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Ischemic preconditioning reduces deep hypothermic circulatory arrest cardiopulmonary bypass induced lung injury

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Abstract. – PURPOSE: Ischemic preconditioning (IP) has been used to reduce ischemia-reperfusion injury in several models. It remains unknown whether IP is sufficient to prevent deep hypothermic circulatory arrest (DHCA) cardiopulmonary bypass (CPB) induced lung injury.

MATERIALS AND METHODS: Twenty-four piglets were randomly divided into four groups: routine CPB (CPB), CPB + DHCA (DHCA), CPB + IP + DHCA (IP-1) and CPB + hypoxia-ischemia preconditioning + DHCA (IP-2). Lung static compliance (Cstat) and pulmonary vascular resistance (PVR) were measured as indicators of lung function at three points during CPB. TNF-α, IL-8 and IL-10 expressions were detected by radioimmunoassay. CD18 expression was determined by flow cytometer. Some lung tissues were excised to measure the wet/dry weight ratio (W/D) and some were fixed to observe pathological changes.

RESULTS: Cstat significantly decreased whereas PVR increased in DHCA group. IP prevented DHCA-induced lung functional impairment, especially IP-2 treatment. More cytokines were produced after CPB in all groups, but with varying level. Left atrium/pulmonary artery ratio of CD18 expression on monocytes decreased only in DHCA group, whereas which on polymorphonuclear neutrophils decreased in DHCA group, IP-1 group at 1h post-CPB and IP-2 group. Although lung W/D was increased in IP-2 group compared with pre-CPB, but significantly lower than that in DHCA group. Histological findings showed less lung injuries in IP groups than DHCA group.

CONCLUSIONS: DHCA aggravates lung inflammatory injury and IP may reverse this injury. Maintaining ventilation with pulmonary artery perfusion in the lung IP process during CPB seems to be more superior to single pulmonary artery perfusion.

Key Words:
Deep hypothermic circulatory arrest, Ischemic preconditioning, Ischemia reperfusion, Inflammatory response, Lung injuries.

Abbreviations

Cstat = lung static compliance; CPB = cardiopulmonary bypass; DHCA = deep hypothermic circulatory arrest; ICAM = intercellular adhesion molecule; IL: interleukin; IP: ischemic preconditioning; LA= left atrium; LFA = lymphocyte function associated antigen; LIRI = lung ischemia reperfusion injury; McAb = monoclonal antibody; PA = pulmonary artery; PBF = pulmonary blood flow; PMN = polymorphonuclear leukocytes; PVR = Pulmonary vascular resistance; PVRI = pulmonary vascular resistance index; TEM = transmission electron microscopy; TNF = tumor necrosis factor; TV = tidal volume; VCAM = vascular cell adhension molecule; W/D = weight/dry.

Introduction

Deep hypothermic circulatory arrest (DHCA) is a common cardiopulmonary bypass (CPB) technique in the surgical treatment of complex congenital heart diseases as well as other conditions that require complete cessation of flow[1]. However, prolonged ischemia (hypoxia) from cross-clamping of the aorta and reperfusion (re-oxygenation) injury upon unclamping can cause severe pulmonary injury, leading to increased morbidity and mortality[2].

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To alleviate lung injury, a number of possible interventions have been proposed, including modification of pump circuit surfaces, ultrafiltration strategies, leukocytes trapping filters, and glucocorticoid administration[3-5]. However, these approaches turn to exogenous mechanism. The endogenous strategy involves in protective function of ischemic preconditioning (IP), which has already been proven to improve lung I/R injury in several animal models[6-9]. Importantly, IP has been demonstrated as an auxiliary measure to significantly reduce clinically relevant markers of myocardial and pulmonary injury after surgery, such as troponin-T[10-12]. In addition, recent studies also suggest low volume[13] or low frequency[14] ventilation during CPB can reduce the postoperative lung injury. However, there are still rare researches on the application of IP with or without ventilation in CPB accompanied with DHCA[15]. Therefore, in this research, different IP treatments have been designed before DHCA in the cardiac surgery to evaluate the protective function of IP on DHCA induced lung damage.

Materials and Methods

Animal Model

All animal studies have been approved by China Ethics Committee and performed in accordance with the ethical standards. Healthy male and female Shanghai piglets of a native stock (aged 3-4 weeks, weighting 7-10 kg), were provided by the Field of Laboratory Animals (Songjiang District, Shanghai, China). All rats were housed at room temperature (18-25°C) with a relative humidity of 65% and a 12 h light/dark cycle. Food and water deprivation was respectively performed 12 h and 8 h before operation. Anesthesia was induced by intramuscular injection of ketamine (7 mg/kg) and atropine (20 μg/kg) and maintained with intravenous thiopentone (6 mg/kg/h) and succinylcholine (3-6 mg/kg/h). Animals were ventilated mechanically (Servo 900C, Siemens, Munich, Germany) after tracheotomy with an inspiratory frequency of 20 breaths/min, a tidal volume (TV) of 10 mL/kg and inspired oxygen concentration of 60-80%. The right femoral artery was cannulated for mean arterial pressure monitoring. Exposure of the heart was achieved after median sternotomy and then the pulmonary artery and the left atrium were cannulated for measuring pulmonary artery pressure (PAP) and left atrium pressure (LAP). The electromagnetic flow meter was used to determine pulmonary blood flow (PBF). Pulmonary vascular resistance index was calculated according to the following formula: PVRI = [(PAP – LAP)/PBF] mmHg/kg/min/mL. Lung static compliance (Csat) was calculated as follows: Csat = TV/P-pause mL/cmH_2O. The PVRI and Csat were recorded at three time points: CPB starting point, CPB endpoint and 1h after the endpoint.

Animal Grouping

Twenty four piglets were randomly divided into 4 groups: routine CPB group (CPB, n = 6), CPB + DHCA group (DHCA, n = 6), CPB + IP + DHCA group (IP-1, n = 6) and CPB + hypoxia-ischemia preconditioning + DHCA group (IP-2, n = 6). Two adult Shanghai piglets (weighting 60 kg) were used as blood donor.

After systemic anticoagulation with heparin (3 mg/kg), piglets were connected to CPB via cannulae in the ascending aorta and the right atrium. Within the first 3 min, when the CPB pump flow rate of all groups was gradually increased to 100-150 mL/kg/min at room temperature (35°C-37°C), mechanical ventilation would be terminated.

In the CPB group, animals were cooled to a rectal temperature of 18°C after 30 min of perfusion, and then perfusion continued for another 60 min with the mechanical ventilation at room temperature.

In the DHCA group, animals were cooled to a rectal temperature of 18°C and the ascending aorta was cross-clamped after 30 min of perfusion. Then, cold crystalloid cardioplegic solution (15 ml/kg) was administrated, and the CPB was stopped for 60 min. Subsequently, the clamp was released. Animals were rewarmed to a rectal temperature of 35°C within 30 min and perfusion continued for another 30 min with the mechanical ventilation.

In the IP-1 group, 5 min after initiation of CPB, the blood in the shunts was perfused into pulmonary artery via the right heart perfusion system at a flow rate of 25 mL/kg and then the blood of the lesser circulation was backflow into the venous reservoir through the left atrium drainage followed by perfusion with oxygenated blood for 10 min. After two cycles of 5 min ischemia and 10 min reperfusion, the ascending aorta was cross-clamped and DHCA was performed with the same steps as the DHCA group.

In the IP-2 group, the right heart perfusion system was also turned on 5 min after initiation of CPB, and simultaneously mechanical ventilation

was also initiated with an inspiratory frequency of 5 breaths/min[14]. After two cycles of 5 min hypoxia-ischemia and 10 min reperfusion, the ascending aorta was cross-clamped and DHCA was performed with the same steps as the DHCA group.

Finally, all groups were mechanically ventilated for 60 min after CPB[16]. The workflow of each group is shown in Figure 1. The rectal temperature, electrocardiogram, heart rate, $PaCO_2$ and PaO_2 were detected continuously using a monitor (Hewlett Packard, 78543C). Perioperative mean arterial pressure was maintained between 30-60 mm Hg, and blood gas analysis (AVL OMNI 3 blood gas analyzer, Roche Diagnostics, Switzerland) was performed every 30 min during CPB, with the $PaCO_2$ 4.66-5.99 Kpa (35-45 mmHg), PaO_2 10.6-20 Kpa (80-150 mmHg) and pH 7.35-7.45. During cooling and re-warming, an α-stat strategy was used[17].

Inflammatory Cytokines and Cells Assay

Blood was drawn from the pulmonary artery (PA) and left atrium (LA) at three time points and then the concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-8, and IL-10 were measured with radioimmunoassay kit (Northern Biological Reagent Institute, Beijing, China). Data were corrected with a standard hemachrome content of 10 g/dl (the corrected value = measured value *10/measured hemachrome content) and the LA/PA ratios of TNF-α, IL-8, and IL-10 expressions were calculated.

CD18 expressions in the neutrophils granulocytes and monocytes at three time points were detected by using the Epics XL flow cytometry (FCM; Beckman Coulter Inc., Brea, CA, USA) with the reagents provided by BD Biosciences Pharmingen (San Diego, CA, USA). CD18 expression was calculated as the expression value of rat anti-pig CD18-PE labeled monoclonal antibody (McAb) minus that of the control rat IgG1-PE McAb. LA/PA ratio of CD18 was believed to negatively related with the number of monocyte and neutrophil granulocyte in the lung[18].

Wet/dry Ratio

Small pieces of lung tissue were excised, rinsed briefly in PBS, blotted, and then weighed to obtain the "wet" weight. Lung tissues were then dried in a thermostat at 60°C for 48 h to obtain the "dry" weight. The wet to dry lung weight ratio was calculated, namely, the W/D.

Histopathological Analysis

After CPB, some lung tissues were dissected and fixed with 4% formalin, dehydrated, embedded in paraffin, sectioned, and hematoxylin-eosin (HE) staining to observe the pathological changes under optical microscope. Some lung tissues were fixed with glutaraldehyde to observe the ultrastructures under transmission electron microscopy (TEM; JEM-200CX, JEOL, Tokyo, Japan).

Statistical Analysis

All data were processed with the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA), and shown as average ± standard deviation. The differences among values obtained at various time points in the same group were analyzed with the repeated measure analysis of variance (ANOVA)

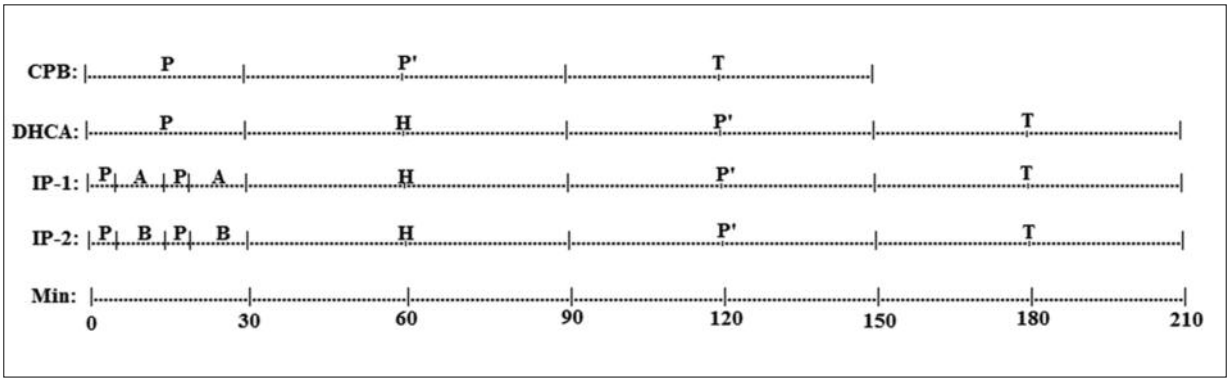

Figure 1. The experiment procedure of four groups. P: Pre-CPB parallel pulmonary artery perfusion (without mechanical ventilation; the animals were cooled to a rectal temperature of 18°C); P': Post-CPB parallel pulmonary artery perfusion (with mechanical ventilation; the animals were cooled to a rectal temperature of 35°C-37°C). ***A,*** Pulmonary artery perfusion with oxygenated blood (without mechanical ventilation). ***B,*** Pulmonary artery perfusion with oxygenated blood (with simultaneous mechanical ventilation); H: Deep hypothermic circulatory arrest (a rectal temperature of 18°C); T: Mechanical ventilation after CPB terminated.

and Bonferroni's test. Differences among groups were analyzed by using One-Way ANOVA and Fisher's LSD test. $p < 0.05$ was considered statistically significant.

Results

The Comparison of Respiratory Function Indexes

Overall, Csat significantly decreased whereas PVRI significantly increased after CPB. IP prevented DHCA-induced lung functional impairment, especially IP-2 treatment (Figure 2).

Comparison within the group. Compared to the pre-CPB value, Csat value in CPB group significantly decreased only at 1h post-CPB ($p = 0.001$) with no remarkable changes at the endpoint of CPB ($p = 0.097$). However, in the other three groups, the Csat decreased notably at the two time points after CPB ($p < 0.005$). The PVRI of all the groups significantly increased after CPB ($p < 0.001$), other than the IP-1 group in which the significant increase of PVRI was not observed right at the endpoint of CPB ($p = 0.118$).

Comparison among the groups. Compared to the CPB group, the Csat showed a significant decrease ($p = 0.0003$ and $p = 0.046$, respectively) at two time points post-CPB in the DHCA group. While the Csat in IP-1 group only decreased at the endpoint of CPB ($p = 0.001$) with no change at 1h post-PCB ($p = 0.182$). A opposite change trend was shown in the IP-2 group ($p = 0.007$ for 1h post-CPB and $p = 0.54$ for the endpoint). Compared to the DHCA group, Csat at two time points post-CPB of the IP-2 group increased significantly ($p < 0.01$) with no notable difference in IP-1 group ($p = 0.14$ and $p = 0.465$, respectively). In addition, the Csat values of IP-2 group at two time points post-CPB were significantly higher than those in the IP-1 group ($p = 0.002$ and $p = 0.004$, respectively).

As for the PVRI, only the DHCA group increased significantly at two time points after CPB compared to the CPB group ($p = 0.002$ and $p = 0.003$). Csat increased in IP-1 group at 1h post-CPB ($p = 0.0007$), with no difference at the endpoint ($p = 0.791$) compared to the CPB group. For the IP-2 group, there was no difference from the CPB group in PVRI at either time point ($p = 0.423$

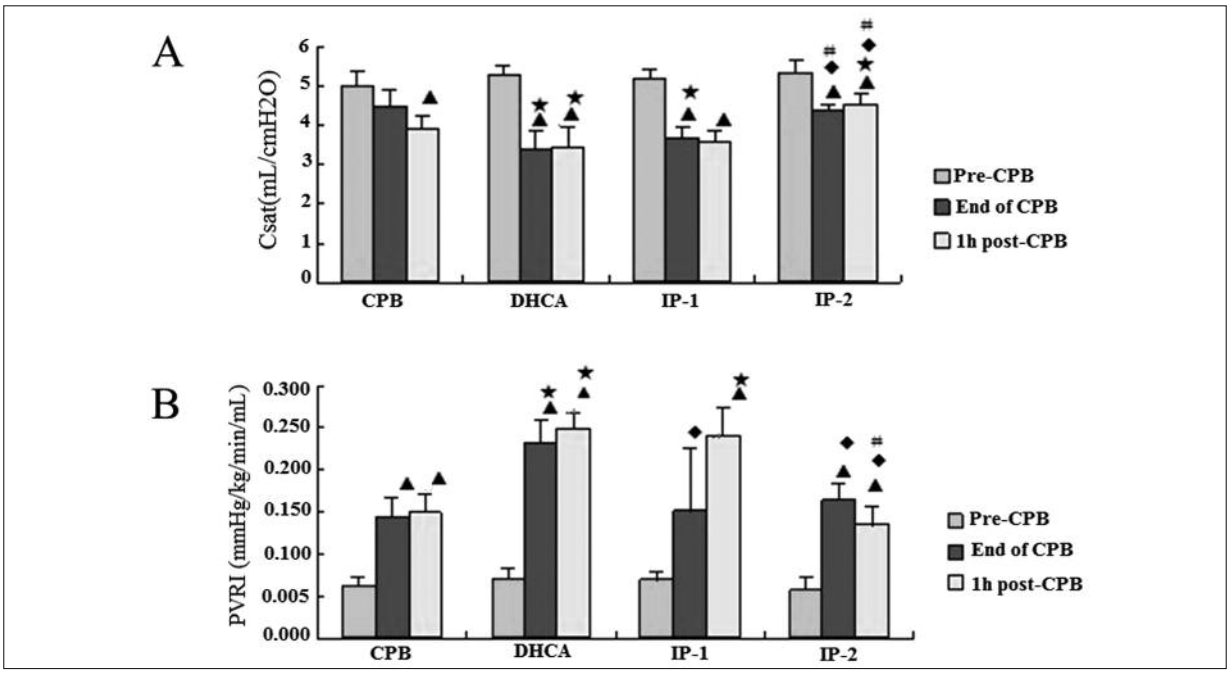

Figure 2. Comparison of respiratory function index in four groups. ***A,*** Csat (Lung static compliance). ***B,*** PVRI (Pulmonary vascular resistance index); CPB group: routine CPB; DHCA group: CPB+DHCA; IP-1 group: CPB + IP + DHCA; IP-2 group: CPB + hypoxia-ischemia preconditioning + DHCA; ▲vs the pre-CPB value, $p < 0.05$; ★vs Baseline group, $p < 0.05$; ◆vs Control group, $p < 0.05$; #vs IP-1 group, $p < 0.05$.

and $p = 0.283$). Compared to the DHCA group, PVRI decreased only at the endpoint of CPB in IP-1 group ($p = 0.004$), with no notable change at 1h post-CPB ($p = 0.591$), while PVRI displayed a decrease at two time points in IP-2 group ($p = 0.013$ and $p = 0.0005$, respectively). The PVRI of IP-2 group was similar to that of the IP-1 group at the endpoint of CPB ($p = 0.589$), with a remarkable decrease at 1h post-CPB ($p = 0.0007$).

LA/PA Ratios of Lung TNF-α, IL-8 and IL-10 expression

Comparison within the groups (Figure 3). The expression of TNF-α, IL-8 and IL-10 in each group increased significantly at two time points after CPB ($p < 0.05$).

Comparison among the groups (Figure 3). Compared with CPB group, LA/PA ratios of TNF-α in both DHCA group and IP-1 group significantly increased at 1h post-CPB ($p < 0.05$); the ratios for IL-8 both in DHCA group at two time points after CPB and in IP-1 group at the endpoint of CPB were significantly higher than those in CPB group ($p < 0.05$); the ratios for IL-10 in other three groups all increased. Meanwhile, compared with DHCA group, LA/PA ratios for TNF-α in IP-2 group greatly decreased at 1h post-CPB ($p < 0.05$), and the proportion for IL-8 and IL-10 notably decreased in the two IP groups ($p < 0.05$). There was no statistical difference between IP-1 group and IP-2 group ($p > 0.05$).

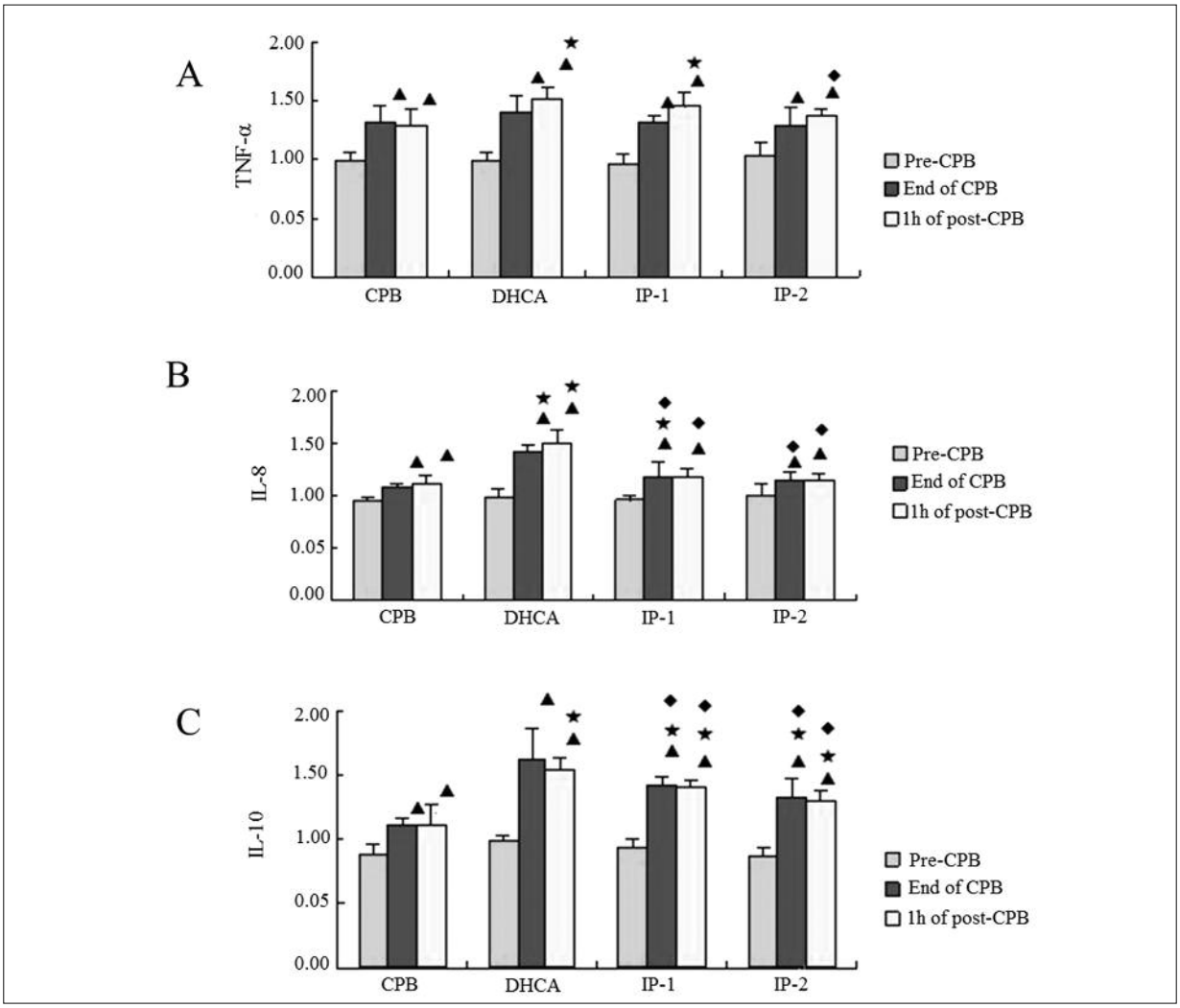

Figure 3. Comparison of LA/PA ratios of TNF-α, IL-8 and IL-10 in four groups. ***A,*** TNF-α. ***B,*** IL-8; C: IL-10; CPB group: routine CPB; DHCA group: CPB + DHCA; IP-1 group: CPB + IP + DHCA; IP-2 group: CPB + hypoxia-ischemia preconditioning + DHCA; ▲vs pre-CPB, $p < 0.05$; *vs CPB group, $p < 0.05$; ♦vs DHCA group, $p < 0.05$; #vs IP-1group, $p < 0.05$.

LA/PA Ratios of CD18 Expression on Monocytes and Neutrophils

Comparison within the groups (Figure 4). Compared with the value of pre-CPB, only the LA/PA ratios of CD18 on monocytes in the DHCA group decreased significantly after CPB; whereas ratios of CD18 on polymorphonuclear leukocytes (PMNs) significantly decreased in IP-1 group at 1h post-CPB, DHCA group as well as IP-2 group at two time points post-CPB ($p < 0.05$).

Comparison among the groups (Figure 4). The CD18 expression on monocytes was obviously lower in the DHCA group at two time points post-CPB and in the IP-1 group at 1h post-CPB compared with the CPB group ($p < 0.05$); that expression of IP-2 group increased greatly compared to DHCA group ($p < 0.05$). In contrast to the CPB group, the CD18 expression on PMNs was significantly reduced in all the other groups ($p < 0.05$) except that of the IP-2 group at 1h post-CPB ($p = 0.241$); CD18 expressions of the two IP groups at 1h post-CPB were much higher than that of the DHCA group ($p < 0.001$) while there was no difference between them ($p > 0.05$).

The Ratios of W/D

Comparison within the groups (Figure 5). Compared with the pre-CPB values, the W/D ratio of the DHCA group and IP-2 group increased greatly while the IP-1 group only showed a remarkable increase at 1h post-PCB ($p < 0.05$).

Comparison among the groups (Figure 5). Compared with CPB group, DHCA group had a much higher value of post-CPB W/D ($p < 0.05$) while W/D of IP-1 group was only significantly increased at 1h post-CPB ($p < 0.05$); Compared with DHCA group, W/D ratio in IP-2 group significantly decreased at two time points ($p < 0.05$); There was no notable difference between the two IP groups ($p > 0.05$).

Histological Findings of the Lung Injury

It could be detected that DHCA group suffered the most from pulmonary edema while CPB group suffered the least. However, there was no

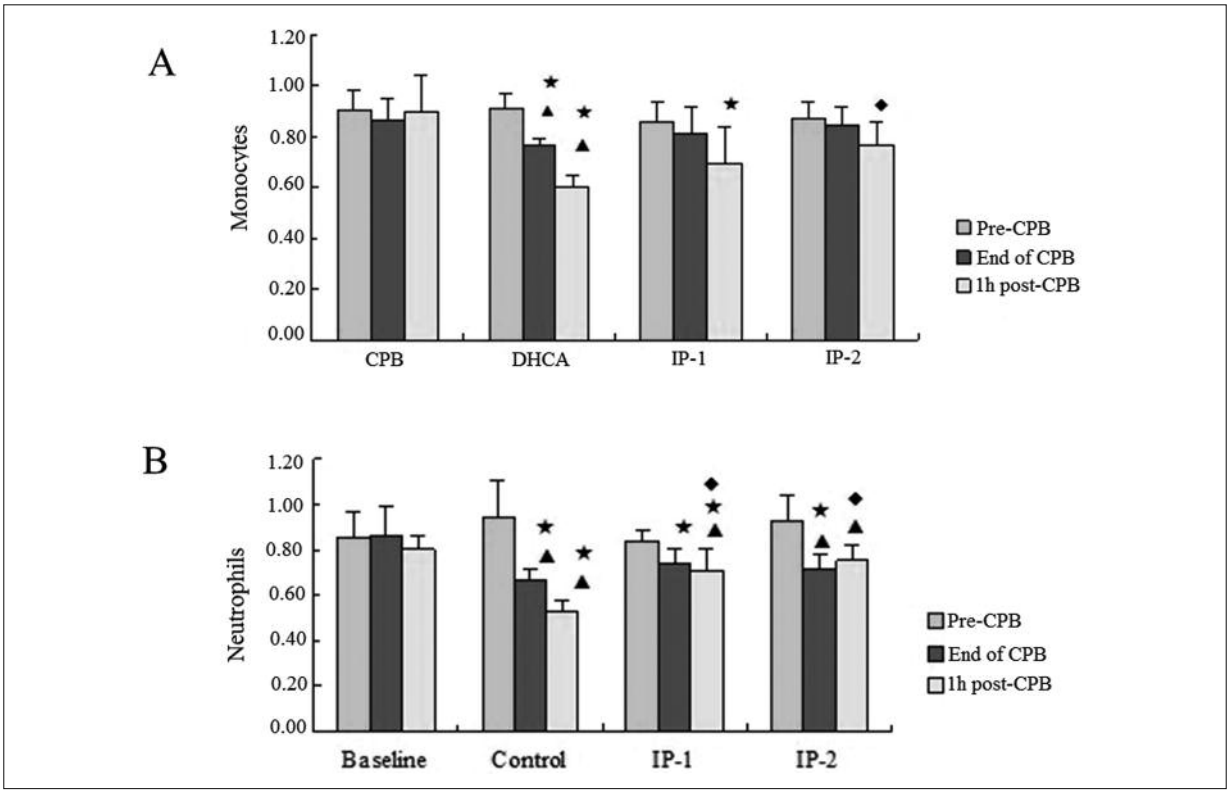

Figure 4. Comparison of LA/PA ratio of CD18 expression in monocytes and neutrophils. ***A,*** CD18 expression in monocyte; ***B,*** CD18 expression in neutrophils; CPB group: routine CPB; DHCA group: CPB + DHCA; IP-1 group: CPB + IP + DHCA; IP-2 group: CPB + hypoxia-ischemia preconditioning + DHCA; ▲vs pre-CPB, $p < 0.05$; *vs CPB group, $p < 0.05$; ♦vs DHCA group, $p < 0.05$; #vs IP-1 group, $p < 0.05$.

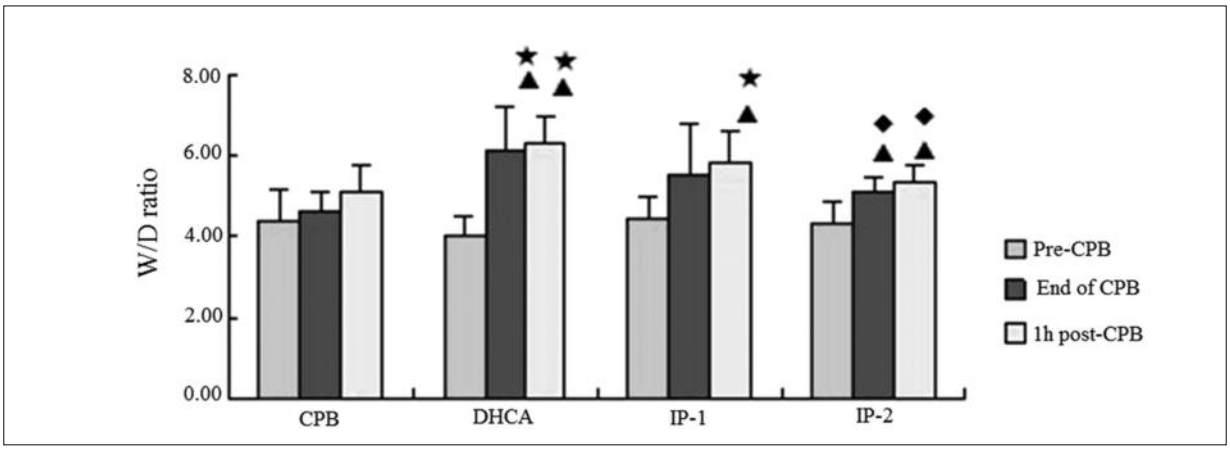

Figure 5. Lung W/D ratio in four groups. W: wet weight; D: dry weight; CPB group: routine CPB; DHCA group: CPB+DHCA; IP-1 group: CPB + IP + DHCA; IP-2 group: CPB + hypoxia-ischemia preconditioning + DHCA;▲vs pre-CPB, $p < 0.05$; *vs CPB group, $p < 0.05$; ♦vs DHCA group, $p < 0.05$; #vs IP-1group, $p < 0.05$.

difference between the two IP groups. Under the optical microscope, no obvious morphological change was observed in the pulmonary tissues of CPB group. The alveolar septum and capillary vessel were intact, without distinct pulmonary edema and leukocytes infiltration, only with occasional alveolar macrophage and erythrocyte extravasation (Figure 6A). However, the pulmonary tissue of the DHCA group showed significant thickening of the alveolar wall, microthrombosis in capillaries, and pulmonary interstitial edema accompanied with abundant

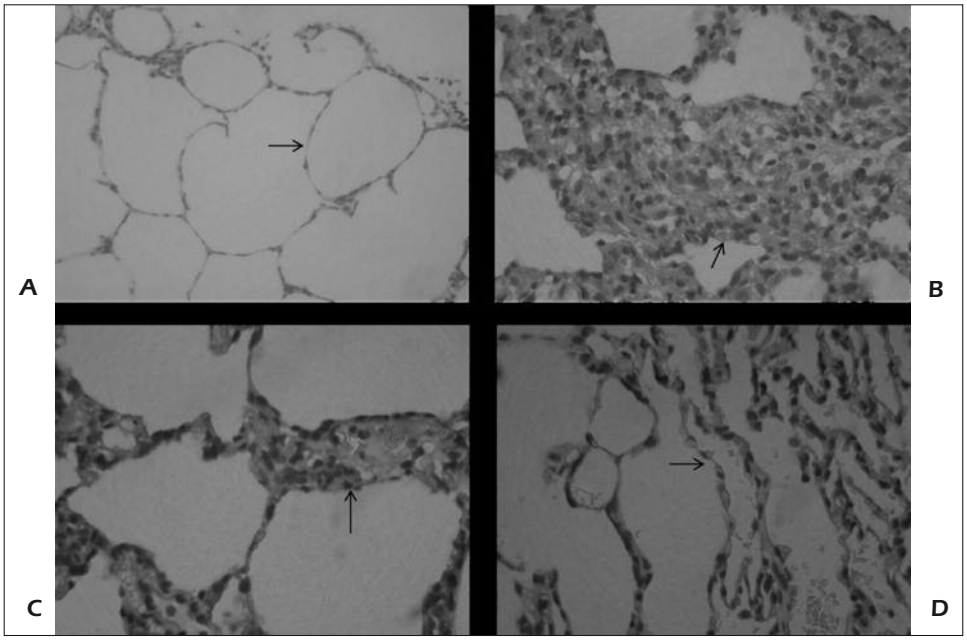

Figure 6. Lung histologic changes under optical microscope after four different treatments. ***A,*** CPB group, routine CPB. No obvious histologic changes were observed. The alveolar septum and the capillary vessel were intact (*shown as arrow*). ***B,*** DHCA group, CPB+DHCA. There was significant thickening of the alveolar wall, microthrombosis in capillaries, and pulmonary interstitial edema; ***C,*** IP-1 group, CPB + IP + DHCA. Compared with baseline group, the alveolar septum suffered from severe edema and there were much more PMNs (*shown as arrow*) in the pulmonary interstitium. Compared with control group, the pulmonary edema and alveolar damage were alleviated (*shown as arrow*); ***D,*** IP-2 group, CPB + hypoxia-ischemia preconditioning + DHCA. Pulmonary edema, alveolar damage, and PMNs infiltration (shown as arrow) were further relieved, although there was no significant difference compared with IP-1G.

PMNs and mononuclear phagocytes (Figure 6B). Compared with the CPB group, the alveolar septum suffered from severe edema and there were much more PMNs in the pulmonary interstitium in the IP-1 group (Figure 6C). While compared with the DHCA group, the pulmonary edema and alveolar damage were alleviated to some extent, showing less PMNs infiltration and clustered cell extravasation in the pulmonary interstitium (Figure 6C). Similar morphological change was also present in IP-2 group. Pulmonary edema, alveolar damage, and PMNs infiltration in the pulmonary interstitium were further relieved in IP-2 group, although there was no significant difference between two IP groups (Figure 6D). In brief, it could be concluded that the trend of pulmonary injury in those four groups were as follows: the DHCA group > IP-1 group > IP-2 group > CPB group.

Furthermore, all the tissue samples of the four groups were observed under the TEM. In the CPB group, type II cellular lamellar bodies decreased in both the number and density, while cells were in normal state, with intact nucleus and mitochondria (Figure 7A). However, in the DHCA group, the capillaries in the alveolar walls showed significant degeneration, necrosis and broken wall, with several erythrocytes and degenerative inflammatory cells accumulated in the lumen (Figure 7B). After IP-1 treatment, erythrocytes only showed mild stasis, a few inflammatory cells were observed in the alveoli, and type II cells lacked of lamellar body inclusions (Figure 7C). This condition was further mitigated after IP-2 treatment. The neutrophils were reduced, with only one in the alveoli cavity. There was no obvious erythrocyte stasis. Mitochondrial swelling was observed in one metamorphic type I cell (Figure 7D).

Discussion

Cardiac surgery using CPB leads to a global cardiac ischaemia-reperfusion injury and induces a systemic inflammatory reaction through activation of the complementary system, production of cytokines and interleukins, and release of mediators[19,20]. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1

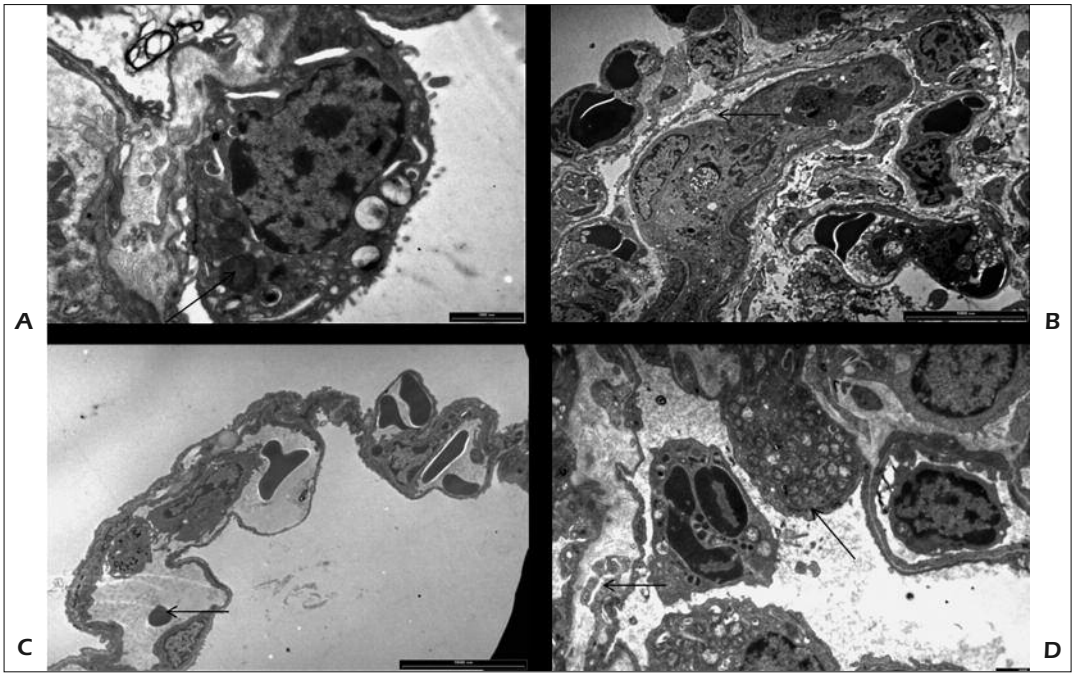

Figure 7. TEM observation of lung histologic changes after four different treatments. ***A,*** CPB group, routine CPB. Type II cellular lamellar bodies decreased in both the number and density (*shown as arrow*), while cells were in normal state, with intact nucleus and mitochondria. ***B,*** DHCA group, CPB + DHCA. The capillaries in the alveolar walls showed degeneration, necrosis and broken wall (shown as arrow), with many erythrocytes and degenerative inflammatory cells in the lumen. ***C,*** IP-1 group, CPB + IP + DHCA. There was mild stasis of erythrocytes, and a few inflammatory cells in the pulmonary alveoli cavity. ***D,*** IP-2 group, CPB + hypoxia-ischemia preconditioning + DHCA. There was one neutrophils in the alveoli cavity (shown as arrow), with no obvious erythrocyte stasis, and mitochondrial swelling (*shown as arrow*) was observed in one metamorphic type I cell.

(VCAN-1) belong to the adhesion molecule immunoglobin superfamily, with low expression by the endothelial cells in normal condition. However, the expression of ICAM-1 can be found in the endothelial cells, epithelial cells and monocytes when there is inflammatory stimulation, such as IL-8 and TNF, which are released from I/R-induced leukocytes. ICAM-1 can combine specifically with lymphocyte function associated antigen (LFA-1), CD11a/CD18 and CD43, promoting the adhesion of leukocytes to the vascular endothelium cells[21]. Finally, migrated leukocytes release cytotoxic proteases resulting in increased capillary permeability. Capillary leak and edema are the result and organ dysfunction may follow[22,23]. As expected, our present study revealed induction of an inflammatory reaction after CPB, showing high expression of cytokines (such as TNF-α, IL-8 and IL-10), especially in CPB with DHCA group. The CD18 expression on monocytes and neutrophils of vessels near the pulmonary circulation increased greatly, but the ratio of LA/PA was significantly decreased after CPB with DHCA, implying that more numbers of those two cells were activated and detained in the pulmonary tissues, which was in accordance with previous clinical studies[24,25] and our pathological observation. The LA/PA ratio of CD18 expression also increased in the routine CPB group, but there was no significant difference between pre- and post-CPB, which is partially explained by shorter extracorporeal circulation and lacking of complete hypothermic I/R. W/D ratio is an indicator of pulmonary edema, which was also significantly increased in the DHCA group compared with CPB group. Due to the above reasons, the two parameters of pulmonary function, Csat and PVRI, were deteriorated in our results. These findings suggest that CPB with DHCA aggravates lung inflammatory injury compared with routine CPB.

IP describes the phenomenon by which transient ischemia induces tissue protection against subsequent I/R injury. This has been well investigated during myocardial ischemia[26] and proved to be a potent, endogenous protective strategy for heat[27], brain[28] and lung injury[29]. In this study, we also explored the protective mechanism of IP treatment for DHCA –induced lung injury. The results showed that IL-8 and IL-10 were significantly decreased at the endpoint of CPB or 1h post-CPB compared with DHCA group. The monocytes activated in the IP-1 group after DHCA showed no increase compared with the pre-CPB. However, the neutrophils were still significantly retained in the IP-1 group (lower LA/PA ratio of CD18 expression) at post-CPB. The possible reason is that there is a strong relationship between the inhibition of adhesion molecules on both monocytes and neutrophils and the double-peaks model of pulmonary damage. The pulmonary cellular response to I/R is mediated by macrophage in the early phase of reperfusion, while the neutrophils-dependent response is only limited to the later phase[30]. Our research focused on the early phase of reperfusion, in which the monocytes' invasion or proliferation resulting in an increase in the number of cells can activate macrophage, thus IP had more obvious effect on monocytes inhibition.

Atelectasis is considered as a major cause of decreased arterial oxygenation after CPB and then pulmonary dysfunction[31]. Recent studies also recommend to using low-frequency mechanical ventilation (hypoxic preconditioning) during CPB, which has been demonstrated to reduce tissue metabolic and histologic damage in the lungs and is associated with improved postoperative gas exchange[14]. Thus, we also designed the hypoxia-ischemia preconditioning and hypothesized this treatment may be more effective for protection of DHCA-induced injury. As expected, our results indicated that TNF-α expression, the number of monocytes, W/D ratio, and PVRI were significantly decreased in IP-2 group compared with DHCA, but not changed in the IP-1 group. Although it seemed that the IP-2 group exhibited lower level of capillary erythrocytes in pulmonary alveoli and inflammatory cells infiltration than the IP-1 group, but no difference was observed between the two groups in mitochondria swelling and vacuolization of type I and II cell, which is in accordance with the report by Featherstone RL et al[7]. Therefore, other protective strategies are needed to be further investigated.

Conclusions

Our research suggests that pulmonary IP can activate the endogenous protective function during DHCA, and then prevent the tissue from further pulmonary I/R injury. Interestingly, maintaining ventilation with pulmonary artery perfusion in the lung IP process during CPB seems to be more superior to single pulmonary artery perfusion. However, there were still some limitations in this study. We only investigated the changes of various indicators 1h after post-CPB, not 24 or 48h post-CPB; our study is very fo-

cused on the anti-inflammatory mechanism of IP in protecting tissues against ischemia, the apoptosis mechanisms are not explored[32,33]. Thus, further investigation is still needed to confirm our conclusion.

Statement of Conflict of Interest

The Authors state no conflict of interest.

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