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The Journal of Heart and Lung Transplantation

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Protection against lung graft injury from brain-dead donors with carbon monoxide, biliverdin, or both

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KEYWORDS:
brain death;
lung transplantation;
carbon monoxide;
biliverdin

BACKGROUND: The process of brain death can induce acute lung injury in donors and aggravate ischemia-reperfusion injury in grafts. Carbon monoxide (CO) and biliverdin (BV) have been shown to attenuate ischemia-reperfusion injury. We therefore examined if the administration of both CO and BV provide enhanced cytoprotection against lung graft injury from brain-dead (BD) rat donors.

METHODS: Brain death was induced in all donors, after which they were observed for 1.5 hours and then underwent lung transplantation. The recipients were ventilated with 40% oxygen (control group), ventilated with 250 ppm CO in 40% oxygen (CO group), treated with BV (35 mg/kg) intraperitoneally (BV group), or treated with CO and BV conjointly (COBV group) before transplantation ($n = 8$ each group). The recipients were sacrificed 2 hours after lung transplantation by exsanguination. Serum levels of interleukin (IL)-8 and tumor necrosis factor (TNF)-α were measured by enzyme-linked immunosorbent assay.

RESULTS: CO and/or BV treatment attenuated partial pressure of arterial oxygen (Pao_2)/fraction of inspired oxygen (Fio_2) aggravation in the recipients after reperfusion, reduced the wet weight/dry weight ratio, decreased the lung injury score, inhibited the activity of myeloperoxidase in grafts, and decreased serum levels of IL-8 and TNF-α compared with the control group ($p < 0.05$). The COBV group had significantly decreased malonaldehyde levels and increased superoxide dismutase levels in lung grafts compared with the CO group ($p < 0.05$). The static pressure-volume curve of the lungs was ameliorated in the CO group, BV group, and COBV group compared with the control group ($p < 0.05$).

CONCLUSIONS: CO and BV exert protective effects through anti-inflammatory and anti-oxidant mechanisms, and dual treatment provided enhanced cytoprotection against lung graft injury from BD rat donors.
J Heart Lung Transplant 2011;30:460–6

Lung transplantation is limited by the universal shortage of suitable donors.[1] Brain-dead donors (BDD) are a major source of lungs for transplantation, but donation after brain death can cause inflammatory responses in the lungs.[2,3] In addition, early hemodynamic disturbances during the brain death process and progressive systemic inflammatory responses induced by brain death may further aggravate reperfusion injury and chronic rejection.[3,4] In our previous study, the severity of lung injury in grafts from BDDs was significantly higher compared with normal rats.[5]

Heme oxygenase-1 (HO-1) degrades heme into carbon monoxide (CO), Fe^{2+}, and biliverdin (BV). HO-1 provides protection in several models of lung injury, and CO and BV act as mediators of the beneficial effects.[6–8] We observed that the inhalation of CO (250 ppm) in BDDs reduced lung graft injury by inhibiting apoptosis and downregulating inflammatory mediators.[5] Using the same model, we found that BV effectively ameliorated lung graft injury mainly by its anti-oxidant and anti-inflammatory capabilities.[9] Taken

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1053-2498/$ -see front matter
doi:10.1016/j.healun.2010.11.020

together, CO and BV provide cytoprotection through distinct mechanisms. Thus, in the current study, we hypothesized that CO and BV, if administered conjointly, would provide enhanced cytoprotection against lung graft injury from BDD.

Materials and methods

All procedures in this study were approved by the Institutional Animal Care and Use Committee of Harbin Medical University.

Study design

Male pathogen-free Wistar rats (weight, 250–300 g) were purchased from the Animal Experiment Center of Harbin Medical University. Rats were randomized into 4 groups of 8 rats each. Brain death was induced in the donor rats, and they were ventilated with 40% oxygen. Recipient rats were ventilated with 40% oxygen in the control group, ventilated with 250 ppm CO in 40% oxygen in the CO group, ventilated with 40% oxygen and treated with BV (35 mg/kg in 1 ml saline) intraperitoneally before transplantation in the BV group, and treated with CO and BV conjointly in the COBV group with the same specifications as the CO group and the BV group.

Model of brain death and lung transplantation

The BD model was produced as previously described.[5,10] Briefly, the rats were anesthetized by 60 mg/kg sodium pentobarbital intraperitoneally and intubated through a tracheostomy. Brain death was produced by injecting saline (20 μl/min) into a balloon catheter (Fogarty 4F, Baxter Health Care Corp, Irvine, CA), which was introduced into the intracranial cavity through an occipital burr hole. Brain death was confirmed by observing apnea, a sudden decrease and increase in mean arterial blood pressure (MAP), the disappearance of electroencephalogram activity, and maximally dilated and fixed pupils. The MAP was maintained between 80 and 120 mm Hg by infusing a mixture of norepinephrine (0.01 μg/kg per minute) and saline. The animals were ventilated (Harvard Apparatus, South Natick, MA) at a rate of 50 breaths/min, a tidal volume of 10 ml/kg was maintained, and 40% oxygen fraction was inspired.

After 1.5 hours, an orthotopic left lung transplantation was performed using the cuff technique.[11] Anesthesia in the recipient rats was maintained by sodium pentobarbital and pipecuronium bromide (0.4 mg/kg per hour) intraperitoneally. The recipient rats were sacrificed 2 hours after lung transplantation by exsanguination. Arterial blood gases were analyzed every 30 minutes (Rapid Lab 248, Bayer, Medfield, MA). Normal saline was infused at an hourly rate of 10 ml/kg through the femoral vein in all rats.

CO and BV treatment

An original gas containing 500 ppm CO in 40% oxygen and 60% nitrogen was purchased (XueLong Gas Corporation, Daqing, China). Then, mixtures of 250 ppm CO (T40 Rattler, New York, NY) in 40% oxygen and 60% nitrogen were made by diluting it with a gas containing 40% oxygen and 60% nitrogen.

BV (Frontier Scientific, Logan, UT) was dissolved in 0.2 N NaOH and adjusted to a final pH of 7.4 with 1 N HCl. Finally, it was diluted in saline. Both preparation of the solution and the experiment were completed in dim light because BV is light-sensitive. Bilirubin levels in the serum were measured at 0, 5, 30, 60, 90, and 120 minutes after the solutions were administrated (Roche Diagnostics, Mannheim, Germany).

Measurement of inflammatory cytokines and oxidation-reduction indicators

The upper lobe of the lung graft, which was desiccated at 70°C for 1 week, was used to measure the wet weight (W)/dry weight (D) ratio. The inferior lobe, which was stored at −80°C, was homogenized to measure myeloperoxidase (MPO) activity using a special Regent-Box (Jiancheng Bio-Technology, Nanjing, China) and a spectrophotometer. One unit of MPO was defined as the quantity that degraded 1.0 mole of peroxide per minute at 37°C. The results were expressed as units per gram of lung tissue.

The levels of IL-8 and TNF-α in the serum were measured by an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN). The malonaldehyde (MDA) activity, which was expressed as nanomoles per milligram of protein (prot), and superoxide dismutase (SOD) activity, which was expressed as units per milligram of protein (prot), in the supernatants of the homogenate of the grafts were determined using MDA and SOD assay kits (Jiancheng Bio-Technology, Nanjing, China), respectively.

Histologic examination and scoring

Lung grafts were fixed in the paraformaldehyde, embedded in paraffin, sectioned (6-μm thick), and stained with hematoxylin and eosin. All sections were evaluated by a pathologist who was blinded to the study. The evaluation was based on (1) neutrophil infiltration, (2) airway epithelial cell damage, (3) interstitial edema, (4) hyaline membrane formation, and (5) hemorrhage. Each section had 5 scores that corresponded to the 5 criteria and was determined from their degree of deterioration (normal = 0, minimal change = 1, mild change = 2, moderate change = 3, and severe change = 4). A total histologic lung injury score (LIS) was calculated by summing the 5 scores.[12]

Measurement of the static pressure-volume curves of the lung grafts

A midthoracotomy was performed immediately after sacrifice, and the animals were connected to an apparatus to measure the static pressure-volume (P-V) curves of the lungs. Lung volumes were measured by raising the airway pressure to 30 cm H_2O and then decreased to 0 cm H_2O in a stepwise manner with 1 minute of equilibration at each 5-cm H_2O interval.[13] The volume measurements were corrected for gas compression in the apparatus.

Statistical analysis

Data were expressed as mean ± SD. Differences in the measured variables between groups were determined by a 2-way analysis of variance (ANOVA), followed by a Student-Newman, Keuls test. Continuous data were analyzed by repeated-measures ANOVA. The significant difference level was set at $p < 0.05$.