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Protection of donor lung inflation in the setting of cold ischemia against ischemia-reperfusion injury with carbon monoxide, hydrogen, or both in rats

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ABSTRACT

Aims: Lung ischemia-reperfusion injury (IRI) may be attenuated through carbon monoxide (CO)'s anti-inflammatory effect or hydrogen (H_2)'s anti-oxidant effect. In this study, the effects of lung inflation with CO, H_2, or both during the cold ischemia phase on graft function were observed.
Materials and methods: Rat donor lungs, inflated with 40% oxygen (control group), 500 ppm CO (CO group), 3% H_2 (H_2 group) or 500 ppm CO + 3% H_2 (COH group), were kept at 4 °C for 180 min. After transplantation, the recipients' artery blood gas and pressure-volume (P-V) curves were analyzed. The inflammatory response, oxidative stress and apoptosis in the recipients were assessed at 180 min after reperfusion.
Key findings: Oxygenation in the CO and H_2 groups were improved compared with the control group. The CO and H_2 groups also exhibited significantly improved P-V curves, reduced lung injury, and decreased inflammatory response, malonaldehyde content, and cell apoptosis in the grafts. Furthermore, the COH group experienced enhanced improvements in oxygenation, P-V curves, inflammatory response, lipid peroxidation, and graft apoptosis compared to the CO and H_2 groups.
Significance: Lung inflation with CO or H_2 protected against IRI via anti-inflammatory, anti-oxidant and anti-apoptotic mechanisms in a model of lung transplantation in rats, which was enhanced by combined treatment with CO and H_2.

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1. Introduction

Lung transplantation (LTx) is an effective treatment for patients with terminal lung disease. However, lung transplant-related mortality remains high. Non-optimal donor lung quality is the most common contributor to early mortality and primary graft dysfunction (PGD) after LTx [1].

Currently, hypothermic preservation plays a key role in the maintenance of donor lung quality by decreasing the metabolic rate by reducing the activity of cellular components and enzyme systems [2]. However, hypothermic preservation also has harmful effects, such as oxidative stress and sodium pump inactivation, among others [3].

Protective measures used to attenuate deleterious effects include lung preservation solutions (e.g.: low-potassium dextran solution, LPD), ex vivo lung perfusion, and flush perfusion [4–5]. Additionally, lung inflation during the cold ischemia phase was more effective than lung deflation for the protection of donor lung quality, and inflation with oxygen was more effective than inflation with nitrogen [6]. Therefore, lung inflation in the setting of cold ischemia with gas that protects against lung graft injury via inhalation may provide similar protective effects.

Carbon monoxide (CO) and molecular hydrogen (H_2), biologic gases that are similar to nitric oxide and hydrogen sulfide, inhaled by donors and/or recipients have exhibited protective effects against lung ischemia-reperfusion injury (IRI) in a lung transplantation model [7–8]. CO mainly alleviates microcirculation derangement mediated by reperfusion and provides anti-inflammatory function, whereas H_2 is mainly used as an anti-oxidant [8–9]. We previously found that lung inflation with H_2 or CO during cold ischemia protected against lung IRI

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through anti-inflammatory and anti-oxidant functions [10,11]. Nakao et al. [12] found that inhalation of both CO and H_2 showed better therapeutic efficacy against cardiac IRI in rats than CO or H_2 alone. Therefore, we hypothesized that lung inflation with both CO and H_2 during the cold ischemia phase may exert superior effects against lung IRI in a rat lung transplantation model.

2. Materials and methods

All protocols were carried out in accordance with the Institutional Animal Care and Use Committee of Harbin Medical University and the Guide for the Care and Use of Laboratory Animals (NIH, revised 1996).

2.1. Experimental protocol

Adult male pathogen-free Sprague-Dawley rats, including donors and recipients, weighing 260–300 g, were purchased from Vital River Laboratories (Beijing, China) and randomly assigned to 5 groups. After their removal from the donors, the donor lungs were inflated with 40% oxygen (O_2) + 60% nitrogen (N_2) in the control group, 500 parts per million (ppm) CO + 40% O_2 + N_2 in the CO group, 3% H_2 + 40% O_2 + N_2 in the H_2 group or 500 ppm CO + 3% H_2 + 40% O_2 + N_2 in the COH group, and were then kept in 4 °C LPD solution (prepared by Harbin Medical University) for 180 min. The mixed gases were purchased from Liming Gas Corporation and Xuelong Gas Corporation (Hei Longjiang, China). Inflation gas was replaced every 20 min (5 ml/kg) with an airtight injector (Agilent Corporation, California, USA). Rats in the sham group underwent left thoracotomies with no transplantation. Recipients were sacrificed by exsanguination at 180 min after reperfusion.

2.2. Orthotopic left lung transplantation

The donors were anesthetized with sodium pentobarbital intraperitoneally (60 mg/kg), intubated endotracheally via tracheotomy, and ventilated (Model 683, Harvard Apparatus, MA, USA) with 40% O_2 + 60% N_2 at a tidal volume of 10 ml/kg. After intravenous injection of sodium heparin (200 U/kg), a median sternotomy was performed, and the lungs were flushed with 20 ml LPD solution. Then, the donor lungs were removed from the donor and inflated with the gas mixtures according to the protocol. Recipients were anesthetized and ventilated as the donors. A left thoracotomy was performed, and the lung graft was implanted using the cuff technique, as previously described [13]. The breathing rate was adjusted to keep arterial carbon dioxide tension ($PaCO_2$) within 35–45 mm Hg. Anesthesia was maintained with additional intraperitoneal doses of pentobarbital sodium and pipecuronium bromide (0.4 $mg \cdot kg^{-1} \cdot h^{-1}$).

2.3. Blood gas analysis

Arterial blood gases were measured before transplantation and every 60 min following reperfusion using a conventional analyzer (Rapid Lab 348, Bayer, Medfield, USA). Time points were recorded as T0–T4 in recipients; the points corresponded to baseline (10 min before transplantation) and 3 min, 60 min, 120 min, and 180 min following reperfusion. At the end of the experiment, blood from the left pulmonary vein was obtained for analysis.

2.4. Measurement of the static compliance of lung grafts

Median sternotomies were performed immediately after the recipients were euthanized; then, the lungs were connected to an apparatus to measure static compliance by the pressure-volume (P-V) curves [14]. Airway pressure was increased from 0 to 30 cm H_2O and then decreased to 0 cm H_2O in 5-cm stepwise intervals. Lung volumes were recorded following 1 min of stabilization.

2.5. Assessment of graft oxidative injury and inflammatory cytokines in serum

The upper section of each lung graft was desiccated at 80 °C for 72 h to detect the wet-to-dry weight ratio (W/D). The inferior section was used to measure the myeloperoxidase (MPO) activity, malondialdehyde (MDA) level, and superoxide dismutase (SOD) level using the respective kit (Jiancheng Bio-Technology, Nanjing, China). Interleukin (IL)-8 and tumor necrosis factor (TNF)-α in serum were measured with an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA).

2.6. Histological examination

The middle section of each lung graft was fixed in 10% formaldehyde, embedded in paraffin, cut into 6-μm thick sections, and stained with hematoxylin and eosin. The lung injury scores (LISs) were based on the neutrophil infiltration, hemorrhage, interstitial edema, hyaline membrane formation, and airway epithelial cell damage by standard criteria (normal = 0, minimal change = 1, mild change = 2, moderate change = 3, and severe change = 4) [15]. A pathologist, using a single-blind method, examined all sections.

2.7. Cell apoptosis detection

Alveolar epithelial cellular apoptosis was detected using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (Zhongshan Golden Bridge Biotechnology, Beijing, China) according to the manufacturer's protocol. The apoptosis index (AI) was a measure of the positive cell number in each 100 cells, and five high-power (×40) fields were randomly selected from each section for counting [16]. A pathologist examined all sections using a single-blind method.

2.8. Immunohistochemistry of lung grafts

Caspase-3 protein expression was measured by immunohistochemical staining using a Power Vision two-step immunohistochemistry detection kit (Zhongshan Golden Bridge Biotechnology, Beijing, China). The immunohistochemical score (IHS) was evaluated in five randomly selected, high-power (×40) fields from each section. The IHS was determined by multiplying the quantity score (no staining = 0; 1%–10% of

Table 1
Experiment-related data (mean $\pm$ SD, n = 8).

Group	Number of rats excluded		Duration (min)		
	Donor	Recipient	Cold ischemia	Warm ischemia	Transplant operation
Sham group	–	0	0	0	0
Control group	0	0	205 $\pm$ 3	2.2 $\pm$ 1.2	25 $\pm$ 3
CO group	0	0	207 $\pm$ 4	2.4 $\pm$ 0.9	27 $\pm$ 4
H_2 group	0	1	209 $\pm$ 2	2.5 $\pm$ 1.1	29 $\pm$ 2
COH group	0	0	204 $\pm$ 3	2.3 $\pm$ 1.0	24 $\pm$ 3

The time of cold ischemia, warm ischemia, and transplant operation in the control group, CO group, H_2 group, and COH group had no significant difference.