamic

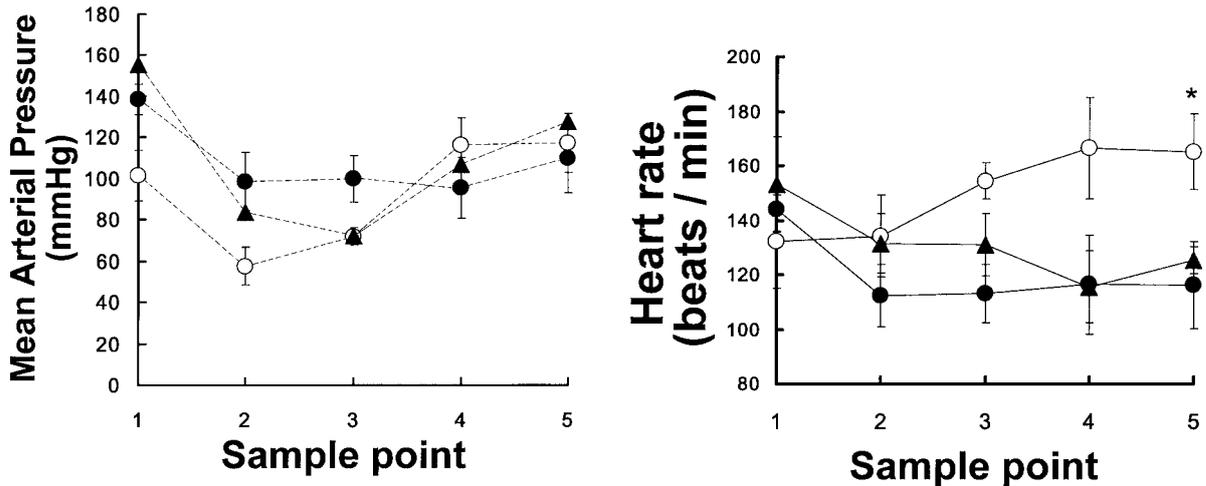
There was no difference among the three groups in mean arterial pressure (MAP) (Fig. 2A). Although heart rates (HRs) were not different among the three groups before CPB, the hemodilution group showed significantly elevated HRs compared to PFOB group at just after weaning from CPB ( $p < 0.05$ ) (Fig. 2B). The amount of 7% sodium bicarbonated used during the experiment was no different among three groups (data not shown).

### Blood cell count and plasma lactate concentration

There were no marked differences among the three groups in WBC and PLT counts. RBC count and Ht were significantly lower in hemodilution and PFOB groups compared to control group ( $p < 0.05$ ) (Table 2). Although there was no difference among the three groups at pre-operative point, lactate concentration of the hemodilution group was significantly increased compared to PFOB and control groups during the late phase of CPB and just after weaning from CPB ( $p < 0.05$ ) (Fig. 3).

### Complement and vasoactive substances (histamine and bradykinin)

Pre-operative values of CH50 were no different among the three groups and CH50 of PFOB group were similar



**Fig. 2.** Changes in mean arterial blood pressure (A) and heart rate (B) during the experiment among three groups; control (●), hemodilution (○) and perfluoro-octyl bromide (PFOB, ▲) groups. Values are expressed as mean±SEM. \* vs. PFOB group  $p < 0.05$ , by Bonferroni  $t$  test for post hoc analysis. HR = heart rate; MAP = mean arterial pressure.

to that of control group during experiment. However, that of the hemodilution group was significantly elevated compared to PFOB group ( $p < 0.05$ ) in the late phase of CPB (Fig. 4A).

There was no difference in the level of bradykinin among the three groups at the pre-operative point. The plasma concentrations of bradykinin in PFOB group were similar to that of the control group. However, that of the hemodilution group was significantly elevated compared to the control group ( $p < 0.05$ ) during CPB and just after weaning from CPB (Fig. 4B).

Similarly, there was no difference in the level of histamine among three groups preoperatively. However, that of the hemodilution group was markedly elevated compared to the control and PFOB groups ( $p < 0.05$ ) just after CPB (Fig. 4C).

**Table 2. Peak values of blood cell counts and blood chemistry**

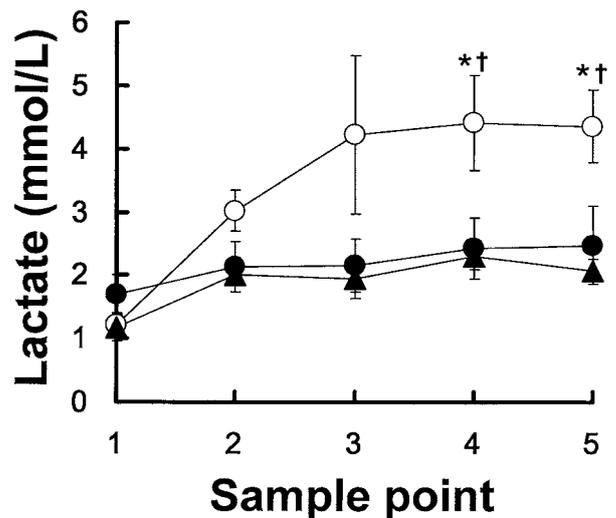
	Control	Hemodilution	PFOB
RBC ( $10^6/\text{mm}^3$ )	4.8±0.3	3.7±0.3 <sup>†</sup>	3.3±0.4 <sup>†</sup>
Ht (%)	30.8±8.0	17.2±4.4 <sup>†</sup>	19.8±5.1 <sup>†</sup>
WBC ( $10^3/\text{mm}^3$ )	4.5±0.6	5.9±1.2	6.6±0.6
PLT ( $10^3/\text{mm}^3$ )	246.1±9.9	341.6±39.7	265.3±34.6

Values are expressed as mean±SEM. <sup>†</sup> vs. control group  $p < 0.05$ , \* vs. PFOB group  $p < 0.05$ , by Bonferroni  $t$  test for post hoc analysis. BUN = blood urea nitrogen; Ht = hematocrit; PLT = platelet; RBC = red blood cell; WBC = white blood cell.

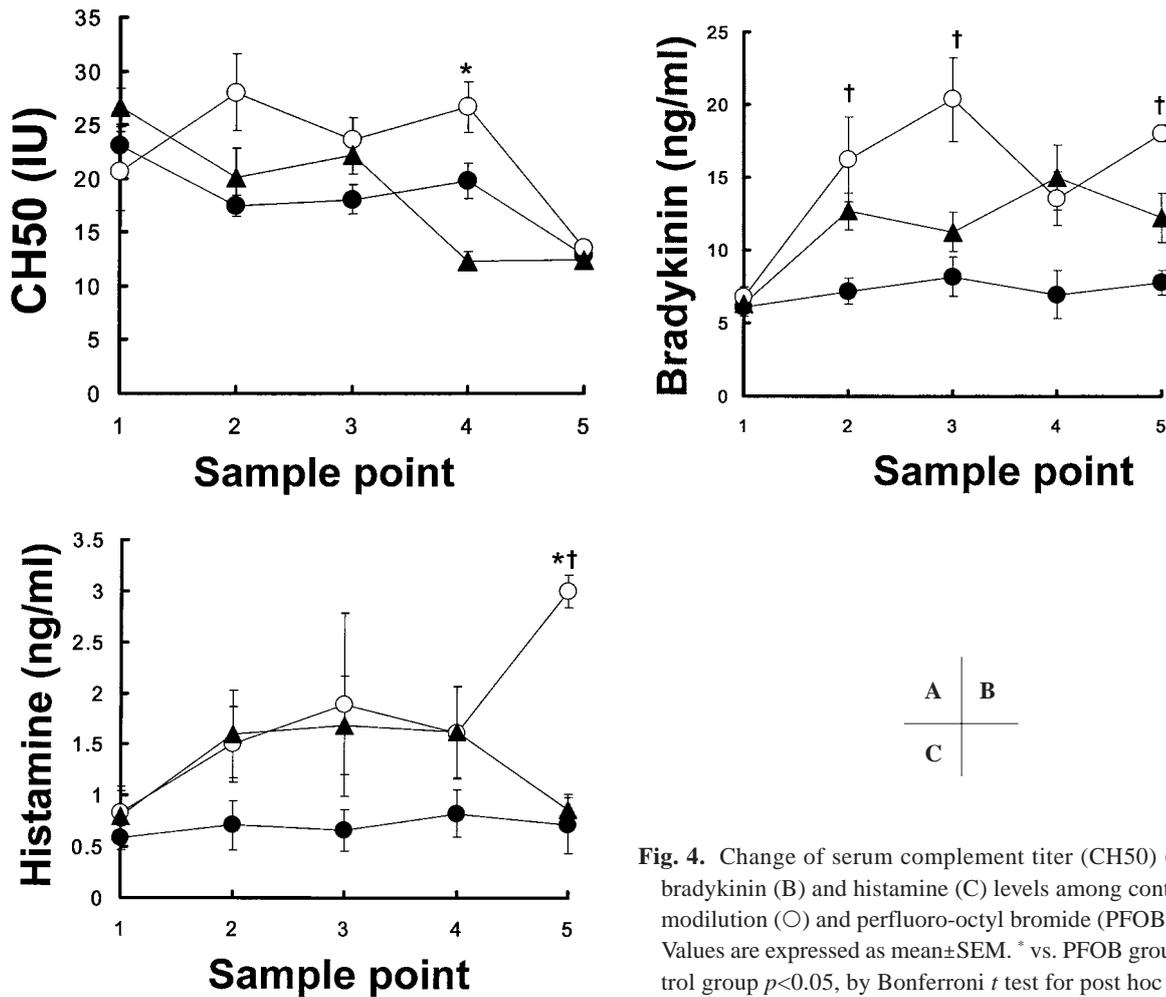
**Comment**

Our findings indicate that the new artificial blood was able to reduce the inflammatory reaction including complement activation, liberation of vasoactive substances (bradykinin and histamine) and plasma lactate level caused by hemodilution during CPB.

Blood transfusion is not completely risk free. The complications of autologous transfusion include viral transmission and immunological reactions. Transfusions are



**Fig. 3.** Change in plasma lactate among control (●), hemodilution (○) and perfluoro-octyl bromide (PFOB, ▲) groups. Values are expressed as mean±SEM. \*\* vs. control and PFOB groups  $p < 0.05$ , by Bonferroni  $t$  test for post hoc analysis.



**Fig. 4.** Change of serum complement titer (CH50) (A), plasma bradykinin (B) and histamine (C) levels among control (●), hemodilution (○) and perfluoro-octyl bromide (PFOB, ▲) groups. Values are expressed as mean±SEM. \* vs. PFOB group, † vs. control group  $p < 0.05$ , by Bonferroni *t* test for post hoc analysis.

also reported to be a major risk factor for postoperative infections.<sup>13</sup> Blood may not be available for patients with rare blood types and some patients may refuse for religious reasons. Therefore, in order to avoid blood transfusion many approaches including drugs, autotransfusion, limitation of priming volume of the CPB circuit, and tolerance of hemodilution are used in clinical practice.

The lowest safe Ht during CPB has not been conclusively established. Fang and colleagues demonstrated that mortality risk significantly increased when Ht levels are less than 14% during all cardiac surgery especially in high-risk patients and children.<sup>14</sup> In addition, the CPB with hemodilution (approximate Ht 20%) produces hypotension resulting from decrease total peripheral resistance.<sup>15</sup>

Studies in canine models of hemodilution during CPB have also shown conflicting results. Systemic oxygen consumption was maintained on CPB until the Ht was diluted to less than 20%.<sup>16</sup> However, other studies dem-

onstrated that a critical Ht level of 9-14% maintains adequate oxygen delivery during normothermic CPB.<sup>17</sup> In our study Ht lower than 20 and CPB duration of 2 hours were used to simulate the adverse effects of hemodilution. Our experiment demonstrated the detrimental effects of hemodilution during CPB from the standpoint of lactate level and vasoactive substances release, although hypotension was not shown. Additionally, although the increase in HR was shown in the hemodilution group, which might be caused by systemic anemia, the elevation of that was not shown in the PFOB group.

The use of artificial blood is one possible means of avoiding the adverse effects of hemodilution during cardiac surgery. It may allow the avoidance of transfusion and enhance the safety of non-transfusion cardiac surgery. There are two types of artificial blood, modified hemoglobin and PFC emulsion. Many modified hemoglobins have been developed and are well studied.<sup>18,19</sup> However, since they originate from human or bovine he-

moglobin, they still have the potential for infectious disease transmission. In contrast, PFC emulsions have no potential of disease transmission, because they are entirely artificial organic compounds consisting of carbon and fluorine.

Recently, new refining and modification processes have lead to the development of a second generation PFCs, which are smaller and more stable in vivo, and possess increased ability for oxygen transport than the first generation PFCs.<sup>20)</sup> Our PFC has PEG linked phospholipids around the particle to extend blood retention time by preventing entrapment by the reticuloendothelial system, and contains fatty acid fluoride to bind PFOB and emulsifier more firmly.<sup>11)</sup> PFC emulsion seems suitable for cardiac surgery for two reasons. First is its oxygen dissolution property with a linear oxygen solubility curve which is different from that of hemoglobin. Thus, a high oxygen concentration is required to carry enough oxygen. In cardiac surgery, a high oxygen concentration is easily obtained by mechanical ventilation. The second reason is the short duration for requirement of supplement. Since in cardiac surgery the patient after bypass receives the diluted blood from the CPB circuit, the period of CPB is the most critical due to maximal hemodilution. The short half-life of PFC emulsion in blood is about 1-2 days and is one of its main drawbacks. However, this short half-life will not be problematic because the PFC emulsion is only used to counteract the hemodilution during the period of CPB.

Our study showed that PFC emulsion infusion could attenuate a hypoxic insult. In this study, although there was no elevation of plasma lactate concentration in PFOB group, there was significant elevation of that in the hemodilution group. Plasma lactate concentration is one of the indices of anaerobic metabolism. The elevation of plasma lactate in hemodilution group might indicate insufficient tissue oxygen delivery due to tissue anaerobic metabolism. The lack of elevated plasma lactate level secondary to hemodilution suggests adequate oxygen delivery with the use of PFC emulsion.

The necessary dose of PFC is still controversial. In this experiment considering priming volume of the CPB circuit, the dogs received 600 ml (about 60 ml/kg) of our 28% of PFOB, which is between 10 and 12% of PFC concentration. Johnson and associates have reported a safe dose of PFC, which is similar to the dose in the present experiment.<sup>20)</sup> Of course, further investigation for the safety of PFC and its effects on various organs is warranted.

In general, CPB activates complement, vasoactive substances and various cytokines.<sup>5,6)</sup> Activated inflammatory modulators including complement and histamine during CPB may produce a capillary leak syndrome associated with high morbidity.<sup>7,8)</sup> Therefore, the attenuation of this inflammatory response may be an important factor in reducing the morbidity associated with CPB. In this study the elevation of vasoactive substance release with hemodilution during CPB was attenuated with the new PFC. The mechanism of this effect will need to be elucidated in the future. Saeed and associates showed the inhibition of the effect of vasoactive agents including histamine, serotonin and norepinephrine by PFC emulsion in rabbit thoracic aorta.<sup>21)</sup> In addition, previous in vitro reports demonstrated that PFC attenuated neutrophil-endothelial cell interactions<sup>22)</sup> and release of proinflammatory mediators from stimulated macrophages.<sup>23,24)</sup> These reports support our findings that the newly developed PFOB emulsion reduced the production of vasoactive substances, although the mechanisms of these inhibitory effects have not been fully evaluated.

In conclusion, the present study demonstrated the possible clinical benefits of a newly-developed PFC emulsion during cardiac surgery by ameliorating the adverse effects of hemodilution during CPB. This may allow cardiac surgery to be performed with limited or no transfusion of blood.

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