Limb remote ischemic preconditioning on lung protection in patients with thoracoscopic lobectomy: a randomized controlled trial

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Research Article

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Abstract

Background

One-lung ventilation (OLV) is often used during lobectomy anesthesia. Inflammation and oxidative stress during OLV can aggravate lung tissue injury, which is an important factor causing postoperative acute lung injury. Studies have confirmed that remote ischemic preconditioning (RIPC) can initiate endogenous protective mechanisms and alleviate injury to target organs. However, whether RIPC has a lung protective effect in patients undergoing lung resection remains unknown. Therefore, this randomized controlled clinical study investigated whether RIPC could reduce OLV-related lung injury, improve intraoperative oxygenation parameters, and induce lung protection in patients with thoracoscopic lobectomy.

Methods

We adopted a single-center, randomized, single-blind clinical controlled trial method and selected 54 patients who underwent inferior lobectomy with OLV and were randomly divided into a preconditioning group (RIPC group) and a blank control group (NC group). The preconditioning group received lower limb RIPC before OLV, while the blank NC group did not receive RIPC. RIPC was used to perform 5 cycles of 5 min ischemia /5 min reperfusion on the opposite lower limb via a limb ischemia preadaptor. Four time points after anesthesia induction (T0), 30 min after single-lung ventilation (T1), 90 min after single-lung ventilation (T2), and 30 min after double-lung ventilation restoration (T3) were used as the data-recording and blood-collection points. The main outcome measure was the oxygenation index (OI), while the secondary outcome measures included Clara cell secreted protein CC16, inflammatory cytokines (IL-6), serum malondialdehyde (MDA), lung-related variables, and length of hospital stay.

Results

There was no significant difference in the OI in the RIPC group compared with the NC group at each time point of T0-T3 (P>0.05), but in general, the OI value of the RIPC group at the T1-T3 time points showed an increasing trend compared with that of the NC group. There were significant differences in plasma CC16 levels between T1-T3 (P<0.05), and the plasma CC16 level in the RIPC group was significantly decreased. IL-6 and MDA levels at T1-T3 were lower than those in the NC group (P<0.05). However, there were no significant differences in blood pH and pulmonary-related variables (respiratory index (RI), alveolar-arterial oxygen partial pressure (A-aDO2), and arterial-alveolar oxygen partial pressure (a/A ratio)) (P>0.05), and the length of hospital stay was not significantly different (P>0.05).

Conclusions
Remote limb ischemic preconditioning can reduce lung injury during lobectomy and can protect lung tissue mainly by reducing the inflammatory and oxidative-stress responses.

**Introduction**

According to statistics, the incidence of lung cancer shows an increasing trend year by year, with an estimated 2 million new cases and 1.76 million deaths annually, and lung cancer is one of the main causes of disease-related death (1). At present, the surgical modalities represented by lobectomy have achieved satisfactory results in the early stage of lung cancer. However, in lung cancer patients undergoing surgery, the incidence of acute lung injury (ALI) after thoracic surgery is 2–8%, and further development of ALI can lead to acute respiratory distress syndrome (ARDS), respiratory failure and even multiple organ failure or death (2). One-lung ventilation (OLV) is an important factor in ALI (3). OLV is often necessary in lobectomy to avoid bilateral lung cross-infection and facilitate surgical operation. During OLV, the tissue of the operated lung undergoes varying degrees of damage, produces a large number of oxygen free radicals, and releases a large number of inflammatory factors. Subsequently, it is further damaged due to redilation and ischemia–reperfusion injury, which further leads to the production and release of oxygen free radicals and various inflammatory factors. The above adverse factors induce exacerbation of the systemic inflammatory response and oxidative stress response, which is highly likely to lead to ALI after pulmonary lobectomy (4–6). Therefore, the strategy of perioperative lung protection has gradually attracted the attention of anesthesiologists. Reducing perioperative lung injury and protecting lung function have become urgent problems to be solved in surgical anesthesia.

At present, studies on perioperative lung protection strategies mainly include drug interventions (such as sevoflurane preconditioning, dexmedetomidine preconditioning, glucocorticoids, protease inhibitors, propofol, etc.) or changes in ventilation parameters (such as small tidal volume, small tidal volume combined with positive end-expiratory pressure ventilation and allowable hypercapnia, etc.) (7–10). All these methods can alleviate perioperative lung injury to a certain extent, improve lung oxygenation function and improve prognosis. A large number of studies have confirmed (11–13) that limb remote ischemic preconditioning (RIPC) has an organ-protective effect against ischemia, hypoxia and reperfusion injury, and is noninvasive and easy to implement; thus, it has attracted the attention of many scholars in organ protection research.

RIPC refers to the repeated, transient and nonfatal ischemia reperfusion (I/R) of skeletal muscle far away from the target organ, performed several times to activate the endogenous protective mechanism of the body and improve the tolerance of the target organ to subsequent ischemic or hypoxic injury (14). Limb RIPC causes no trauma to skeletal muscle, is simple and feasible, and has broad clinical application prospects. Although most studies have focused on heart, kidney and brain patients, relatively little research has been done on lung injuries.

Whether RIPC has a direct lung protective effect on patients undergoing lobectomy and the possible mechanism of action are not fully understood. This study investigated whether RIPC can reduce lung
injury in patients with thoracoscopic lobectomy and improve oxygenation parameters during surgery. By exploring the lung protective effect and mechanism of RIPC, we can provide a clinical reference for perioperative lung protective strategies.

**Materials and methods**

**Study Design and Participants**

A single-center, randomized and single-blind clinical trial was conducted in lung cancer patients undergoing elective thoracoscopic lobectomy. Written informed consent was given to by each participant and the study content was explained in detail to the participants. This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Gannan Medical College. The trial has been registered on the Chinese Clinical Trial Registry website (ChiCTR2100049712, date of registration: 08/08/2021).

From November 2021 to July 2022, 54 patients with lung cancer undergoing elective thoracoscopic lobectomy were enrolled. Prior to the trial, random assignments were generated by an independent person using a computerized random number generator, and patients were randomized (1:1 assignment) to either the RIPC or the NC groups. Eligible patients were between the ages of 30 and 80 years. The inclusion criteria were as follows: (1) American Society of Anesthesiologists (ASA) grade ~ ; (2) good cognitive function, no surgical contraindications; (3) no history of long-term use of antioxidants (such as multivitamins, etc.), long-term use of cortisol hormone and nonsteroidal anti-inflammatory drugs; and (4) complete clinical data, informed consent of patients and their families, and signed consent for surgery and anesthesia. Exclusion criteria were as follows: (1) moderate-to-severe anemia and hypoproteinemia; (2) severe cardiac and renal dysfunction, uncontrolled hypertension (> 160/100 mmHg), diabetes mellitus and coronary heart disease; (3) severe respiratory dysfunction before surgery: arterial oxygen pressure (PaO₂) < 60 mmHg or forced expiratory volume in the first second < 50% predicted value; (4) preoperative coagulation dysfunction and/or thrombocytopenia; (5) history of preoperative radiotherapy and chemotherapy; (6) active infection, such as body temperature > 38°C, increased C-reactive protein increase, etc. (7) history of asthma; (8) history of peripheral vascular and muscle diseases and limb thrombosis. The exclusion criteria were as follows: (1) intraoperative blood loss > 15% of body weight; (2) intraoperative SpO₂ still lower than 90% after correction; and (3) interruption of the operation, or its conversion to thoracotomy.

**Remote Ischemic Preconditioning Protocol**

Remote Ischemic Preconditioning Protocol

The limb RIPC protocol was applied after anesthesia induction and before the operation. Limb RIPC was applied to the thigh of the patient on the opposite side of the operation with a limb ischemia preadapator (Shenzhen City, model RIP-809S), and the lower margin was inflated to 200 mmHg from 4 ~ 5 cm away
from the knee joint. Five cycles were performed, each consisting of five minutes of ischemia (aeration) followed by five minutes of cuff venting. At the same time, the limb ischemia preadaption instrument was also placed on the thigh of the NC group, but it was not inflated.

Routine preoperative prohibition of drinking and fasting was instituted. The venous channel of the upper extremity was routinely established after the patient entered the room. Routine monitoring of heart rate, noninvasive blood pressure, oxygen saturation and electrocardiogram was performed. Invasive blood pressure monitoring was performed by radial artery puncture and catheterization. The anesthetic induction drugs sufentanil (0.4–0.5 µg/kg), propofol (2.5-3 mg/kg), and midazolam (0.04 mg/kg) were injected intravenously, and rocuronium bromide (0.6–0.9 mg/kg) was injected after sleep. After rapid induction, a suitable two-lumen bronchial catheter was inserted orally (F37 for males and F35 for females). With the assistance of bronchoscopy, the tracheal catheter was positioned and adjusted in the appropriate position. After the anesthesia machine was connected, volume control breathing was performed. The tidal volume ($V_T$) during double-lung ventilation was set to 8 ml/kg, the respiratory rate ($f$) was 12 times/min, and the inspiratory/expiratory ratio was 1:2. The time for OLV was 5 min before the operation. During OLV, $V_T$ was 6 mL/kg, $f$ 14 ~ 16 times/min, the inhaled oxygen concentration ($FiO_2$) was adjusted to 100%, and the oxygen flow was adjusted to 2 L/min. An end-expiratory partial pressure of carbon dioxide at 35 ~ 45 mmHg was maintained. After the location of the catheter was determined, right internal jugular vein puncture and catheterization were performed under ultrasound guidance. Anesthesia was maintained using a combination of static and inhalation anesthesia: propofol 4 ~ 8 mg•kg⁻¹•h⁻¹, remifentanil 0.1 ~ 0.2 µg•kg⁻¹•min⁻¹, sevoflurane concentration of 0.8% ~ 1.5%, BIS of 40 ~ 60, intermittent intravenous administration of rocuronium 0.1 ~ 0.2 mg/kg to maintain muscle relaxation. The intraoperative $SpO_2$ decreased to < 90% of the healthy lung and was restretched. At the end of the operation, ventilation was restored in both lungs. At the end of the procedure, 5~10 µg of sufentanil was administered for analgesia, after which the patient was admitted to the PACU with an inhaled oxygen concentration adjusted to 0.5 L/min. After the catheter was removed, an intravenous analgesic pump was connected (80 ~ 100 µg sufentanil, 8 mg ondansetron and 100 ml normal saline mixture, 2 ml/h infusion, 10 min locking time).

**Study Outcomes and Blood Specimen Collection Methods**

The primary outcome in this study was the oxygenation index ($OI = \frac{PaO_2}{FiO_2}$), while the secondary outcomes were biomarkers of lung injury, human Clara cell secreted protein CC16, inflammatory cytokines (IL-6), and serum malondialdehyde (MDA). The selected pulmonary variables respiratory index ($RI$), alveolar-arterial oxygen partial pressure A-a$DO_2$, arterial-alveolar oxygen partial pressure ratio ($a/A$ ratio) and pH were compared between the two groups. Arterial blood samples were taken at four time points after anesthesia induction (T0), 30 min after OLV (T1), 90 min after OLV (T2), and 30 min after the resumption of double-lung ventilation (T3). pH, blood partial oxygen pressure (PaO2), blood partial carbon dioxide pressure (PaCO2) and lactate (Lac) concentrations were measured. According to the formula, the OI, RI, A-a$DO_2$ and $a/A$ ratio were calculated. Two milliliters of venous blood was collected.
from the internal jugular vein at the above four time points and centrifuged in a 3000r centrifuge for 15 min, and the upper plasma was taken and frozen at -80°C. According to the literature (15), we randomly selected 20 blood samples from the NC group and 20 blood samples from the RIPC group. Clara cell secreted protein (CC16), inflammatory cytokine (IL-6) and serum malondialdehyde (MDA) concentrations were determined by enzyme-linked immunosorbent assay (rB, Atlanta, USA). The postoperative extubation time, PACU time and hospital stay were also recorded.

Statistical Analysis

RI was statistically significant according to the relevant literature. The mean difference between the two groups was 0.23, and the standard deviation was 0.28. Bilateral $\alpha = 0.05$ and $\beta = 0.8$ were set. Twenty-four patients were needed for each group. To compensate for the 10% of cases that might drop out, 27 patients in each group were enrolled. SPSS 26.0 statistical software was used for analysis. Measurement data were expressed as the mean ± standard deviation (mean ± SD). Repeated measure analysis of variance was used to compare the mean values of plasma biochemical markers between groups and within groups. The statistical data were compared by $\chi^2$ test, and the $P$ values were all bilateral; $P < 0.05$ was considered statistically significant.

Results

Baseline and Intraoperative Characteristics

We enrolled 54 patients between November 2021 and July 2022. Patients who were scheduled to undergo thoracotomy ($n = 3$) and who suffered severe intraoperative bleeding ($n = 1$) were excluded, consequently, 50 patients were enrolled in the study. The CONSORT diagram is shown in Fig. 1. Table 1 shows demographic data and perioperative parameters, with no significant differences in the demographic data (age, sex, body mass index, etc.), anesthesia duration, one-lung ventilation duration, operative duration, urine volume, PACU duration, and length of hospital stay ($P > 0.05$).
Table 1
Comparison of demographic data and perioperative parameters between the Control group (n = 25) and RIPC group (n = 25)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n = 25)</th>
<th>RIPC group (n = 25)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>60.40 ± 8.54</td>
<td>58.28 ± 11.45</td>
<td>0.78</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>23.22 ± 2.21</td>
<td>23.62 ± 3.19</td>
<td>0.61</td>
</tr>
<tr>
<td>Sex, males</td>
<td>11 (44%)</td>
<td>10 (40%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Left-side surgery</td>
<td>7 (28%)</td>
<td>9 (36%)</td>
<td>0.76</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>160.60 ± 40.27</td>
<td>163.80 ± 56.22</td>
<td>0.82</td>
</tr>
<tr>
<td>Intraoperative fluid load (ml)</td>
<td>1492 ± 232.90</td>
<td>1702 ± 502.60</td>
<td>0.06</td>
</tr>
<tr>
<td>Estimated blood loss (ml)</td>
<td>156.00 ± 69.70</td>
<td>184.00 ± 98.66</td>
<td>0.25</td>
</tr>
<tr>
<td>Urine (ml)</td>
<td>738.40 ± 201.43</td>
<td>818.00 ± 189.78</td>
<td>0.16</td>
</tr>
<tr>
<td>Duration of anesthesia (min)</td>
<td>204.40 ± 37.29</td>
<td>219.60 ± 59.56</td>
<td>0.29</td>
</tr>
<tr>
<td>OLV duration (min)</td>
<td>142.60 ± 35.77</td>
<td>154.40 ± 45.79</td>
<td>0.32</td>
</tr>
<tr>
<td>Length of PACU stay, (min)</td>
<td>90.20 ± 36.01</td>
<td>91.00 ± 36.14</td>
<td>0.94</td>
</tr>
<tr>
<td>Length of hospital stay (d)</td>
<td>8.00 ± 1.61</td>
<td>8.00 ± 1.9</td>
<td>0.94</td>
</tr>
</tbody>
</table>

In our main outcome, there was no significant difference in OI between the two groups, but the RIPC group showed an increasing trend at the T1 and T2 time points compared with the NC group (Fig. 2A).

As shown in Table 2, arterial pH, PaCO\(_2\), lactic acid and lung-related variables (RI, A-aDO\(_2\), a/A ratio) were similar between the two groups at each observation point (\( P > 0.05 \)). Although there were no significant differences in RI and A-aDO\(_2\) between the two groups (Fig. 2B and 2C), A-aDO\(_2\) showed a downward trend at three time points in the RIPC group compared with the NC group. Compared with that in the NC group, the RI at the T1 and T3 time points of the RIPC group showed a downward trend. There was no significant difference in the a/A ratio between the two groups (Fig. 2D), but compared with the NC group, the a/A ratio in the RIPC group showed a downward trend at the T1 and T2 time points.
Table 2
Comparison of blood gas analysis-related indicators and lung respiration-related variables between the two groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>baseline (T0)</th>
<th>30 min after OLV (T1)</th>
<th>90 min after OLV (T2)</th>
<th>30 min after DLV (T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial PH</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control group</td>
<td>7.41 ± 0.06</td>
<td>7.40 ± 0.06</td>
<td>7.39 ± 0.06</td>
<td>7.30 ± 0.05*</td>
</tr>
<tr>
<td>RIPC group</td>
<td>7.39 ± 0.05</td>
<td>7.37 ± 0.05</td>
<td>7.36 ± 0.05</td>
<td>7.31 ± 0.07*</td>
</tr>
<tr>
<td>PaCO2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>41.00 ± 6.30</td>
<td>40.12 ± 6.65</td>
<td>40.40 ± 5.83</td>
<td>47.24 ± 8.50</td>
</tr>
<tr>
<td>RIPC group</td>
<td>43.48 ± 6.63</td>
<td>43.12 ± 5.42</td>
<td>41.88 ± 5.81</td>
<td>45.55 ± 9.50</td>
</tr>
<tr>
<td>Lac</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control group</td>
<td>0.86 ± 0.33</td>
<td>0.75 ± 0.31</td>
<td>0.70 ± 0.22</td>
<td>0.76 ± 0.28</td>
</tr>
<tr>
<td>RIPC group</td>
<td>0.91 ± 0.57</td>
<td>0.78 ± 0.43</td>
<td>0.71 ± 0.30</td>
<td>0.83 ± 0.42</td>
</tr>
<tr>
<td>a/A ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>0.59 ± 0.12</td>
<td>0.21 ± 0.12*</td>
<td>0.29 ± 0.12*</td>
<td>0.60 ± 0.27</td>
</tr>
<tr>
<td>RIPC group</td>
<td>0.54 ± 0.13</td>
<td>0.25 ± 0.13*</td>
<td>0.30 ± 0.15*</td>
<td>0.72 ± 0.24</td>
</tr>
<tr>
<td>A-aDO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>270.11 ± 76.67</td>
<td>524.25 ± 80.76*</td>
<td>471.70 ± 74.99*</td>
<td>117.89 ± 79.51*</td>
</tr>
<tr>
<td>RIPC group</td>
<td>306.37 ± 85.40</td>
<td>497.82 ± 89.09*</td>
<td>460.17 ± 96.50*</td>
<td>81.18 ± 69.91*</td>
</tr>
<tr>
<td>RI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>0.76 ± 0.38</td>
<td>5.20 ± 2.77*</td>
<td>3.21 ± 2.13*</td>
<td>1.21 ± 1.35</td>
</tr>
</tbody>
</table>

Continuous data are presented as means ± SD

Abbreviations: OLV: one-lung ventilation; DLV: double-lung ventilation; PaCO₂: arterial carbon dioxide partial tension; PaO₂: oxygen partial pressure; a/A ratio: arterial–alveolar oxygen tension ratio; A-aDO₂: alveolar to arterial difference of oxygen tension; RI: respiratory index; OI:oxygenation index

*P < 0.05 vs. baseline
<table>
<thead>
<tr>
<th>Variable</th>
<th>baseline (T0)</th>
<th>30 min after OLV (T1)</th>
<th>90 min after OLV (T2)</th>
<th>30 min after DLV (T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIPC group</td>
<td>1.01 ± 0.64</td>
<td>4.34 ± 2.68*</td>
<td>3.32 ± 2.45*</td>
<td>0.57 ± 0.53</td>
</tr>
<tr>
<td>OI</td>
<td>391.64 ± 79.95</td>
<td>138.6 ± 83.54*</td>
<td>190.8 ± 77.36*</td>
<td>359.12 ± 16.29</td>
</tr>
<tr>
<td>Control group</td>
<td>352.28 ± 86.22</td>
<td>161.28 ± 88.68*</td>
<td>200.48 ± 96.37*</td>
<td>437.04 ± 153.3</td>
</tr>
</tbody>
</table>

Continuous data are presented as means ± SD

Abbreviations: OLV: one-lung ventilation; DLV: double-lung ventilation; PaCO\(_2\): arterial carbon dioxide partial tension; PaO\(_2\): oxygen partial pressure; a/A ratio: arterial–alveolar oxygen tension ratio; A-aDO\(_2\): alveolar to arterial difference of oxygen tension; RI: respiratory index; OI:oxygenation index

*P < 0.05 vs. baseline

Regarding the serological indicators, changes in plasma CC16 over time were significantly different between the two groups at various time points (Fig. 3A). Plasma CC16 levels in the RIPC group were significantly lower than those in the NC group at time points T1-T3 (all P< 0.05). Similarly, plasma MDA and IL-6 levels were significantly lower than those of the NC group at time points T1-T3 (Fig. 3B and 3C), showing a significant difference between the two groups (all P< 0.05).

In addition, as shown in Table 3, there were no significant differences in heart rate, mean arterial pressure, plateau pressure or peak airway pressure between the two groups at each observation point (P> 0.05).
Table 3
Comparison of the heart rate, mean arterial pressure, airway plateau pressure and peak airway pressure between the two groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>baseline (T0)</th>
<th>30 min after OLV (T1)</th>
<th>90 min after OLV (T2)</th>
<th>30 min after DLV (T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>67.72 ± 5.86</td>
<td>69.72 ± 5.78</td>
<td>67.88 ± 4.95</td>
<td>68.44 ± 3.23</td>
</tr>
<tr>
<td>RIPC group</td>
<td>71.96 ± 9.00</td>
<td>71.72 ± 8.16</td>
<td>70.96 ± 8.23</td>
<td>69.88 ± 7.45</td>
</tr>
<tr>
<td>95% CI</td>
<td>[-1.68 to 10.16]</td>
<td>[-3.92 to 7.92]</td>
<td>[-2.84 to 9.00]</td>
<td>[-4.48 to 7.36]</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>81.52 ± 14.11</td>
<td>79.44 ± 14.53</td>
<td>88.20 ± 11.75</td>
<td>86.36 ± 10.40</td>
</tr>
<tr>
<td>RIPC group</td>
<td>85.20 ± 14.47</td>
<td>85.20 ± 13.30</td>
<td>90.36 ± 12.85</td>
<td>90.00 ± 9.01</td>
</tr>
<tr>
<td>95% CI</td>
<td>[-14.82 to 7.46]</td>
<td>[-16.90 to 5.38]</td>
<td>[-13.30 to 8.98]</td>
<td>[-14.78 to 7.50]</td>
</tr>
<tr>
<td>Peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>14.96 ± 3.13</td>
<td>24.12 ± 3.64</td>
<td>24.88 ± 2.77</td>
<td>15.80 ± 2.45</td>
</tr>
<tr>
<td>RIPC group</td>
<td>14.8 ± 2.86</td>
<td>25.2 ± 3.98</td>
<td>25.64 ± 3.70</td>
<td>16.2 ± 2.60</td>
</tr>
<tr>
<td>95% CI</td>
<td>[-2.60 to 2.92]</td>
<td>[-3.84 to 1.68]</td>
<td>[-3.52 to 2.00]</td>
<td>[-3.16 to 2.36]</td>
</tr>
<tr>
<td>Pplat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>13.6 ± 2.60</td>
<td>23.00 ± 3.64</td>
<td>23.68 ± 3.04</td>
<td>15.00 ± 2.68</td>
</tr>
<tr>
<td>RIPC group</td>
<td>13.52 ± 2.90</td>
<td>23.64 ± 4.06</td>
<td>24.04 ± 3.58</td>
<td>15.36 ± 2.97</td>
</tr>
<tr>
<td>95% CI</td>
<td>[-2.71 to 2.87]</td>
<td>[-3.43 to 2.15]</td>
<td>[-3.15 to 2.43]</td>
<td>[-3.15 to 2.43]</td>
</tr>
</tbody>
</table>

Continuous data are presented as means ± SD

Abbreviations: OLV: one-lung ventilation; DLV: double-lung ventilation; HR: heart rate; MAP: mean arterial pressure; CI: confidence interval; RIPC: remote ischemic preconditioning.

Discussion

In this single-center, randomized and single-blind clinical trial, we observed that the application of RIPC to the limb before OLV did not significantly improve oxygenation in patients but reduced lung injury, inflammatory responses, and oxidative stress levels in patients undergoing lobotomy. Specifically, after
the start of OLV, the OI in the RIPC group was not significantly improved, and there was no significant difference from that in the NC group. However, the plasma CC16 level in the RIPC group was significantly lower than that in the NC group at all time points of T1-T3, and the levels of MDA and IL-6 were also significantly lower than those in the NC group at all time points of T1-T3. We found no significant difference between the two groups in terms of lung-related variables and the length of hospital stay.

In thoracoscopic lobectomy, oxidative stress and the inflammatory cascade caused by OLV are prone to damage lung vascular endothelial cells and lung epithelial cells, causing injury to the alveolar capillary wall and increased permeability of the alveolar vascular wall (16, 17). The combination of these factors may lead to ALI and even induce postoperative ARDS or death. Currently, the mechanisms of OLV-associated lung injury may involve hypoxic lung injury (hypoxemia and oxidative stress response) and mechanical stretch lung injury (4). First, hypoxia and hypoxemia can lead to the mass production of oxygen free radicals and the destruction of blood vessel walls (18). Second, hypoxia and hypoxemia can indirectly cause the release of inflammatory factors (TNF-α and IL-6, etc.), leading to the occurrence of inflammatory cascades (18). The main mechanism of oxidative stress lung injury is the imbalance of oxidative/antioxidant capacity in vivo (4). On the one hand, during OLV, ischemia and hypoxia release a large number of oxygen free radicals, which destroy alveolar epithelial cells and vascular endothelial cells and produce a large number of inflammatory factors. On the other hand, ischemia and hypoxia can also damage alveolar type II cells, reducing alveolar surface active substances, increasing alveolar surface tension, and water in capillaries entering the pulmonary interstitium and alveoli, causing pulmonary edema and lung injury (16).

The latest evidence shows that RIPC can initiate endogenous protective mechanisms, prevent endothelial cell injury and neutrophilic granulocyte activation, reduce the inflammatory response and oxidative stress response, and thus alleviate the injury to target organs (19). In 1986, RIPC was first proposed by Murry et al in myocardial cells of dogs (20). At present, the protective effect of RIPC involves the heart, kidney, liver, brain, lung and other organs, and satisfactory therapeutic effects have been achieved (13, 21–24). At present, RIPC has rarely been reported in relevant studies in the field of pulmonology. Therefore, we conducted this study to further verify the protective effect of RIPC against lung injury.

OI is the ratio of PaO$_2$ to FiO$_2$, which reflects the changes in pulmonary oxygenation function and ventilation function to a certain extent. For patients with a pulmonary ventilation/blood flow imbalance or pulmonary dysfunction, OI decreases (25). A-aDO$_2$ is an important indicator reflecting lung diffusion function, which can reflect the degree of lung injury. The higher the A-aDO$_2$ value, the worse the lung function may be (21). In our results, there was no significant difference between the two groups in term of OI and A-aDO$_2$, which may be related to the relative inadequacy of our sample size and other uncontrollable interfering factors (patients' underlying diseases and smoking, etc.). However, the OI value of the RIPC group showed an increasing trend after OLV compared with that of the NC group, and the A-aDO$_2$ of the RIPC group showed a decreasing trend compared with that of the NC group. This may also
indicate that RIPC can improve the oxygenation state of the body to a certain extent and protect the normal diffusion function of lung tissue.

In addition, we found that after the implementation of RIPC, the plasma CC16 level was significantly reduced. With the extension of OLV time, the CC16 level in the RIPC group was always lower than that in the NC group, and the degree of lung injury in the RIPC group was lower than that in the NC group. This suggests that RIPC can induce lung protection and reduce the degree of lung injury. Clara cell secreted protein (CC16) is secreted by Clara cells of bronchial and terminal nonciliated epithelial cells. As a homodimer with a molecular weight of 15.8 kDa, its biological activity and pathway are not fully understood (26), but a study has shown that CC16 has anti-inflammatory and antioxidant effects. It plays an important protective role in oxidative stress and respiratory inflammation and has lung tissue specificity (27). Another study has shown that acute exposure to pulmonary irritants can lead to transient elevation of CC16 in serum, which is mainly related to increased airway permeability (28). Especially in acute lung injury, the serum CC16 level increases significantly, and a high serum CC16 concentration predicts the severity of infection and the development of disease, which is due to the leakage of the bronchoalveolar/blood barrier, resulting in a significant increase in the serum CC16 concentration (26). Therefore, CC16 may not only reflect the pathogenesis of lung diseases but also serve as a potential biomarker of lung diseases.

Regarding the inflammatory response, the plasma IL-6 level in the RIPC group began to increase after 90 min of OLV, while that in the NC group immediately increased after the start of OLV and was significantly higher than that in the RIPC group, these findings indicated that RIPC can inhibit the inflammatory response of the body and produce certain lung protection. A study on OLV in rabbits showed that a series of inflammatory reactions similar to lung ischemia reperfusion injury occurred from collapse to reexpansion, in which proinflammatory cytokines, such as IL-6 and TNF, were significantly increased, suggesting that a nonventilated lung could undergo lung injury during reexpansion (29). Lung reperfusion injury is different from other postischemic reperfusion injuries. It is the result of the interaction and mutual promotion of multiple pathological processes, and its initiating factors are the acute inflammatory response and oxidative stress response, thus leading to the massive release of related inflammatory factors, oxygen free radicals and lipid mediators (4, 30). Studies have shown that IL-6 plays an important role in acute reactive protein synthesis and inflammatory cell aggregation. The IL-6 concentration is increased in all posttraumatic cycles, and its increased level is related to the degree of trauma, operation time and postoperative complications, which is a reliable indicator of the surgical stress response (31). Our results suggest that RIPC may provide lung protection by reducing serum IL-6 levels and the degree of inflammatory response.

In addition, it was found that serum malondialdehyde (MDA) levels were significantly higher after collapsed side lung reexpansion than before OLV. With extension of the OLV time, the level of MDA increases significantly, indicating that oxidative stress can occur in the collapsed lung after OLV and that its severity is related to the duration of OLV (32). Our results showed that MDA increased to varying degrees after OLV, and the level of MDA increased gradually with extension of the OLV time, however, the
level of MDA in the RIPC group was always lower than that in the NC group. This indicates that after RIPC is implemented, the body generates an antioxidant mechanism, and RIPC can reduce the oxidative stress response generated during OLV.

The advantage of our study is its demonstration that RIPC can reduce lung injury caused by OLV in lobectomy to a certain extent through CC16, a marker associated with lung injury. Previous studies have mainly used 3-cycle RIPC. By using 5-cycle RIPC for the test, we can further supplement the effect of the RIPC cycle on lung injury.

Our study also had several limitations. First, the number of patients was small. Although we found differences in some biomarkers of the inflammatory response, our sample size was insufficient to detect clinical differences related to lung function during lobectomy (e.g., oxygenation index, respiratory index, alveolar arteriovenous oxygen differential pressure, etc.). Therefore, a larger sample size might yield more reliable results. Second, our study was limited to 30 min after surgery, and we did not collect other postoperative indicators, which may have obscured the protective effect of RIPC on lung function. Finally, we did not investigate other possible protective mechanisms associated with RIPC, such as TNF, HIF-1α, and other relevant markers such as interleukin-10.

**Conclusion**

In conclusion, our study showed that RIPC can alleviate lung injury from OLV during lobectomy, moreover, RIPC exerts a protective effect on lung tissue mainly by reducing the inflammatory response and oxidative-stress responses. Due to the limited sample size, clinical randomized controlled trials with multicenter and larger sample sizes should be conducted in the future to determine the impact of limb RIPC on clinical outcomes.

**Declarations**

**Ethics approval and consent to participate**

The study conformed to the guidelines of the Declaration of Helsinki and was approved by the Research Ethics Committee of the First Affiliated Hospital of Gannan Medical College (LLSC-2021071901) and was registered retrospectively in the Chinese Clinical Trial Registry (ChiCTR2100049712). All participants gave written informed consent.

**Consent for publication**

Not applicable

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.
Competing interests

The authors declare no competing financial interests.

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Authors’ contributions

WFZ helped design and conduct the study, analyze the data, and write the manuscript. SCZ, MWZ, YC, JC, YYZ and CWZ helped design and conduct the study. JY, XLL, MLZ, HYX and LQY participated in the analysis of data and critical review of the manuscript. WDL contributed to the design of the study, collection and analysis of data, and revise the manuscript.

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**Figure 1**

CONSORT diagram depicting the recruitment of participants
Figure 2

Time course of OI, RI, A-aDO$_2$ and the a/A ratio in the control group and RIPC group. Note: #: compare with T0, $P<0.05$

Abbreviations: OI: oxygenation index; RI: respiratory index; A-aDO$_2$: alveolar to arterial difference of oxygen tension; a/A ratio: arterial–alveolar oxygen tension ratio
Figure 3

Time course of CC16, IL-6 and MDA in the control group and RIPC group. Note: #: compare with the NC group, \( P<0.05 \)

Abbreviations: CC16: Clara cell secreted protein CC16; IL-6: interleukin-6; MDA: serum malondialdehyde