Effects of pulmonary artery perfusion with urinary trypsin inhibitor as a lung protective strategy under hypothermic low-flow cardiopulmonary bypass in an infant piglet model

W Li,1 X Wu,1 F Yan,1 J Liu,1 Y Tang,2 K Ma1 and S Li1

Abstract
Objective: This study aimed to evaluate the effects of pulmonary artery perfusion with a urinary trypsin inhibitor (UTI) as a lung protective strategy in order to provide an experimental basis for immature lung clinical protective strategies on deep hypothermia with low-flow (DHLF) cardiopulmonary bypass (CPB)-induced pulmonary injury in an infant piglet model.

Methods: The piglets (\( n=15 \)), aged 18.7±0.3 days, weight 4.48±0.21 kg, were randomly divided into 3 groups, with 5 piglets in each group: the control group, the pulmonary artery perfusion without UTI group (Group P) and the pulmonary artery perfusion with UTI group (Group U). The levels of the cytokines tumour necrosis factor-\( \alpha \), myeloperoxidase, malondialdehyde and interleukin-10 (TNF-\( \alpha \), MPO, MDA and IL-10) in pulmonary venous serum and lung tissue and the activity of NF-kappa B in lung tissue were measured by enzyme-linked immunosorbent assay (ELISA) and electrophoresis mobility shift assay (EMSA), respectively.

Results: After DHLF-CPB, all of the piglets demonstrated a state of lung injury as a deterioration of lung function indices, lung injury scores, pulmonary ultrastructure changes, expression of TNF-\( \alpha \), MPO, MDA and IL-10 and the activities of nuclear factor-kappa B (NF-\( \kappa \)B), while pulmonary artery perfusion with UTI significantly ameliorated lung function and histopathological changes, with greatly decreased serum levels of TNF-\( \alpha \) and MPO compared to the other two groups. Also, we found an increase in the level of IL-10 in Group U lungs compared with that in Group P lungs, which correlated with a strong inhibition in the activity of NF-\( \kappa \)B.

Conclusion: Pulmonary artery perfusion with UTI ameliorated the DHLF-induced immature pulmonary injury in the lungs via a reduction of pro-inflammatory cytokine expression and up-regulated levels of IL-10 by inhibiting the activity of NF-\( \kappa \)B.

Keywords
deeep hypothermia with low flow; urinary trypsin inhibitor; pulmonary artery perfusion; nuclear factor kappa B; immature lung protection

Introduction
Deep hypothermia with low flow (DHLF) cardiopulmonary bypass (CPB) has been commonly used in the repair of complex congenital heart defects in neonates and infants in order to gain a bloodless field of vision for surgeons. However, the premature lungs are more vulnerable to DHLF-CPB and acute lung injury (ALI) is more severe than that in older children,1 which is still a major cause of infant death after CPB. The aetiology is multifactorial; blood is continuously in contact with the CPB circuit, which results in the activation of the cellular and humoural inflammatory pathways and, also, pulmonary ischaemia-reperfusion (IR) injury, which

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are considered the main causes of pulmonary dysfunction after CPB.2

Recent studies have reported the potential benefits of additional pulmonary artery (PA) perfusion to alleviate lung injury during cardiac surgery with CPB.3 Ulinastatin, a urinary trypsin inhibitor (UTI), was first isolated and purified from human urine in 1982.4 The severity of pulmonary IR injury can be reduced with an ulinastatin intravenous infusion in animal models and clinical practice5,6 and, adding ulinastatin in the pulmonary preservation solution can attenuate lung IR injury, inhibiting the inflammatory response.7

As a common transcription factor, NF-κB is abundant in the cytoplasm. Increasing evidence suggested that NF-κB plays a key role in the signal transduction pathways associated with the onset and development of immune and inflammatory reactions.8 To date, no studies have reported the effect of NF-κB activity on DHLF-CPB-induced immature lung injury.

Therefore, the aim of our study was to investigate the role of NF-κB in ALI induced by DHLF-CPB in a piglet model and assess the protective effect of UTI administered by pulmonary perfusion on DHLF-CPB-ALI.

Material and Methods

Animals

A total of 15 healthy piglets of both sexes, aging from 14 to 21 days (18.7±0.3 days) and weighing 2.4 to 7 kg (4.48±0.21 kg), were used in this study. All the piglets were randomly divided into 3 groups, the control group (n=5), the PA perfusion without UTI group (Group P, n=5) and the PA perfusion with UTI group (Group U, n=5). All animal experimental procedures were approved by the Institutional Animal Care and Use Committee, Fu Wai Hospital and the Beijing Council on Animal Care, Beijing, China. The animals received humane care in compliance with the "Guide for the care and use of laboratory animals" published by the US National Institutes of Health (publication no. 85-23, revised 1996).

Anesthesia and Monitoring

All the piglets were premedicated with intramuscular ketamine (10 mg/kg bodyweight [BW]) and given atropine (0.05 mg/kg BW) and midazolam (0.5 mg/kg BW) and anaesthesia was maintained by an intravenous infusion of fentanyl (10 to 15 µg/kg BW/h) and vecuronium (0.1 to 0.12 mg/kg BW/h) and, then, they were endotracheally intubated.

Volume-controlled ventilation was used at a frequency of 25 to 35 breaths per minute, a tidal volume of 8 ml/kg BW and a positive end-expiratory pressure of 4 cmH2O. The inspired oxygen fraction (FiO2) was constantly 0.8 and the inspiratory expiratory ratio (I:E) was 1:1.5. Meanwhile, all the piglets inhaled 1% sevoflurane, using a Dräger Primus anaesthetic machine (Dräger, Lübeck, Germany).

The right carotid artery and jugular veins were catheterized to monitor blood pressure, blood gas analysis and central venous pressure and to administer medication. During surgery, the electrocardiograph (ECG) and nasopharyngeal temperature were also monitored. Appropriate treatment was administered based on the indicators from the monitor data.

Experimental design conduct of CPB

Following median sternotomy and heparinization [400-500 IU/kg (4-5 mg/kg) BW], a non-pulsatile systemic CPB flow was established at 2.4 L/min/m2 via a 10F aortic cannula and a 22F venous cannula in the right atrial appendage. The CPB circuit was equipped with a roller blood pump (Stöckert, Munich, Germany) and a CAPiox RX05 Baby-RX Oxygenator (Terumo, Tokyo, Japan). A new and sterile circuit was used for each experiment. The CPB circuit (Tianjin Plastics Research Institute, Tianjin, China) was primed with 400 mls of porcine blood, 200 mls 6% hydroxyethyl starch and heparin (400 IU/kg BW). Blood gas management was conducted according to the principle of alpha-stat management.

When the nasopharyngeal temperature reached 30°C, cardiac arrest was obtained by the use of an aortic cross-clamp and infusion of antegrade 4°C St. Thomas' cardioplegia solution through the aortic root. Deep hypothermia (20°C-25°C, nasopharyngeal temperature) and a low flow rate of 25–50 ml/kg/min were used during DHLF. The mean systemic pressure was maintained at approximately 50 mmHg. After 120 min of cardiac arrest, the piglets were rewarmed to a nasopharyngeal temperature of 35°C in about 30 minutes. Then, assisted circulation lasted 30 minutes before weaning from CPB and all the piglets received a modified ultrafiltration procedure during this time. After that, the piglets were given standard ventilation for 120 minutes before being sacrificed, using an intravenous hypokalaemic injection.

Study protocol

During the period of cardiac arrest, the main pulmonary artery was clamped and perfused with a lung protective solution via an 18G I.V. catheter in Group P and Group U, which was drained from the left atrium. The solutions were given at 4°C and 35-40 cmH2O gravity pressure. The initial perfusion was 15 ml/kg and half the amount of perfusion was given every 30 minutes during in the remaining cardiac arrest time.
In Group U, the pneumo-protective solution consisted of ulinastatin (12,000 IU/kg), NaHCO$_3$ (2 g/L), methylprednisolone (30 mg/kg), anisodamine (1 mg/kg), L-arginine (10 g/L) and 6% dextran. In Group P, the pneumo-protective solution consisted of the same solution, but without ulinastatin.

**Physiologic measurements and specimen collection**

The following variables were recorded at four defined time-points: before CPB (T1), at the end of CPB (T2) and at 60 minutes (T3) and 120 minutes (T4) after CPB: lung compliance (LC) and peak airway pressure (PAP) (obtained from the anaesthetic machine). Arterial blood samples were sampled and analyzed with a blood gas analyzer (Nova Biomedical, Waltham, MA, USA). Respiratory index (RI), oxygenation index (OI) and the M index (Ratio of PaO$_2$ to PAO$_2$) were calculated as:

\[
RI = \frac{A-aDO_2}{PaO_2} = \frac{PaO_2 - PaO_2}{PaO_2}, \quad OI = \frac{PaO_2}{FiO_2} \quad \text{and} \quad M \text{ index} = \frac{PaO_2}{PAO_2},
\]

PAO$_2$ = FiO$_2$[(BP − PH$_2$O) − (PaCO$_2$ / RQ)]

FiO$_2$ is the fractional concentration of oxygen in the inspired air and the value is obtained from the anaesthetic machine. BP: the barometric pressure; PH$_2$O: the saturated vapor pressure; PaCO$_2$: the arterial carbon dioxide tension pressure; and RQ: the respiratory exchange ratio.

Arterial blood gas analysis was performed and pulmonary venous blood was sampled at the same time intervals. Serum was collected by centrifugation at 4°C with 3000 g for 15 minutes and stored at -80°C until cytokine analysis was performed. At the completion of the 120 minutes observation, the tissue from the right lower lung lobe was harvested and cut into two pieces and flushed with cool phosphate buffered saline (PBS) solution. One piece was stored in liquid nitrogen for further examination, the second piece was fixed with 10% neutral buffered formalin for 24 h and embedded in paraffin; sections of 4 µm were stained with hematoxylin and eosin (HE staining) for light microscope observation.

**TNF-α, MPO, MDA and IL-10 levels**

Frozen lung tissues were homogenated in cold normal saline and centrifuged to obtain the supernatant for measurement. The levels of TNF-α and IL-10 in the serum and lung tissue were measured using the ELISA kits supplied by the Qiagen Biological Technology Co. Ltd., Beijing, China.

**NF-κB DNA binding activity**

The expression of NF-κB was examined by using a non-radioactive electrophoretic gel mobility shift assay (EMSA) kit (Pierce, Thermo Fisher Scientific Inc., Rockford, IL, USA), following the manufacturer's instructions. The nuclear extracts were obtained from lung tissue by using a cytoplasmic and nuclear protein extraction kit and the concentration was determined by BCA Protein Assay Kit. Incubation of 300 fmol of biotin-probe labelled double-stranded NF-κB oligonucleotide with the nuclear extracts (20 µg) at room temperature for 20 min. Two microlitres of specific competitor (cold oligonucleotide) was added in a 100x competing system. Nuclear extract from THP-1 cells stimulated by 50 ng/ml TNF-α for 45 min was used as the positive control. The mixture was electrophoresed on 6.5% polyacrylamide hydrophilic gels and transferred to Whatman paper, after the membrane was fixed and dried, the DNA-protein complexes were visualized by the imager apparatus (Alpha Fluorochemical) and the optical density of each band were quantified by ImageQuant software (GE Healthcare Life Sciences, Shanghai, China). For the supershift assay, antibodies against NF-κB/P65 and NF-κB/P50 were added 20 min after the beginning of the reaction and probe labelled; incubation was continued for another 20 min.

**Histopathology**

For each paraffin section sample, 20 random fields were examined at ×400 magnification. Within each field, scoring of histological sections was done in compliance with the recommendation of the American Thoracic Society by two pathologists who were blinded to the groups. Lung injury was scored, based on 5 parameters: (1) neutrophils in the alveolar space, (2) neutrophils in the interstitial space, (3) hyaline membranes, (4) proteinaceous debris filling the airspaces and (5) alveolar septal thickening. Each of the five histological findings was graded using a three-tiered schema, resulting in a possible score by a specific equation as reported previously.

**Statistical analysis**

All values are given as mean±standard deviation (SD). Data were analyzed using SPSS 18.0 software for Windows (SPSS Inc, Chicago, IL, USA). Two-way analysis of variance for repeated measurements was used to compare variables within and between groups.
Differences between the groups were tested for significance by the Student's t-test for unpaired samples or repeated measures of analysis of variance (ANOVA). A value of $p<0.05$ was considered statistically significant.

**Results**

**Lung function**

The blood gas analysis for the three groups are presented in Table 1. At the end of the study, the partial pressure of oxygen was improved in both perfused groups, especially the partial pressure of the carbon dioxide in Group U, which was significantly lower than in the other two groups. We found no significant group differences or group differences over time in pH, haemoglobin (Hb), lactate and standard base excess in this study.

When lung function indices were considered, lung compliance, oxygenation index, respiratory index and M index significantly worsened after the CPB procedure in the three groups; until T3, the degree of the decline was attenuated (time $- p<0.0001$). This was more noticeable in the control Group (group $- p<0.05$; time$^* \text{group} - p<0.05$, Table 2). The value of these indices in Group U was significantly higher than that of the control group. Of interest is that, at the end of CPB, the value of the peak airway pressure (PAP) in Group U group was significantly lower than in the others.

DHLF-CPB increased the levels of TNF-α, MPO, MDA and IL-10, which could be attenuated by UTI perfusion, except for the increase of IL-10

The levels of TNF-α, MPO and MDA in Group P and Group U were significantly lower than the control group at T3 and T4 (Figures 1A-C). The concentrations of TNF-α and MPO were significantly lower in Group U compared with the values of the other groups at the postbypass period and at the end of the study, respectively. There was no significant difference in IL-10 expression between the control group and Group P. In Group U, the concentration of IL-10 was significantly higher than in the other two groups at T3 and T4 (Figure 1D).

Urinary trypsin inhibitor perfusion significantly decreased the values of TNF-α, MPO and MDA in lung tissues compared to the control group ($p<0.05$) and were also significantly different compared to Group P except for the level of TNF-α and MDA. No significant difference existed between Group P and the control group in the level of IL-10 in lung tissues (Table 3).
Inhibition of NF-κB DNA binding activity by UTI perfusion

The EMSA indicated that DHLF-CPB induced higher DNA binding activity of NF-κB in the control group compared with the two perfusion groups as the shift banding was evidently visible (87.48±3.36, p<0.05; vs. P and U; Figure 2C). The NF-κB activation was significantly inhibited by UTI perfusion compared with Group P (U 57.73±1.81 vs. P 63.16±2.25 p<0.05; Figure 2C).

The specificity of the observed NF-κB DNA complex was shown by the assay of cold competition (lane 10, Figure 2A). Supershifting experiments showed that the complexes observed in the nuclear protein extracts from lung specimens contained p65 and p50 subunits of the NF-κB family, as shown, that were displaced by A1P65 antibody and A1P50 antibody (lanes 4 and 5, Figure 2B).

UTI perfusion alleviated lung injury shown in histopathological changes

The observation indicated that more severe lung injury happened in piglets which received DHLF-CPB without pulmonary perfusion. Lung injury scores supported that the lung injury level of piglets treated with UTI perfusion was significantly lower than that of the controlled group and Group P (U 0.30±0.04 vs. C 0.78±0.03 and P 0.57±0.04, p<0.05). The lungs in Group P showed moderate lung injury. (Figure 3A-C)

Discussion

CPB is a contributor to the inflammatory response which results in major organ dysfunction after cardiac surgery. On the other hand, the lung ischaemic re-perfusion injury also causes inflammatory activation and subsequent lung injury, a common post-operative complication of CPB. In the studies of Skaryak and Schultz, they found that DHLF was associated with worse pulmonary function when compared with DHCA in animal models. Due to the incomplete anatomical differentiation, poor functional capacity and biochemical maturity, the infant's premature lungs are highly susceptible to injury during DHLF CPB. The previous studies have shown that the use of PA perfusion may partially alleviate lung injury, which is consistent with a previous study. While the LC, RI, OI and MI of Group U at T4 compared with the control group, it also revealed that UTI perfusion alleviated lung injury. The UTI may alleviate lung

Table 2. Lung function indices.

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>p&lt;0.0001</th>
<th>0.007</th>
<th>0.221</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung compliance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Controls</td>
<td>4.42±0.57</td>
<td>2.36±0.50</td>
<td>1.98±0.46</td>
<td>1.90±0.41</td>
<td>&lt;0.0001</td>
<td>0.067</td>
<td>0.221</td>
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<tr>
<td>Group P</td>
<td>4.70±0.73</td>
<td>2.48±1.43</td>
<td>2.28±1.28</td>
<td>2.40±1.19</td>
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<tr>
<td>Group U</td>
<td>4.72±0.62</td>
<td>3.34±0.27</td>
<td>3.38±0.31*</td>
<td>3.30±0.39*</td>
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<tr>
<td><strong>Peak airway pressure (cmH2O)</strong></td>
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<tr>
<td>Controls</td>
<td>14.2±1.64</td>
<td>26.0±3.74</td>
<td>26.8±2.58</td>
<td>27.8±2.49</td>
<td>&lt;0.0001</td>
<td>0.033</td>
<td>0.083</td>
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<td>Group P</td>
<td>12.2±3.27</td>
<td>26.8±1.64</td>
<td>27.0±1.58</td>
<td>25.8±4.66</td>
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<tr>
<td>Group U</td>
<td>13.0±2.34</td>
<td>21.2±2.16*</td>
<td>22.4±4.27</td>
<td>22.0±4.06</td>
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<tr>
<td><strong>Respiratory index</strong></td>
<td></td>
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<tr>
<td>Controls</td>
<td>0.84±0.25</td>
<td>4.66±2.13</td>
<td>7.09±1.11</td>
<td>6.55±1.07</td>
<td>&lt;0.0001</td>
<td>0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Group P</td>
<td>1.03±0.39</td>
<td>4.20±2.94</td>
<td>3.63±2.63*</td>
<td>3.41±2.14*</td>
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<tr>
<td>Group U</td>
<td>0.86±0.48</td>
<td>1.60±0.90</td>
<td>1.77±0.80*</td>
<td>1.81±0.69*</td>
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<tr>
<td><strong>Oxygenation index</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Controls</td>
<td>365.1±51.1</td>
<td>134.8±61.9</td>
<td>80.4±12.1</td>
<td>86.5±12.5</td>
<td>&lt;0.0001</td>
<td>0.025</td>
<td>0.012</td>
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<tr>
<td>Group P</td>
<td>332.6±61.6</td>
<td>166.1±90.8</td>
<td>189.9±109.3</td>
<td>182.1±82.7</td>
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<tr>
<td>Group U</td>
<td>373.1±90.8</td>
<td>283.6±115.8</td>
<td>250.7±68.8*</td>
<td>243.0±56.1*</td>
<td></td>
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<tr>
<td><strong>M index</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.55±0.08</td>
<td>0.20±0.09</td>
<td>0.13±0.02</td>
<td>0.13±0.02</td>
<td>&lt;0.0001</td>
<td>0.024</td>
<td>0.014</td>
</tr>
<tr>
<td>Group P</td>
<td>0.51±0.10</td>
<td>0.25±0.13</td>
<td>0.28±0.16</td>
<td>0.27±0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group U</td>
<td>0.57±0.14</td>
<td>0.43±0.18</td>
<td>0.39±0.11*</td>
<td>0.37±0.09*</td>
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</table>

Pa=Within Groups; Pb= Between Groups; Pc=time*group. *p<0.05 vs time-matched values in the Control group.
Δp<0.05 vs time-matched values in Group P.
oedema to some extent, as the PaCO₂ of Group U was significantly lower than Group P at T4. Simultaneously, according to the histological findings, we confirmed that the extent of neutrophil infiltration, proteinaceous fluid, alveolar septal thickening and other pathological signs in lung tissue markedly decreased with UTI perfusion.

Previous studies showed that neutrophil aggregation and activation plays an important role in the development of the occurrence of lung IR injury.

Our results revealed that DHLF-CPB caused rapid elevation of MPO and MDA in serum, which correlated with the accumulation of activated neutrophils in the injured lung. UTI is recognized as being generated from pre-α-/inter-α-trypsin inhibitors, induced by neutrophil elastase during inflammation.

As a broad-spectrum, non-selective serine protease, it can inhibit various inflammatory proteases, such as trypsin, α-chymotrypsin, plasmin and neutrophil elastase. The animal experiments and clinical studies have shown that UTI can protect multiple organs from inflammation or injury-induced dysfunction via inhibiting the activity of these proteases.

Our results showed that pulmonary perfusion with UTI suppressed the expression of MPO and MDA in pulmonary venous serum and lung tissue, which demonstrated a reduction in neutrophil accumulation in the injured lung.

In unstimulated cells, NF-κB mainly consists of p50 and p65, which is sequestered in the cytoplasm by the IκB protein family. When exposure to TNF-α or other appropriate stimuli, IκB kinases (IκKs) are activated, leading to phosphorylation and ubiquitination of IκBs. The ubiquitination degrades IκB, releasing NF-κB, allowing it to enter the nucleus and activate transcription of the target gene, which is also important in modulating the expression of TNF-α. TNF-α is an important inflammatory factor released in a sequential manner to produce an inflammatory cascade and has improved an important mediator in CPB-induced lung injury.

IL-10 was considered as a potent anti-inflammatory cytokine that inhibits macrophage activation, inhibiting the expression of inflammatory mediators during the inflammatory response. A number of studies have demonstrated that IL-10 may suppress NF-κB activation through the preservation of IκB by inhibiting IκK activity.

As expected, perfusion with UTI further counteracted the inflammatory response in the lungs and had a significantly greater affect than on lungs perfused with non-UTI perfusate. In our study, the activation of NF-κB was consistent with the severity of lung injury, which is also consistent with the levels of TNF-α in the pulmonary venous serum and tissue and MPO and MDA, as described above. The production of TNF-α in Group U was down-regulated; the UTI in pulmonary perfusion may inhibit the activation of NF-κB by blocking the TNF-α positive feedback pathway. Furthermore, pulmonary perfusion with UTI resulted in significantly increased levels of IL-10, measured post CPB, which may be a result of increased synthesis and secretion of IL-10. These results suggest that UTI in the pulmonary perfusate may inhibit production of TNF-α and increase production of IL-10 to a considerable extent, which may regulate the activation of NF-κB, resulting in a greater anti-inflammatory effect than seen in lungs perfused with non-UTI solution during DHLF-CPB in the infant piglet.
Table 3. Lung tissue levels of TNF-α, MPO, MDA and IL-10.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group P</th>
<th>Group U</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>37.54±5.46</td>
<td>28.94±4.29*</td>
<td>25.88±3.55*</td>
<td>0.004</td>
</tr>
<tr>
<td>MPO (IU/L)</td>
<td>21.07±1.68</td>
<td>12.48±1.65*</td>
<td>9.97±1.06*,Δ</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MDA (nmol/L)</td>
<td>1.24±0.20</td>
<td>0.96±0.08*</td>
<td>0.93±0.06*</td>
<td>0.005</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>17.38±1.08</td>
<td>17.47±0.63</td>
<td>19.58±1.85*,Δ</td>
<td>0.031</td>
</tr>
</tbody>
</table>

TNF-α: Tumour necrosis factor alpha; MPO: myeloperoxidase; MDA: malondialdehyde; IL: interleukin.
*p<0.05 vs Control group. Δp<0.05 vs Group P.

Figure 2. (A) The activity of NF-κB complexes in different groups are shown in lanes 1-9. Lanes 1-3: Control group, lanes 4-6: Group P, lanes 7-9: Group C. The specificity was confirmed by competition with a 100-fold excess of unlabeled NF-κB in lane 10: cold competition assay, lane 11: positive control, lane 12: negative control. (B) Supershift analysis was conducted with antibodies directed against p65 (lane 4), p50 (lane 5). Lane 1: free probes, lane2: sample, lane3: 100x cold competition assay. (C) Amount of NF-κB DNA complexes in different groups. The activity of NF-κB in Control group was significantly higher than in P and U groups. *p<0.05 vs Control group. The NF-κB activation was significantly inhibited in Group U compared with Group P. Δp<0.05 vs the Group P.

Study limitation

This study was performed in a short-term animal model with a short post-bypass time and limited measurements. Due to the low bodyweight of the piglets used in our study, it was not possible to collect lung tissue samples sequentially at each checkpoint. In addition, the dose of ulinastatin, the flow of the perfusate and whether lung perfusion should be started prior to aortic cross-clamping are areas of future possible research. The aim of our study was solely to compare pulmonary inflammation and pulmonary function between pulmonary perfusion with and without UTI in DHLF-CPB, so the change observed in the pulmonary venous serum cannot be related to systemic inflammatory response.
Pulmonary perfusion with UTI may prove to be a valuable tool in preventing pulmonary complications in infants undergoing operations requiring DHLF-CPB; the application for a short duration of CPB needs to be studied.

Conclusion

In conclusion, this study showed that, in DHLF-CPB-induced lung injury, PA perfusion with UTI resulted in better lung protection in an infant piglet model than when non-UTI PA perfusion was used. The mechanism of this seems to be a reduction in the inflammatory reaction by inhibition of NF-κB activity, up-regulation of IL-10 and down-regulation of TNF-α. We believe that this procedure warrants further study.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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