Modified ultrafiltration attenuates pulmonary-derived inflammatory mediators in response to cardiopulmonary bypass

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Abstract

Cardiopulmonary bypass (CPB) stimulates systemic and pulmonary inflammation. Modified ultrafiltration (MUF) mitigates deleterious CPB effects by unclear mechanisms. We evaluated pulmonary inflammation in piglets undergoing CPB followed by MUF. Twenty-four piglets underwent 60 min of hypothermic CPB. MUF subjects (n = 12) underwent hemoconcentration postCPB to the target hematocrit. Pulmonary vascular resistance (PVR), proinflammatory cytokine concentrations, and transpulmonary thromboxane gradients were determined at baseline, following CPB, and at end of the study (EOS) in MUF and control (n = 12) groups. PVR significantly increased postCPB in both groups but decreased after MUF. MUF and control groups were similar in regards to systemic cytokine concentrations. Bronchoalveolar lavage concentrations of IL-6 and IL-8 significantly increased in controls throughout the study. Alveolar IL-6 and IL-8 were unchanged at EOS in MUF subjects, and IL-6 concentrations were significantly less than controls at EOS (P < 0.015). Similarly, transpulmonary thromboxane gradient was significantly less at EOS in MUF subjects compared with controls (P < 0.04). MUF removed circulating inflammatory mediators, lessened pulmonary hypertension, and reduced pulmonary-derived inflammatory markers, providing further evidence that MUF ameliorates pulmonary-based inflammation. These findings lend insight into mechanisms behind salutary clinical benefits of MUF after CPB.

Keywords: Ultrafiltration; Cardiopulmonary bypass; Inflammation; Perfusion; Pulmonary vascular resistance

1. Introduction

Cardiopulmonary bypass (CPB) induces inflammation associated with hemodilution, ischemia/reperfusion, and other effects [1]. The inflammatory reaction is typically transient, but severely damaging effects are possible due to complement activation, pro-inflammatory cytokines, neutrophil stimulation, and endothelial activation [1]. Post-CPB inflammation is especially pronounced in the lungs [2], leading to pulmonary edema and pulmonary hypertension (pHTN), both significant components of cardiopulmonary dysfunction seen after CPB [3]. Increased concentrations of various inflammatory mediators within pulmonary secretions have been noted after CPB and other injury models and are correlated with adverse clinical outcomes [4, 5].

Modified ultrafiltration (MUF) was introduced in neonates and children undergoing cardiac surgery with CPB in order to reduce postoperative edema [6]. In a prospective, randomized trial, MUF reduced total body water accumulation and the need for postoperative blood transfusions [6]. In addition, MUF has been associated with reduced morbidity following CPB [3]. However, the specific mechanisms by which MUF exerts positive clinical benefits remain incompletely defined. Based on previous studies, we hypothesized that CPB induces the expression of inflammatory mediators within the lungs. Furthermore, we expected inflammatory mediators to be affected by MUF, which would help explain observed clinical benefits.

2. Materials and methods

Twenty-four neonatal piglets (mean weight 8.4 ± 0.4 kg) were randomized in two equal groups, controls (n = 12) and MUF (n = 12) prior to experimentation. All animals received humane care in compliance with the ‘Principles of Laboratory Animal Care’ (National Society for Medical Research) and the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86–23, revised 1996).
2.1. Anesthesia and surgical preparation

Each subject was sedated (ketamine (15 mg/kg, i.m.) and atropine (0.02–0.05 mg/kg, i.m.) before orotracheal intubation. Fentanyl (30–200 μg/kg/h i.v.) and ketamine (4–10 mg/h i.v.) infusions maintained anesthesia throughout the study. Subjects were paralyzed (pancuronium, 0.7 mg/kg i.v. bolus and 0.1 mg/kg/h i.v. infusion). After median sternotomy and pericardiotomy, pressure transducer catheters were placed in the right atrium (RA), left atrium (LA), and pulmonary artery (PA). A 10-mm flow probe (Transonic System, Inc, Ithaca, NY, USA) encircled the aorta proximal to the innominate artery, allowing cardiac output (CO) determination. After recording baseline measurements, the RA and innominate artery were cannulated for CPB.

2.2. Conduct of cardiopulmonary bypass

The CPB circuit was primed with crystalloid solution and fresh donor porcine whole blood, maintaining a circuit hematocrit (HCT) percentage of 18–22%. Each subject was anticoagulated (unfractionated heparin, 300 U/kg) and placed on hypothermic (28 °C) CPB at calculated flows of 120 ml/kg/min. The aorta was not clamped. After 60 min of CPB, subjects were rewarmed to 36 °C and weaned from CPB. During rewarming, neither group underwent ultrafiltration.

2.3. Technique of modified ultrafiltration

A size-appropriate, polyarylethersulfone hemoconcentrator (COBE HC 700 Midi COBE Cardiovascular Arvada, CO, USA) was placed between the arterial and venous limbs of the CPB circuit. The hemoconcentrator inlet was clamped during CPB. After CPB, the inlet was unclamped, permitting perfusion from the arterial CPB limb through the filter (10–15 ml/kg/min) before returning to the subject via the venous limb. Additional blood from the venous reservoir was returned to the venous cannula as needed to maintain intravascular volume and stable hemodynamics. Inotropic support after separating from CPB was avoided. Arteriovenous MUF was performed until the HCT reached the goal of 35%.

2.4. Hemodynamic data collection

CO was estimated by integrating aortic flow probe data over 15 s. Mean pulmonary artery (MPa) pressures and left atrial (MLA) pressures were recorded directly. Pulmonary vascular resistance (PVR) was determined as:

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PVR = (\frac{MPa - MLA}{CO}) \times 80 \text{ dynes/s/cm}^5.
\]

2.5. Inflammatory cytokine assessment

At each study time point, 10 ml of sterile saline was injected via the endotracheal tube, followed by gentle suctioning to retrieve bronchoalveolar lavage (BAL) specimens for alveolar cytokines IL-6, IL-8, and tumor necrosis factor (TNF)-α. Systemic venous blood was collected at each study time point for determination of IL-6, IL-8, and TNF-α concentrations. Ten milliliter aliquots of MUF were collected to quantify the presence of pro-inflammatory cytokines. The concentration of each cytokine was determined using enzyme-linked immunosorbent assays (ELISA, R&D Systems, Minneapolis, MN, USA). Samples were stored at –90 °C and batch processed.

2.6. Transpulmonary thromboxane gradient

Serum samples for transpulmonary TxB2 gradients were taken from the PA and left atrium immediately prior to instituting CPB, immediately prior to separating from CPB, and at the end of study (EOS). TxB2 concentrations were determined using ELISA (Cayman Chemical, Ann Arbor, MI, USA), with samples diluted 1:10 as per the manufacturer’s instructions.

2.7. Study endpoint

Following separation from CPB (control group) or completion of MUF, subjects were euthanized 4 hours later (EOS). Physiological data were recorded prior to EOS. Euthanasia was performed by intravenous overdose of sodium pentobarbital (Euthanal® solution®, 100 mg/kg).

2.8. Data analysis

Student’s t-tests compared data between groups at various study time points, while analysis of variance (ANOVA) with repeated measures compared data within individual groups at different study time points. A P<0.05 was considered significant. Analyses were carried out using Intercooled STATA Version 9.0 software (College Station, TX, USA). All data are reported as mean ± standard deviation unless otherwise noted.

3. Results

Baseline weights and baseline and postCPB %HCT were similar between groups. After MUF, %HCT was significantly increased (postCPB HCT 21.7 ± 3.3% vs. postMUF HCT 33.6 ± 2.3%, P<0.001). Mean arterial pressure (MAP) was similar between groups at all study time points (Table 1), but postMUF MAP was significantly increased relative to the postCPB state in MUF subjects (postCPB MAP 40.2 ± 6.4 mmHg vs. postMUF MAP 51.7 ± 11 mmHg, P=0.005). Following CPB, PVR was significantly increased in both groups. PVR increased among controls to EOS, but MUF was associated with significantly reduced PVR relative to postCPB data, and was significantly less at EOS compared with controls (MUF PVR 5500 ± 1900 dynes/s/cm² vs. control PVR 10,100 ± 5100 dynes/s/cm²; P=0.03; Fig. 1).

Plasma concentrations of proinflammatory cytokines were evaluated at baseline, after CPB, and EOS in both groups. In both groups, TNF-α concentrations postCPB were significantly elevated compared with baseline (Table 2). Subsequently, TNF-α plasma concentrations did not change in either group. IL-6 and IL-8 concentrations were unchanged postCPB relative to baseline in both groups. However, IL-6 and IL-8 concentrations were significantly elevated at EOS relative to baseline in both groups; no differences between
MUF and control were observed (Table 2). Examination of the ultrafiltrate collected during MUF was notable for significant concentrations of proinflammatory cytokines, indicating successful removal of each of these inflammatory mediators during MUF (Table 2).

Alveolar TNF-α concentrations were not different between groups or within groups at any time point (Fig. 2). Conversely, alveolar IL-8 concentrations increased in both groups following CPB relative to baseline. In addition, BAL IL-6 concentration was significantly increased in both groups following CPB time point (P = 0.036), while BAL IL-8 did not increase further after MUF (Fig. 3). Similarly, CPB increased alveolar IL-6 concentrations in both groups relative to baseline (Fig. 4). BAL IL-6 was significantly increased in controls at EOS compared with postCPB (Fig. 4, P = 0.01). In contrast, BAL IL-6 did not increase after MUF and at EOS BAL IL-6 concentration was significantly less among MUF subjects compared with control subjects (Fig. 4, P = 0.015).

The transpulmonary TxB2 gradient was determined at baseline, after CPB, and at EOS in both groups. In the MUF group, transpulmonary TxB2 gradient was significantly reduced at EOS compared with the postCPB state [208 ± 200 pg/ml postCPB vs. 141 ± 120 pg/ml EOS, P = 0.04; (Fig. 5)]. In contrast, transpulmonary TxB2 gradient was significantly increased in controls at EOS relative to postCPB state [63 ± 60 pg/ml postCPB vs. 730 ± 360 pg/ml EOS, P = 0.04; (Fig. 5)]. The difference in TxB2 gradients between MUF subjects and controls at EOS was also significant (P = 0.04).

4. Discussion

This study assessed the pulmonary inflammatory response to CPB and its potential attenuation with MUF in a neonatal piglet model. Following CPB, subjects undergoing MUF demonstrated significantly reduced pulmonary-based inflammation, as reflected by reduced pHTN, reduced alveolar concentrations of IL-6 and IL-8 relative to controls, and decreased transpulmonary TxB2 concentrations. These observations are among the most informative details describing the physiological effects of MUF and lend insight into the possible mechanisms behind the well-recognized clinical benefits of MUF [3, 6]. These results suggest that MUF exerts beneficial postCPB effects by mitigating the consequences of upregulated mediators of inflammation, reducing effective pulmonary concentrations of inflammatory cytokines, and limiting the cascade of inflammatory events leading to adverse clinical outcomes.

Table 2. Plasma concentrations of proinflammatory cytokines IL-6, IL-8, and TNF-α are shown for control and modified ultrafiltration (MUF) groups at baseline, after cardiopulmonary bypass (postCPB), and at the end of study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>PostCPB</th>
<th>Ultra-filtrate</th>
<th>End of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>14 ± 30</td>
<td>140 ± 200</td>
<td>1800 ± 1000*</td>
<td>1800 ± 1000*</td>
</tr>
<tr>
<td>MUF</td>
<td>70 ± 90</td>
<td>30 ± 40</td>
<td>34.6 ± 48</td>
<td>1400 ± 2000*</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>25 ± 60</td>
<td>12 ± 40</td>
<td>23.4 ± 37</td>
<td>1100 ± 1400*</td>
</tr>
<tr>
<td>MUF</td>
<td>80 ± 90</td>
<td>20 ± 40</td>
<td>23.4 ± 37</td>
<td>1100 ± 2000*</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>140 ± 200</td>
<td>800 ± 700*</td>
<td>80 ± 80</td>
<td>850 ± 600</td>
</tr>
<tr>
<td>MUF</td>
<td>40 ± 50</td>
<td>1140 ± 1000*</td>
<td>80.8 ± 80</td>
<td>1140 ± 600</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. baseline conditions.
The proinflammatory cytokines evaluated in this study are notorious in CPB-induced inflammation. Following institution of CPB, various systemic inflammatory mediators are released and activate circulating neutrophils. In turn, neutrophils stimulate locally-active proinflammatory cytokines in tissues, such as the lung to propagate the inflammatory response [1]. For example, cytokines released within the pulmonary alveoli may diffuse into the pulmonary interstitium to ‘prime’ the pulmonary endothelium for neutrophil adherence and inflict further tissue damage [7]. These processes most likely underlie heightened inflammatory responses within the lung [2, 6, 7].

It is interesting to note that plasma TNF-α concentrations peaked earliest after CPB in the present study (Table 1), and TNF-α appeared in greatest concentrations within the ultrafiltrate. Since TNF-α is recognized as the chief proinflammatory cytokine activating neutrophils early in the inflammatory response [8], it one potential mechanism to explain reduced inflammation in the present study (reduced alveolar IL-6 and IL-8 concentrations) could be reduced TNF-α – neutrophil interactions after MUF, but confirmatory studies are required.

An intervention which reduces concentrations of alveolar cytokines may have direct clinical relevance since elevated alveolar levels of IL-8 and IL-6 are prognostic markers for adverse clinical outcomes associated with pulmonary inflammatory disorders [4, 5] through neutrophil activation and interference with neutrophil apoptosis, the primary method for resolving neutrophil-mediated inflammation [9]. In addition, IL-8 is a strong chemoattractant factor, leading...
to transmigration of neutrophils from the bloodstream and into tissues, especially in the lung [10]. This phenomenon has been noted after CPB [1]. No study has directly evaluated the effect of MUF on pulmonary neutrophil activity or pulmonary neutrophil elastase concentrations, but these appear to affect pulmonary outcomes in other injury models [5].

The observations of the present study regarding transpulmonary TxB2 concentrations corroborate TxB2 as a key mediator in postCPB pHTN [11]. Once again, activated neutrophil-endothelial interactions stimulate the release of various cytokines-induce thromboxane elaboration [11]. Therefore, preventing neutrophil adherence to vascular endothelium may blunt postCPB inflammation via reduced thromboxane production. In fact, Friedman et al. noted that neutrophil integrin blockade reduced thromboxane synthesis in sheep, resulting in less pulmonary injury after CPB [12]. Further studies are required to help define these potential mechanisms by which MUF may be involved with reduced thromboxane elaboration.

This study has several limitations. For example, we noted significant biological variability in the raw data and in several outcome variables (see Figs. 1 and 5). In addition, the study time points did not exactly match for the two groups studied secondary to the time required to perform MUF. However, this was typically limited to 15–30 minutes. Some of the recognized benefits of ultrafiltration may be realized regardless of the particular strategy for volume removal [13]. Therefore, we cannot specifically attribute the observed benefits to the version of ultrafiltration used in the present study. In fact, ultrafiltration performed prior to or during CPB may actually be more effective than MUF for removing inflammatory mediators [13, 14]. We studied MUF since it appears to be more uniformly applied in current clinical practice [15].

In summary, we evaluated the effects of CPB and MUF on pulmonary-derived inflammatory mediators. For the first time, MUF is correlated with reduced alveolar concentrations of proinflammatory cytokines IL-6 and IL-8, and transpulmonary thromboxane concentrations, implying that pulmonary-based inflammation is reduced. These results lend insight into possible mechanisms by which beneficial clinical effects have been noted. However, due to the complex nature of the systems involved, additional studies are required to further characterize the biological effects by which MUF confers recognized clinical advantages.

References