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Original Article

Experimental Swine Lung Autotransplant Model to Study Lung Ischemia–Reperfusion Injury☆

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ABSTRACT

Introduction: Ischemia–reperfusion (IR) lung injury has been investigated extensively on clinical and experimental models of cold ischemia. However, relatively few studies examine the detailed biochemical changes occurring during normothermic (warm) IR.
Animals and methods: Six large-white pigs underwent a lung autotransplant which entailed left pneumonectomy, ex situ cranial lobectomy, caudal lobe reimplantation and its reperfusion for 30 min. Throughout the procedure, several parameters were measured in order to identify hemodynamic, gasometric and biochemical changes. Non-parametric statistical analyses were used to compare differences between periods.
Results: After ischemia, a significant increase ($p < .05$) in lipid peroxidation metabolites, proinflammatory cytokines and chemokines (TNF-α, IL-1β and MCP-1), neutrophil activation, inducible nitric oxide synthase activity and protein kinase MAPK p38 levels were observed in lung tissue. However, constitutive nitric oxide synthase activity in lung tissue and carbon monoxide plasma levels decreased. The same held true throughout the reperfusion period, when an increase in the constitutive heme-oxygenase activity was also shown.
Conclusions: An experimental model of normothermic lung IR injury is presented and detailed changes in hemodynamic, gasometric and biochemical parameters are shown. Both the model and the studied parameters may be clinically useful in future investigations testing new therapies to prevent normothermic IR induced lung injury.

Procedimiento de autotrasplante pulmonar en el cerdo como modelo experimental para el estudio del síndrome de isquemia-reperfusión

Palabras clave:
Daño pulmonar agudo
Isquemia caliente
Daño por reperfusión
Modelo experimental
Autotrasplante
Mediadores inflamatorios

RESUMEN

Introducción: El daño pulmonar agudo por isquemia reperfusión (IR) ha sido estudiado fundamentalmente en modelos experimentales y clínicos con IR fría. Son limitados los estudios que profundizan en las alteraciones bioquímicas durante la IR normotérmica (caliente). El objetivo del este trabajo es presentar un modelo de autotrasplante pulmonar en cerdo para el estudio de las fases más precoces del síndrome de IR normotérmica pulmonar.
Animales y métodos: Seis cerdos de la raza Large-White fueron sometidos a neumonectomía izquierda, lobectomía craneal *ex situ*, reimplantación del lóbulo caudal y reperfusión del mismo durante 30 min. Durante el procedimiento se analizaron diferentes parámetros para identificar cambios hemodinámicos, gasométricos y bioquímicos en el modelo. El estudio estadístico se realizó con pruebas no paramétricas.

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Resultados: Tras la isquemia, se observó en tejido pulmonar un aumento significativo ($p < 0,05$) de metabolitos de peroxidación lipídica, de citoquinas y quemoquinas proinflamatorias (TNF-α, IL-1β y MCP-1), de actividad leucocitaria (mieloperoxidasa o MPO), de actividad óxido nítrico sintasa inducible y de la proteína quinasa MAPK p38, mientras que se observó un descenso de actividad tisular de las formas constitutivas de NOS y de monóxido de carbono sérico. Estas alteraciones se mantuvieron o acentuaron durante la reperfusión, donde se observó también una mayor actividad tisular hemo-oxigenasa constitutiva.
Conclusiones: Se presenta un procedimiento experimental de IR normotérmica pulmonar describiendo en profundidad cambios hemodinámicos, gasométricos y bioquímicos. Tanto el modelo como los parámetros analizados podrían ser útiles en el estudio de nuevas terapias moduladoras del daño pulmonar agudo en situaciones clínicas de IR normotérmica.

Introduction

Several clinical situations require subjecting lung tissue to more or less prolonged periods of ischemia, with the consequent risk of acute lung injury after reperfusion. Most of the studies on how to preserve the lung tissue from the effects of ischemia-reperfusion (IR) are done in experimental or clinical models of lung transplantation with cold ischemia.[1] There are clinical situations, however, in which the progressive cooling of the lung is not possible, nor is it possible to perfuse it with a preservation solution before interrupting blood circulation. Among these situations are lung resections with angioplasty of the pulmonary artery[2–4] and lung lobe transplants from living donors. Less frequent, almost anecdotal situations are the ex situ pulmonary resections of central tumors with reimplantation of the viable pulmonary lobe or lobes.[5] In these cases, part of the lung tissue suffers a more or less prolonged period of warm (normothermic) ischemia, while reperfusion edema and the need for prolonged post-op ventilation are frequent.[6]

In this context, the study of lung injury due to IR and potential modulating therapies requires experimental models in which ischemia is initiated without cooling or previous lung preservation. An experimental model reproducing this situation is lung autotransplantation in animals. The objective of this present study is to present a model of lung autotransplantation in pigs in order to study the earliest phases of the IR syndrome and analyze the hemodynamic, gasometric and biochemical changes that take place during this period.

Animals and Methods

The study was done with the approval of the Animal Experiment and Research Committee of the institution, following all times the European and Spanish guidelines for the manipulation and care of experimental animals.

In six large-white pigs, we carried out a procedure of orthotopic lung autotransplantation. Mean animal weight was 42.8 kg. The intervention consisted of left pneumonectomy with cranial lobectomy ex situ, reimplantation of the caudal lobe and reperfusion for 30 min. Mean procedure time was 289 min (range: 232–325 min) and the mean pulmonary ischemia time was 90 min (range: 84–97 min). At the beginning of the procedure and during the periods of ischemia and reperfusion, different parameters were analyzed to identify hemodynamic, blood gas and biochemical changes in the model.

Surgical Procedure

The animals received no solid food for 18 h before the procedure, with water available ad libitum. The premedication was done with intramuscular ketamine at 10 mg/kg. During surgery, we placed a peripheral catheter, and previous 100% oxygenation was established as monitored with electrocardiogram (ECG) and pulse-oximetry. Anesthesia was induced with propofol (4 mg/kg; Diprivan®, Fresenius K), fentanyl (3 μg/kg; Fentanest®, Kern Pharma) and atracurium besilate (0.6 mg/kg; Tracrium®, Glaxo Smith Kline) through a dorsal vein of the ear. Intubation was done with an orotracheal tube measuring 6–7 mm in internal diameter. Respiratory assistance included a Dräger SA 1 ventilator. Ventilation was controlled by volume (tidal volume 8 ml/kg, 12-15 breaths/min, ratio between inspiration and expiration of 1:2) and it was adjusted during surgery in order to maintain in arterial blood between 35 and 40 mmHg of carbonic anhydride; meanwhile, the inspired fraction of oxygen (FiO_2) was maintained at 1 during the whole procedure. Surgical tracheotomy was performed and, after withdrawing the orotracheal tube, a 6 mm ringed tube was introduced, which allowed easier selective intubation of the right bronchi during surgery. The anesthesia was maintained with propofol at continuous perfusion (8-10 mg/kg/h), with fentanyl and atracurium in bolus, as needed. Intravenous perfusion was maintained with lactated Ringer's solution at 5-6 ml/kg/h as well as a colloid substance, hydroxyethyl starch, as needed. During the intervention, the animal subjects were monitored with three-lead ECG, pulse-oximetry, capnography, invasive arterial pressure and central venous pressure, therefore the femoral vein and artery were catheterized. Through the femoral vein, we introduced a pulmonary artery catheter (thermodilution catheter 7.5 F, Edwards, Irving, California, USA). In order to control diuresis, suprapubic cystostomy was carried out.

After these preliminary procedures, the animal was situated in right lateral decubitus and left thoracotomy was performed with resection of the fourth or fifth costal arch. For the pneumonectomy, we successively dissected the pulmonary artery, the pulmonary cranial vein, the pulmonary caudal vein and the left bronchus. Then, the left bronchus was cut and, with direct vision, the orotracheal tube was advanced towards the right bronchus, initiating the period of one-lung ventilation. The left pulmonary artery was occluded with a protected clamp near the fork of the main pulmonary artery and was cut distally, leaving a margin of 5-10 mm for arterial anastomosis of the reimplantation. The pulmonary cranial vein was ligated near the auricle and cut. To complete the pneumonectomy, the pulmonary vein of the caudal lobe was clamped near the opening of the vein of the mediastinal lobe, cut 1 or 2 mm from the clamp and sutured with continuous proline 6/0 suture. With this maneuver, we were able to keep enough length of the caudal lobe vein for venoatrial anastomosis of the reimplant. In order to prevent thrombosis of the pulmonary artery, which remained clamped during the bench surgery and the reimplant, it was heparinized with 300 UI/kg in bolus at the moment of its occlusion.

This was followed by the bench surgery, and cranial lobectomy was performed. The left lung received anterograde and retrograde perfusion with University of Wisconsin solution at 10-15 °C while manually ventilated (FiO_2: 0.21) until a clear effluent was reached through the pulmonary artery and veins. We dissected the pedicle of the caudal lobe that was going to be reimplanted: the left pulmonary artery (after ligature and sectioning of the cranial branches), the caudal pulmonary vein (liberated from the pleural adherences until the segmental branches) and the left main bronchus (after cutting and suturing the cranial bronchus).