



Effect of modified ultrafiltration on cytokines and hemoconcentration in dogs undergoing cardiopulmonary bypass

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ABSTRACT. Cardiac surgery using cardiopulmonary bypass (CPB) generates severe inflammatory reactions secondary to hemodilution and surgical stress. This study was conducted to evaluate whether modified ultrafiltration (MUF) could be performed safely and to clarify its effects during mitral valve repair in dogs in terms of hemodilution and the status of inflammatory cytokines. We retrospectively studied 38 dogs with mitral valve disease who underwent MUF immediately after mitral valve repair under CPB. To determine the effect of MUF, we measured the pre- and post-MUF blood dilution and blood cytokine levels. The levels of red blood cells, hematocrit (HCT), and albumin were significantly increased after MUF, whereas interleukin (IL)-6 levels were significantly increased from 24.3 (range 9.6–54.6) to 32.3 (15.9–65.1) pg/ml. The levels of IL-8 and IL-10 declined significantly after MUF, from 368.2 (246.1–669.4) and 45.4 (28.6–76.1) to 272.2 (174.1–414.4) and 28.8 (18.8–44.5) pg/ml, respectively. Our results demonstrated that MUF can be applied in dogs undergoing CPB and is effective in achieving hemoconcentration. Moreover, MUF may be useful for the removal of cytokines. Further studies are needed to validate these findings and clarify the effects of inflammatory cytokines after CPB.

KEY WORDS: cardiopulmonary bypass, cytokine, mitral valvuloplasty, modified ultrafiltration

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Cardiac surgery using cardiopulmonary bypass (CPB) is potentially associated with high surgical invasiveness because of the need for extracorporeal circulation and long operative duration. In human medicine, pediatric patients with a small physique experience specific problems associated with CPB that result in decreased oxygen-carrying capacity and edema because of the higher hemodilution rates of these patients than those in adults. Subjects in veterinary medicine include small dogs that weigh 5 kg or less, who experience problems of CPB-induced hemodilution similar to that in children [11]. The high surgical invasiveness of cardiac surgery is associated with excessive cytokine production and leukocyte activation [7, 17]. Moreover, the post-operative blood cytokine level increases significantly compared to the pre-operative level during cardiac surgery in dogs [14]. Modified ultrafiltration (MUF) is a technique developed to manage CPB-induced hemodilution and alleviate inflammatory reactions caused by increased cytokine levels. MUF is a technique involving the concentration of blood via filtration and further cytokine removal through membrane filtration. In human medicine, MUF-based cytokine removal has shortened the duration of post-operative stay at the intensive care unit [9, 15, 19, 23]. However, MUF-induced changes in hemoconcentration and blood cytokine levels in veterinary medicine have not been reported. Furthermore, there are no clinical case reports of MUF in dogs.

In this study, mitral valvuloplasty (MVP) was performed in dogs with mitral valve disease (MVD), and hemodilution and blood cytokine levels were measured before and after MUF to examine the effect of MUF. This is the first report investigating the effect of MUF on MVP in dogs.

MATERIALS AND METHODS

Study subjects

We retrospectively studied dogs diagnosed with MVD between October 2016 and December 2017 at the Animal Medical Center, College of Bioresource Sciences, Nihon University. These dogs subsequently underwent MVP and received MUF after CPB. In total, 38 dogs with MVD (21 males, 17 females) consisting of 18 Chihuahuas, five Cavalier King Charles spaniels, five mongrels, two Shih Tzu, one Chin, one Yorkshire Terrier, one Maltese, one Pomeranian, one Shetland sheepdog, one Beagle, one Toy Poodle, and one miniature Dachshund were included. According to the American College of Veterinary Internal Medicine consensus

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guideline [2], the subjects were classified based on their severity of heart failure as follows: 18 cases of Stage B2, 18 cases of Stage C, and two cases of Stage D disease. We further classified the subjects in Stage B2 as the non-heart-failure group and those in stages C and D as the heart-failure group (Table 1).

Anesthesia and surgery

The subjects received subcutaneous administration of atropine sulfate (0.04 mg/kg; Mitsubishi Tanabe Pharma Corp., Osaka, Japan) as a pre-anesthetic medication. After sufficient oxygenation, anesthesia was induced intravenously with fentanyl (5 µg/kg; Daiichi Sankyo Co., Ltd., Tokyo, Japan) and propofol (4 mg/kg; Mylan Inc., Canonsburg, PA, USA). After the animals were intubated and placed under inhalational anesthesia with isoflurane (DS Pharma Animal Health Co., Ltd., Osaka, Japan), we undertook additional procedures to ensure the maintenance of anesthesia via continuous infusion of fentanyl at 5 µg/kg/hr and intravenous administration of vecuronium (0.1 mg/kg; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan). The surgical approach involved a left lateral thoracotomy via the fifth intercostal space. After cardiac arrest was induced, we performed MVP, specifically mitral chordal replacement and mitral annuloplasty.

MUF

We conducted vein-artery MUF. After the blood was concentrated by being passed from a venous cannula inserted into the left jugular vein through a hemoconcentrator (BIOCUBE® BHC-030, Nipro, Osaka, Japan), the blood was re-infused to an arterial cannula inserted into the left carotid artery [5]. First, the circuit on the inflow/outflow side was blocked and separated from CPB. Then, MUF was conducted by the passage of residual blood in the reservoir and a circuit through hemoconcentration. Thereafter, with reference to each subject's data including physique and HCT, we collected blood from the venous cannula into the reservoir and pumped it into the common carotid artery during MUF (Fig. 1). After checking the blood pressure and intracardiac volume, we terminated MUF by reinfusing the concentrated blood.

Sampling

To measure the levels of cytokines (interleukin [IL]-6, IL-8, IL-10, and tumor necrosis factor alpha [TNF-α]), red blood cells (RBCs), white blood cells (WBCs), HCT, and platelets (PLTs), we collected pre- and post-MUF blood samples in ethylenediaminetetraacetic acid-treated tubes via an arterial cannula inserted into the right femoral artery. Similarly, samples for albumin (Alb) quantification were collected in heparin-treated tubes.

Assay techniques

The levels of RBCs, WBCs, HCT, and PLTs were quantified using an automated cell counter (Celltac α, Nihon Kohden Co., Ltd., Tokyo, Japan). Blood Alb was measured using an automatic analyzer after the sample was centrifuged at 1,500 rpm for 5 min (LABOSPECT 003, Hitachi High-Technologies Corp, Tokyo, Japan). Blood samples for cytokine measurement were centrifuged at 1,000 × g for 30 min at room temperature within 30 min of blood collection. The plasma was frozen and immediately stored

Table 1. Characteristics of dogs undergoing cardiopulmonary bypass (CPB)

Variable		
Sex	Male	21
	Female	17
Age (years)		10 (8–10)
Weight (kg)		4.1 (2.8–6.6)
Stage	CHF+ (ACVIM Stage B2)	18
	CHF– (ACVIM Stage C, D)	20
Cardiopulmonary bypass time (min)		94 (87–108)
Aortic cross-clamp time (min)		68 (64–74)
Total anesthesia time (min)		393 (359–435)

Data are shown as median and interquartile range. CHF: congestive heart failure, ACVIM: American College of Veterinary Internal Medicine.

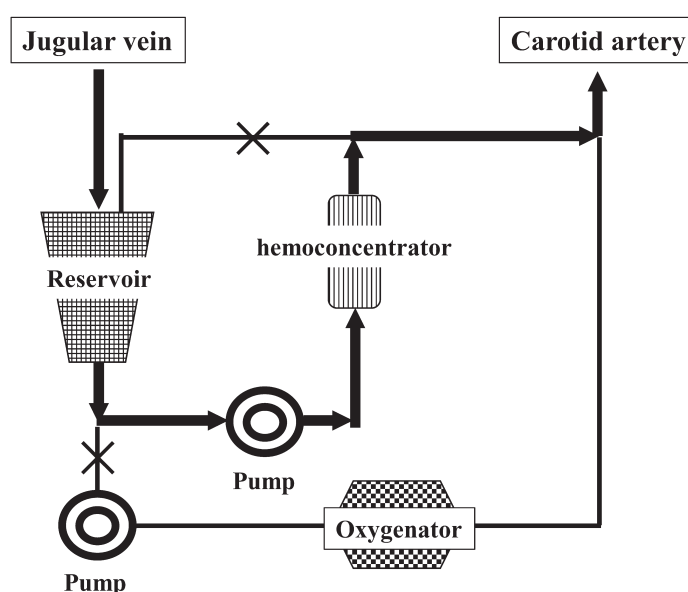


Fig. 1. The modified ultrafiltration (MUF) circuit is installed in the cardiopulmonary bypass (CPB) circuit. After the CPB, the circuit is interrupted at a specific point (marked × in the illustration), and MUF is undertaken.

below -20°C . Blood samples were thawed at room temperature before undertaking measurements. Enzyme-linked immunosorbent assays were conducted using Quantikine Canine Immunoassay kits (R&D Systems, Minneapolis, MN, USA) for IL-6, IL-8, IL-10, and TNF- α . All measurements were performed using a spectrophotometer (Synergy 2 Multi-Mode Microplate Reader; BioTek Instruments Inc., Winooski, VT, USA).

Statistical analysis

Statistical analysis was conducted in GraphPad Prism ver. 5.00 for Windows (GraphPad Software, San Diego, CA, USA) and the results are expressed as the median and interquartile range. Analysis was conducted using the Wilcoxon signed-rank test to compare pre- and post-MUF measurements. The Mann-Whitney U -test was performed for intergroup comparisons between the two groups classified by the presence or absence of heart failure. $P < 0.05$ indicates statistical significance.

RESULTS

The median age of our study population was 10 years (range 8–10) and the median weight was 4.1 kg (2.8–6.6). In post-MUF samples, the levels of RBCs, WBCs, HCT, and Alb were significantly increased compared to their pre-MUF levels, but PLT level declined significantly (all $P < 0.05$; Table 2). No significant difference was observed in cytokine level between groups with or without heart failure (Table 3).

After MUF, IL-6 levels was significantly increased compared to the pre-MUF measurements, whereas those of IL-8 and IL-10 declined significantly (all $P < 0.05$). No significant difference was observed in the levels of TNF- α pre- and post-MUF (Table 4).

DISCUSSION

This study revealed that MUF can be safely and effectively applied in dogs undergoing CPB. In dogs with a small physique, hemodilution and highly invasive surgery have significant impact on the surgical success rate. In this study, RBC and HCT levels were significantly increased after MUF, indicating that MUF achieved sufficient hemoconcentration in dogs similarly as it does in humans. Moreover, no post-operative edema was observed, suggesting that an increase in Alb may reduce the risk of post-operative edema induced by hypo-osmosis. When the HCT decreased to 22% or less during CPB in humans, there was a significant increase in the incidence of post-operative renal failure, myocardial infarction, pulmonary edema, and multiorgan failure [10]. Because of its hemoconcentration effects, MUF after CPB is highly effective in preventing various post-operative complications

Table 2. Changes in hematologic and biologic variables after modified ultrafiltration (MUF)

		Pre-MUF	Post-MUF
Red blood cells (RBCs)	$\times 10^6/\mu\text{l}$	4.6 (4.2–4.8)	5.6 (5.2–6.0) ^{a)}
White blood cells (WBCs)	$/\mu\text{l}$	7,350 (4,300–10,400)	9,600 (6,825–12,400) ^{a)}
Platelets (PLTs)	$\times 10^3/\mu\text{l}$	138 (75.8–181.8)	102.5 (62.3–146.8) ^{a)}
Hematocrit (HCT)	%	29.3 (25.7–35.4)	39.3 (38.1–43.9) ^{a)}
Albumin (Alb)	g/dl	1.6 (1.4–1.7)	1.9 (1.8–2.1) ^{a)}

a) $P < 0.05$. Data are shown as median and interquartile range. The Wilcoxon signed rank test was performed to compare pre- and post-MUF measurements.

Table 3. Comparison of cytokine levels between congestive heart failure (CHF)⁻ and CHF⁺ (without and with heart failure, respectively)

	CHF ⁻ (pg/ml)	CHF ⁺ (pg/ml)
Pre-MUF		
IL-6	28.6 (10.4–41.2)	21.0 (10.0–57.9)
IL-8	418.7 (220.3–714.6)	352.7 (256.0–589.6)
IL-10	47.7 (16.5–61.0)	44.1 (32.2–81.7)
TNF- α	21.0 (11.7–32.0)	8.9 (3.5–12.9)
Post-MUF		
IL-6	34.3 (18.2–61.3)	25.5 (14.5–61.6)
IL-8	324.4 (187.2–456.9)	251.9 (174.1–351.7)
IL-10	21.7 (8.7–44.5)	32.9 (23.2–44.2)
TNF- α	11.3 (6.9–17.6)	6.6 (5.3–12.1)

Data are shown as median and interquartile range. The Mann-Whitney U -test was performed for intergroup comparisons. IL: interleukin, TNF- α : tumor necrosis factor alpha.

Table 4. Changes in cytokine concentration after modified ultrafiltration (MUF)

	Pre-MUF (pg/ml)	Post-MUF (pg/ml)
IL-6	24.3 (9.6–54.6)	32.3 (15.9–65.1) ^{a)}
IL-8	368.2 (246.1–669.4)	272.2 (174.1–414.4) ^{a)}
IL-10	45.4 (28.6–76.1)	28.8 (18.8–44.5) ^{a)}
TNF- α	12.4 (3.6–23.6)	9.0 (5.2–14.7)

a) $P < 0.05$. Data are shown as median and interquartile range. The Wilcoxon signed rank test was performed to compare pre- and post-MUF measurements. IL: interleukin, TNF- α : tumor necrosis factor alpha.

that arise secondary to low HCT. In human medicine, MUF has been shown to significantly reduce blood transfusion volume [21, 22]. However, since MUF was undertaken in all subjects in this study, intergroup comparisons were precluded. We considered it necessary to examine whether blood transfusion volume can be reduced in dogs as in humans.

Furthermore, the PLT count was significantly reduced after MUF. In experiments using dogs, PLTs are consumed when blood comes into contact with artificial objects, such as CPB circuits [8]. The decreased PLT count in our study subjects can be attributed to the passage of blood through the MUF circuit. In humans, previous reports have stated that blood transfusion volume can be reduced even in patients whose PLT levels had decreased after MUF [3]. Similarly, none of the subjects in our study showed obvious bleeding diathesis or increased blood transfusion volume. Despite the lack of a clear consensus regarding the effect of MUF on the level of each cytokine in humans, overall, MUF has been reported to shorten the duration of post-operative stay at the intensive care unit [9, 15, 19, 23].

IL-6 is an inflammatory cytokine responsible for acute-phase inflammatory reactions. In cardiac surgery using CPB, the IL-6 level was increased in both humans and dogs [6, 14]. Moreover, the MUF procedure usually lowers or has no impact on the level of IL-6 in humans [23]. In this study, IL-6 levels were increased significantly after MUF and were elevated in most cases, but the cause of this increase was not elucidated. A study on dogs who did not undergo MUF demonstrated that the IL-6 level at a time equivalent to the “post-MUF” timepoint was 200 pg/ml or higher [14]. In contrast, the post-MUF IL-6 level was as low as 32.3 pg/ml in our study. In the future, we aim to examine whether MUF can suppress the increase in IL-6.

IL-8 is an inflammatory cytokine that induces neutrophilic chemotaxis and activation. In this study, the levels of IL-8 declined significantly after MUF, as the low molecular weight of IL-8 (8–9 kDa) allows it to be removed effectively. IL-8 activates neutrophilic infiltration in the lungs and induces lung injury after CPB in humans [20]. Elevated levels of neutrophils and IL-8 in bronchial washing solution are also correlated with reduced oxygenation levels after CPB in humans [13]. Furthermore, the level of IL-8 in the alveoli was decreased after MUF in piglets, suggesting that MUF suppressed CPB-induced inflammatory response in the lung [1]. Complications of venous pulmonary hypertension were reported for 14–39% of dogs with MVD [4, 18], suggesting that the impairment of pulmonary function occurred before MVP. In fact, 12 of the 38 subjects in this study had developed pulmonary hypertension pre-operatively, but none showed severe post-operative pulmonary dysfunction. Thus, removing IL-8 by MUF improved post-operative lung function. A study of reperfusion injury during cardiac surgery [12] showed that elevated IL-8 levels promoted the release of granulocyte elastase and consequently caused cell injury. Therefore, the removal of IL-8 even in reperfusion injury, a cause of post-operative complications after cardiac surgery, is considered beneficial.

Similar to IL-6 and IL-8, TNF- α is an inflammatory cytokine. We found that there was no significant difference between the pre- and post-MUF levels of TNF- α . The effect of TNF- α is considered to be limited locally and, therefore, it has little impact on remote organs. A study reported that the blood TNF- α levels remained below a detection threshold value throughout CPB [14]. Despite the lack of a distinctive change in the levels quantified in our study, we confirmed that the blood TNF- α levels were below the detection threshold value in many subjects, implying the likelihood of failure in accurately detecting and quantifying changes in TNF- α .

Another anti-inflammatory cytokine, IL-10, suppresses the adhesion of neutrophils to activated vascular endothelial cells, among other functions. In this study, IL-10 levels were significantly decreased after MUF. In humans, the MUF procedure does not produce consistent results, typically either showing no impact on the IL-10 level or lowering it. Therefore, there is currently no consensus among researchers [9, 16] on the significance of IL-10 in the post-operative status after cardiac surgery.

The results of our study should be interpreted in the context of the following limitations. First, we did not compare changes in cytokine levels pre- and post-MUF with perioperative complications and prognosis. Hence, we could not confirm if the changes in cytokine levels were clinically useful. Second, we did not include a control group, and MUF was conducted in all cases. This is because the subjects in this study were small dogs and we assumed that the MUF procedure would be safe as efficient hemoconcentration can be obtained by MUF. Therefore, it was not possible to evaluate the relationship between MUF and surgical outcomes such as aortic cross-clamp time, cardiopulmonary bypass time, total anesthesia time, and blood transfusion volume compared with a control group.

In conclusion, MUF can be safely applied in dogs undergoing CPB and is effective for hemoconcentration. Moreover, MUF may be useful for cytokine removal in such cases, but it was difficult to selectively remove inflammatory and anti-inflammatory cytokines with the MUF procedure. Further studies with larger sample sizes are needed to evaluate long-term post-operative prognosis and thereby clarify the effectiveness of MUF.

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