Modulation of monocyte chemoattractant protein-1 expression by ischaemic preconditioning in a lung autotransplant model†

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Abstract

OBJECTIVES: Monocyte chemoattractant protein-1 (MCP-1) is believed to play a crucial role in lung ischaemia-reperfusion injury (LIRI). Ischaemic preconditioning (IP) has been shown to protect several organs from ischaemia-reperfusion (IR) injury, although less is known about IP’s effect on MCP-1 modulation. The objective of this study was to investigate IP’s effect on MCP-1 expression in lung tissue and its relationship with oxidative stress and proinflammatory cytokine production in an experimental LIRI model.

METHODS: Two groups (IP and control groups) of seven large white pigs underwent a lung autotransplant (left pneumonectomy, ex situ superior lobectomy and lower lobe reimplantation). Before pneumonectomy was performed in the study group, IP was induced with two cycles of 5 min of left pulmonary artery occlusion with a 5 min interval of reperfusion between the two occlusions. Blood samples and lung biopsies were obtained at prepneumonectomy (PPn), at prereperfusion (PRp) and up to 30 min after reperfusion of the implanted lobe (Rp-10’ and Rp-30’). Haemodynamic and blood-gas measurements, evaluation of oxidative stress in lung tissue and MCP-1, tumour necrosis factor-α (TNF-α) and IL-1 protein and mRNA measurements in lung tissue were performed. Nonparametric tests were used to compare differences between groups. Data are expressed as mean ± SEM.

RESULTS: In control lungs, MCP-1 protein levels were found to be higher at PRp, Rp-10’ and Rp-30’ than at PPn (0.59 ± 0.1 vs. 0.21 ± 0.05, 0.47 ± 0.01 vs. 0.21 ± 0.05 and 0.56 ± 0.01 vs. 0.21 ± 0.05, respectively; P < 0.05). These differences were not evident in the IP group. MCP-1 levels at PRp, Rp-10’ and Rp-30’ were significantly higher in the control group than in the IP group (0.59 ± 0.1 vs. 0.15 ± 0.02, 0.47 ± 0.01 vs. 0.13 ± 0.01 and 0.56 ± 0.01 vs. 0.27 ± 0.01, respectively; P < 0.05). MCP-1, TNF-α and IL-1 mRNA expressions were lower at PRp, Rp-10’ and Rp-30’ (control vs. IP group, P < 0.05) when IP was carried out. Lipid peroxidation metabolites and myeloperoxidase activity increase in lung tissue were prevented by IP.

CONCLUSIONS: In this model, LIRI induced the expression of MCP-1 and the proinflammatory proteins TNF-α and IL-1 in control lungs. IP significantly reduced the expression of these chemokines and cytokines. These features may explain the reduction of oxidative stress observed with IP.

Keywords: Reperfusion injury • Ischaemic preconditioning • Monocyte chemoattractant protein-1 • Experimental animal models

INTRODUCTION

Currently, thoracic surgeons perform more and more complex procedures, which often involves inducing lung ischaemia for a given period of time. Lung ischaemia and the ensuing reperfusion precipitate lung tissue injury, which features alveolar damage, lung oedema and hypoxaemia. Different prophylactic and therapeutic measures have been investigated to prevent lung ischaemia-reperfusion injury (LIRI). Ischaemic preconditioning (IP), defined as short periods of ischaemia prior to prolonged ischaemia, has been shown to protect several organs from IR injury; these organs include the heart, liver, brain and kidneys, but the role of IP in LIRI is not fully understood [1–3].

Previous studies have shown that alveolar macrophages contribute to the acute phase of LIRI [4–6]. In response to oxidative stress, activated alveolar macrophages produce various cytokines and chemokines [7, 8]. Monocyte chemoattractant protein-1 (MCP-1), a chemokine that regulates migration and activation of monocytes and macrophages, is believed to play a crucial role in this process, and its expression has been positively correlated with LIRI in an isolated mouse lung model [4]. It is also known that the expression of MCP-1 in several epithelial cells is upregulated through tumour necrosis factor-α (TNF-α) and...
interleukin-1 (IL-1), which are expressed under hypoxaemic conditions and are activated by excess reactive oxygen species (ROS) [9, 10]. Moreover, it has been shown that IP inhibits TNF-α, IL-1 and ROS production in LIRI [1, 11], although to date, no study has related IP to MCP-1 modulation.

The authors have previously shown that MCP-1 concentration is increased in lung tissue after ischaemia and upon early reperfusion in an in vivo LIRI swine model [12]. Therefore, the objectives of the present study were to examine IP's effect on LIRI-induced MCP-1 expression in lung tissue using the same experimental lung autotransplant model. Furthermore, we sought to establish a plausible link among MCP-1, IL-1 and TNF-α expression in lung tissue and to assess the link between the expression of these cytokines and lung tissue oxidative stress.

**MATERIAL AND METHODS**

This study was granted approval by the institution’s Research and Animal Experimentation Committee, and all animals received humane care in compliance with the European Convention on Animal Care.

**Animal model and study groups**

Fourteen large white pigs, weighing 35–45 kg, underwent an orthotopic left lung autotransplantation (left pneumonectomy, ex situ cranial lobectomy and left caudal lobe reimplantation) with a subsequent 30-min graft reperfusion. Animals were grouped by random numbers (Microsoft Excel 2003) to receive lung autotransplantation without IP (control group, CON, n = 7) or with an IP procedure (IP group, n = 7).

**Anaesthesia and surgical protocols**

The anaesthesia protocol and the surgical technique for this lung autotransplant model have recently been described in detail by Simón Adiego et al. [12]. Briefly, premedication was performed with intramuscular ketamine 10 mg/kg (Ketoral, Parker Davis). Once in the operating room, pulsoxymetry and electrocardiographic monitoring were performed. Anaesthesia induction was achieved with propofol (4 μg/kg; Diprivan, Fresenius K), fentanyl (3 μg/kg; Fentanest, Kern Pharma) and atracurium (0.6 mg/kg; Tracrium, Glaxo Smith Kline). Orotracheal intubation was performed and mechanical ventilation was provided with a Drager SA-1 ventilator (tidal volume 8 ml/kg, respiratory rate 12–15 rpm and inspiratory/expiratory ratio of 1:2, to maintain PaCO₂ in the range 35–40 mmHg). FIO₂ was maintained at 1 throughout the procedure. Intraoperative crystalloid infusion was maintained at 6–8 ml/kg per hour. Anaesthesia was maintained with propofol in continuous perfusion (8–10 mg/kg/h) throughout the experiment. Supplemental doses of fentanyl and atracurium were used when required. A surgical tracheotomy was performed, the otracheal tube was removed and a 6 mm cuffed tube was inserted into the trachea through the tracheotomy. A 7-F pulmonary artery catheter (Edwards Lifesciences) was introduced through the femoral vein. A 7-F femoral artery catheter was used for blood-pressure monitoring and blood sampling.

A left thoracotomy was carried out by means of a fourth or fifth rib resection. To perform left pneumonectomy, the pulmonary artery, cranial vein, caudal vein and main left bronchus were progressively dissected. Two-lung ventilation was maintained until the pulmonary vessels were dissected, the main left bronchus was sectioned and the endotracheal tube was placed into the right bronchus. Just before the pneumonectomy was completed, a bolus of intravenous heparin (300 IU/kg; Mayne Pharma Spain, S. L.) was administered to prevent thrombosis in the clamped pulmonary artery. Next, on the back table, the left lung was perfused through the pulmonary artery and veins with University of Wisconsin solution at 10–15°C until a clear effluent was observed coming out of the pulmonary vessels. A cranial lobectomy was carried out and the caudal left lobe was then implanted back into the animal, performing a bronchus-to-bronchus anastomosis, a pulmonary artery-to-pulmonary artery anastomosis and an inferior vein-to-left atrium anastomosis. Graft reperfusion was performed initially in a retrograde direction by unclamping the left atrium, and then the endobronchial tube was pulled back into the trachea, enabling two-lung ventilation. Anastomotic patency of the atrial anastomosis was determined by active bleeding through the pulmonary artery Anastomosis. The left pulmonary artery was then unclamped, and blood flow was maintained for 30 min. At the end of the experiment, the animal was euthanized with a potassium chloride injection under deep anaesthesia. In the experimental group, 5 min before the dissected pulmonary vessels and the main left bronchus were sectioned, two separate 5-min left pulmonary artery clamping attempts were carried out, with a 5 min interval reperfusion between the two occlusions (Fig. 1).

![Figure 1](image_url)
Measurement and sampling time points (Fig. 1)

Baseline (B) haemodynamic and arterial blood gas measurements were performed 30 min after the thoracotomy, with the animal under two-lung ventilation. Haemodynamic and arterial gas measurements were performed, and lung biopsies were collected at the following time points: pre-pneumonec-tomy (PPn)—before completing pneumonec-tomy and with the animal under one-lung ventilation (OLV); prereperfusion (PRp)—before reperfusion and ventilation of the reimplanted left caudal lobe; 10 min postreperfusion (Rp-10)—10 min after the reperfusion of the reimplanted lobe; and 30 min postreperfusion (Rp-30)—30 min after the reperfusion of the reimplanted lobe. In the experimental group, PPn measurements and biopsies were performed after IP manoeuvres.

Haemodynamic measurements. The following variables were determined at the above-mentioned time points: Haemodynamic: A femoral-artery catheter was used to record the mean blood pressure (MBP). The pulmonary-artery catheter recorded the pulmonary artery mean pressure (MPAP). The following variables were determined at the above-mentioned time points:

- Cardiac index (CI) and systolic volume (SV). In addition, the cardiac output monitor (Edwards Lifesciences) and the mean blood pressure (MBP). The pulmonary-artery catheter determined at the above-mentioned time points: CI (L × min⁻¹ × m⁻²).

Bloodgas measurements. Arterial blood gas analyses were performed at the mentioned time points. In addition, bloodgas samples were taken by puncturing the pulmonary vein of the reimplanted lobe at 10 and 30 min after reperfusion.

Biochemical studies in lung tissue. Lung-tissue biopsies were performed for biochemical studies. The first two samples of lung tissue (PPn and PRp) were obtained from the cranial lobe (the PRp lung sample was taken from the cranial lobe left on the back table, just before reperfusion of the reimplanted caudal lobe) and the last two (Rp-10' and Rp-30') from the reimplanted caudal lobe. Every lung sample was placed in a cryotube, flash-frozen in liquid nitrogen and stored at −80°C until biochemical analysis. The preparation of tissue homogenates has previously been described in detail [13].

Table 1: Haemodynamic and gasometric data

<table>
<thead>
<tr>
<th>Haemodynamics</th>
<th>Group</th>
<th>B</th>
<th>PPn</th>
<th>PRp</th>
<th>Rp-10'</th>
<th>Rp-30'</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>CON</td>
<td>99 ± 5</td>
<td>98 ± 5</td>
<td>88 ± 6</td>
<td>88 ± 5</td>
<td>83 ± 6</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>93 ± 2</td>
<td>105 ± 4</td>
<td>95 ± 6</td>
<td>85 ± 3</td>
<td>83 ± 7</td>
</tr>
<tr>
<td>MPAP (mmHg)</td>
<td>CON</td>
<td>25 ± 2</td>
<td>23 ± 2</td>
<td>24 ± 2</td>
<td>28 ± 4</td>
<td>28 ± 3</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>20 ± 1</td>
<td>30 ± 1*</td>
<td>33 ± 7</td>
<td>30 ± 2</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>CON</td>
<td>100 ± 9</td>
<td>92 ± 10</td>
<td>102 ± 10</td>
<td>99 ± 9</td>
<td>96 ± 10</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>100 ± 6</td>
<td>108 ± 10</td>
<td>114 ± 14</td>
<td>125 ± 13</td>
<td></td>
</tr>
<tr>
<td>SV (ml/beat)</td>
<td>CON</td>
<td>51 ± 6</td>
<td>53 ± 6</td>
<td>47 ± 5</td>
<td>51 ± 5</td>
<td>52 ± 6</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>61 ± 9</td>
<td>51 ± 4</td>
<td>48 ± 7</td>
<td>43 ± 6</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>CI (L × min⁻¹ × m⁻²)</td>
<td>CON</td>
<td>5.2 ± 0.6</td>
<td>4.5 ± 0.3</td>
<td>4.7 ± 0.5</td>
<td>4.9 ± 0.7</td>
<td>6.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>6.6 ± 0.8</td>
<td>5.9 ± 0.4</td>
<td>5.2 ± 0.5</td>
<td>7.1 ± 2.5</td>
<td>6.9 ± 1.4</td>
</tr>
<tr>
<td>Femoral artery PO₂ (mmHg)</td>
<td>CON</td>
<td>356 ± 49</td>
<td>200 ± 31</td>
<td>303 ± 45</td>
<td>246 ± 49</td>
<td>302 ± 53</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>331 ± 60</td>
<td>288 ± 63</td>
<td>391 ± 42</td>
<td>315 ± 61</td>
<td>323 ± 60</td>
</tr>
<tr>
<td>Pulmonary vein PO₂ (mmHg)</td>
<td>CON</td>
<td></td>
<td></td>
<td></td>
<td>279 ± 65</td>
<td>253 ± 39</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td></td>
<td></td>
<td></td>
<td>225 ± 65</td>
<td>446 ± 39</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± standard error of the mean. MBP: mean blood pressure; MPAP: pulmonary artery mean pressure; HR: heart rate; SV: systolic (stroke) volume; CI: cardiac index; PO₂: partial pressure of oxygen; CON: control group; IP: ischemic preconditioning group; B: basal; PPn: Pre-pneumonec-tomy; PRp: Prereperfusion; Rp-10': 10 min postperfusion; Rp-30': 30 min postperfusion.

*P < 0.05 vs. CON group.

Evaluation of oxidative stress: Lipid hydroperoxides (LPO) show the degree of membrane lipid oxidation and were determined using a specific kit for application in lung tissue (K-assay LPO-CC, Kamiya Biochemical Company, USA). Malondialdehyde (MDA), an end compound of lipid peroxidation, is a cell injury marker. It was assayed through the formation of thiobarbituric acid derivatives in lung tissue [14]. The myeloperoxidase (MPO) assay was used to quantify lung tissue neutrophil accumulation and was performed using the modified Bradley method [15].

Enzyme-linked immunosorbent assay (ELISA) measurements of MCP-1: MCP-1 protein levels were determined using specific kits (Biosource International) using lung tissue homogenates.

Western blotting analysis: Western blots were used to measure the protein expression of TNF-α and IL-1. Testing was done using specific antibodies: anti-TNF-α (Endogen) and anti-IL-1β (Bio Genesis).

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR): RNA was isolated from heart samples of male mice using the method described by Chomczynski [16], using the TRI Reagent Kit (Molecular Research Center, Inc., Cincinnati, OH, USA), following the manufacturers protocol. The purity of the RNA was estimated with 1.5% agarose gel electrophoresis, and the RNA concentrations were determined with spectrophotometry. Reverse transcription of 2 mg of RNA for cDNA synthesis was performed using the Reverse Transcription System (Promega, Madison, WI, USA), and a pd(N)6 random hexamer. RT-PCR was performed using an Applied Biosystems 7300 apparatus with the SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) and 300 nM concentrations of specific primers. The sequences of the primer transcripts were as follows: MCP-1, forward: ACAGAAGAGTCACCAAGACCA, reverse: GCGGCCGAG GGTCTT; IL-1, forward TGATGAGAAGACGGCCACAC, reverse CTTCTTTTGGGTATTTGGG; and TNF-α, forward: ATGAGAAG TTCCCCAATGGG, reverse CTCCACCTGGTTTGTTGCTA. The housekeeping gene 18s was used as internal control (forward: GGTCATGGCCGCTTTA, reverse: TCGTGCGTTATCGGAATTAA CC). Relative changes in gene expression were calculated using the 2−ΔΔCT method [17].

Reproducibility within the assays was evaluated in three independent experiments. Each assay was performed with three
replicates. The overall intra-assay coefficient of variation was calculated to be <5%. Assay-to-assay reproducibility was evaluated in three independent experiments. The overall interassay coefficient of variation was calculated to be <6%.

### Statistical analysis

The data are expressed as the mean and the standard error of the mean (SEM). Nonparametric tests were used. Accordingly, a Mann–Whitney U-test was applied to establish the differences between the analysed groups. In addition, a Wilcoxon test for paired data was used to study the evolution of the intragroup values. Statistical significance was considered at $P < 0.05$. The SPSS version 14.0 statistical package was used in this study.

### RESULTS

There were no differences between the CON and IP groups in terms of animal weight ($42 \pm 6$ vs. $35 \pm 3$ kg), lung ischaemia time ($102 \pm 8$ vs. $107 \pm 7$ min), OLV time ($117 \pm 4$ vs. $114 \pm 4$ min) or duration of the entire procedure ($285 \pm 15$ vs. $314 \pm 7$ min).

#### Haemodynamics

The haemodynamic variables (Table 1) showed great stability in both groups, and the differences between them were limited to MPAP, which was found to be greater in the IP group before the pneumonectomy was completed.

#### Blood-gas analysis

$PO_2$ measured in the pulmonary vein 30 min after lung reperfusion was significantly higher in the IP group than that in the CON group, but no other significant differences were observed when comparing blood gases obtained from the pulmonary vein or femoral artery throughout the procedure (Table 1).

#### Oxidative stress in lung tissue

Lipid peroxidation metabolites (LPO and MDA) were increased in lung samples obtained upon ischaemia and after reperfusion. However, in the IP group, LPO and MDA levels in reperfused lung tissue were lower than those in the CON group (Tables 2 and 3).

#### Lung tissue myeloperoxidase

Tissue MPO activity in biopsy specimens from reimplanted lungs increased significantly after reperfusion in the CON group; this increase was not observed in the IP group. MPO activity was higher in the CON group than in the IP group after 10 and 30 min of reperfusion (Table 2).

#### Western blot analysis of tumour necrosis factor-α and interleukin-1

In control lungs, the proinflammatory cytokines IL-1 and TNF-α were found to be significantly higher after ischaemia (PRp) and reperfusion (Rp-10′ and Rp-30′) than before definitive pulmonary-artery clamping prior to pneumonectomy (PPn). These increases were not observed in the IP group.
when IL-1 or TNF-α was measured at PRp, Rp-10’ and Rp-30’. When IP took place, both IL-1 and TNF-α protein expression were found to be lower (IP vs. CON, \( P < 0.05 \)) at PRp, Rp-10’ and Rp-30’ (Fig. 2).

### Enzyme-linked immunosorbent assay quantification of monocyte chemoattractant protein-1

In control lungs, MCP-1 protein levels were found to be higher at PRp, Rp-10’ and Rp-30’ than that at PPn. These differences were not evident in the IP group. MCP-1 levels at PRp, Rp-10’ and Rp-30’ were significantly higher in the CON group than in the IP group (Fig. 2).

### Expression of mRNA for tumour necrosis factor-α, interleukin-1 and monocyte chemoattractant protein-1

All three mRNA expressions increased after ischaemia and reperfusion in the CON group but not in the IP group, except for a mild but significant increase in MCP-1 mRNA expression after the ischaemic period (PRp). All three mRNA expressions were lower at PRp, Rp-10’ and Rp-30’ (CON vs. IP, \( P < 0.05 \)) when IP was performed (Fig. 2).

### DISCUSSION

Lung transplant, living donor lobar lung transplantation, arterial sleeve lung resections and pulmonary arterioplasties are
complex surgical procedures, which involve inducing lung ischaemia for a given period of time. Lung ischaemia and the ensuing reperfusion can precipitate LIRI, which is a leading cause of operative morbidity and mortality. A large number of possible treatments have been investigated to prevent or reduce LIRI, one of which is IP. Although the comprehensive mechanism remains unclear, IP seems to increase the tolerance of organs to subsequent sustained ischaemia. IP has been shown to be effective clinically in hepatic resection and in coronary artery bypass graft surgery, but its role remains unproved in clinical LIRI [2].

A more thorough understanding of the factors contributing to IP and how it may be facilitated to reduce the mortality and morbidity associated with LIRI.

In this experimental model of LIRI, haemodynamic, gasometric and biochemical parameters were studied in the early stages of lung IR. An inflammatory response manifested by an increase in oxidative stress, leukocyte activation and proinflammatory cytokines in lung tissue was observed. IP prevented many of these IR-induced effects. The haemodynamic and gasometric measurements remained relatively constant throughout the experiment, both in the control and experimental groups. Transient increase in PPM MPAP could have been related to previous IP manoeuvre. Interestingly, reperfused lungs showed a better ability to oxygenate the circulating blood when IP was performed.

Clinical and experimental studies have suggested that IR injury occurs in a biphasic pattern. It has been shown that alveolar macrophages, which are activated during ischaemia, play a crucial role in the early phase of reperfusion [5], whereas neutrophils and lymphocytes have a more important role in the delayed phase, which occurs over the subsequent 24 h [5, 6, 18]. In addition, numerous studies have documented that IR increases ROS and that macrophages produce pro- and anti-inflammatory cytokines in response to oxidative stress [18]. Some of these cytokines released by macrophages before and after reperfusion will be involved in the recruitment of neutrophils and lymphocytes into the lung during reperfusion [19]. The findings of this study correlate well with the suggested biphasic pattern of IR injury. In control animal lungs, lipid peroxidation metabolites, IL-1 and TNF-α were found to be significantly higher after ischaemia and during early reperfusion compared to baseline, whereas the increase in MPO activity, which indicates neutrophil activation, was observed only after reperfusion. The progressive increase in TNF-α throughout the IR period points to the prominent role of this cytokine in the development of the acute and delayed phases of LIRI, as suggested by Eppinger et al. [5].

It has been proposed that MCP-1, a chemokine that regulates the migration and activation of monocytes and macrophages, plays a crucial role in the recruitment of macrophages in inflammation and tissue injury [20]. MCP-1 is produced by a variety of cell types, either constitutively or after induction by oxidative stress, cytokines or growth factors [10]. Specifically, MCP-1 has been shown to be upregulated in vitro through TNF-α and IL-1 [9]. The involvement of MCP-1 in chronic diseases, such as AIDS, cancer and atherosclerosis, has been widely studied, but little is known about its role in LIRI [10]. The results of this study are consistent with the above suggestion. In addition to the observed increase in IL-1 and TNF-α, both MCP-1 protein levels and mRNA MCP-1 expression increased progressively upon ischaemia and during early reperfusion, suggesting, as in the case of TNF-α, that this chemokine could play a role as an inflammatory modulator during early and late reperfusion. In this regard, Zhao et al. showed that MCP-1 expression is positively correlated with LIRI in an isolated mouse-lung model [4]. Moreover, in a rat model of warm LIRI, Eppinger et al. [5] found that the administration of anti-MCP-1 antibody before reperfusion reduced the lung permeability index at 30 min of reperfusion by 80%. In a similar model, Naidu et al. [21] demonstrated that chemokine inhibition decreased mRNA expression of several early response cytokines and caused a significant decrease in the secretion of TNF-α. Investigators from the same group suggested that MCP-1 could play a relatively minor role in the development of direct LIRI, compared with other chemokines, such as MIP-1α [22]. However, in the latter study, MCP-1 protein in rat lungs was not detectable until after 4 h of reperfusion. In this experiment, an increase in MCP-1 expression was detected at the end of the period of ischaemia. In such a situation, MCP-1 blockade close to reperfusion may result in delayed and ineffective treatment, which could explain discrepancies among studies.

IP prevented the rise of lipid peroxidation metabolites, IL-1, TNF-α and MCP-1. Interestingly, IL-1 was the only cytokine whose baseline expression (5–10 min after completing the IP sequence in the IP group) was significantly elevated in preconditioned lungs compared to control lungs, suggesting that IL-1 may act in the first steps of IP-induced protection against LIRI. Previous studies have incriminated ROS, ATP-sensitive potassium channel openers, protein kinase C, protein tyrosine kinase and nuclear factor-B as potential intracellular signal transduction pathways of IP [23], but more recent reports have placed the ROS production step upstream in the signalling cascade of ischaemia-induced preconditioning [24]. In this experiment, no significant differences were observed before reperfusion when lipid peroxidation metabolite levels in preconditioned lungs were compared with those of control lungs. This finding correlates well with the previous observation made by others that IP does not increase, but actually reduces, overall ROS production during IR and that specific ROS signals may be carried by one specific free radical species and/or in one intracellular compartment [1, 24]. Thus, the protective effect of IP against ROS-mediated LIRI may, in part, be explained by the fact that sublethal oxidative stress triggers natural protective mechanisms and leads to an adaptation [1]. Maybe the transient increase of MDA observed at PRp in the IP group could have been a consequence of this IP-related sublethal oxidative stress (discrepancies between LPO and MDA values may be explained by the fact that LPO tells one about the early stages of peroxidation while MDA is a measurement of a late stage in the peroxidation process).

Finally, to the best of our knowledge, this is the first report to examine IP-induced changes in MCP-1 protein and mRNA expression in a large-animal model of LIRI. IP prevented the ischaemia-induced increase of MCP-1 expression observed in control lungs, whereas mRNA-MCP-1 expression was found to be slightly but significantly higher upon ischaemia in the preconditioned lungs, compared with control lungs. These results are difficult to explain, but they correlate with the current controversial reports concerning MCP-1’s role in IR injury. As noted above, previous studies have suggested MCP-1 inhibition as a potential target for therapeutic intervention in patients with IR injury [5, 21], whereas other authors have reported that MCP-1 prevents experimental cardiac dysfunction after global IR through a ROS-dependent pathway [25]. The slight increase in mRNA-MCP-1 observed upon ischaemia, but not during early reperfusion, may indicate that acceptable amounts of MCP-1, as
in the case of ROS, could be involved in the signalling cascade of ischaemic-induced preconditioning. Thus, further investigations are mandatory to elucidate the precise role of MCP-1 in LIRI.

In summary, in this large-animal model of lung autotransplantation, which closely mimics the clinical scenario of LIRI, IP seemed to attenuate early IR injury by preventing the increase in lipid peroxidation metabolites, leukocyte activation and the proinflammatory cytokines, IL-1, TNF-α and MCP-1, in lung tissue. Moreover, preconditioned lungs showed a better ability to oxygenate the circulating blood. These results highlight the potential role of IP as a useful preventive strategy against LIRI. This study is the first to demonstrate that IP induced changes in MCP-1 protein and mRNA expression in LIRI. Further investigations are needed to evaluate the clinical implications.

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Conflict of Interest: none declared.

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