Annexin V homodimer protects against ischemia reperfusion–induced acute lung injury in lung transplantation

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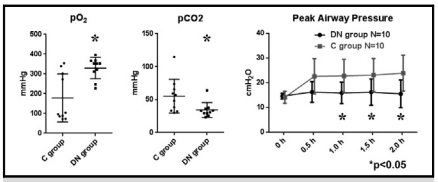
ABSTRACT

Objective: We hypothesized that administration of a homodimer of recombinant annexin V, diannexin, could shield phosphatidylserine on the endothelium, and inhibit leukocyte and platelet adhesion, thereby potentially reducing ischemia reperfusion injury (IRI) in lung transplantation. This hypothesis was tested using a rat syngeneic single left-lung transplant model.

Methods: Rats were randomly assigned to receive diannexin (DN group; n = 10) or normal saline (control group; n = 10). Diannexin (1000 μg/kg) was administered to the donor lung in the pulmonary flush solution, and to the recipient intravenously, 5 minutes after initiation of reperfusion. Grafts were reperfused for 2 hours.

Results: The transplanted grafts in the DN group performed significantly better in gas exchange with higher partial pressure of oxygen (control group: 179 ± 121 vs DN group: 330 ± 54 mm Hg; $P = .007$) and lower partial pressure of carbon dioxide (control: 55.1 ± 26 vs DN: 34.2 ± 11 mm Hg; $P = .04$), as well as lower peak airway pressure (control: 20.5 ± 8.5 vs DN: 12.0 ± 7.9 cm H_2O; $P = .035$) after 2 hours of reperfusion. Wet-to-dry lung weight ratio ($P = .054$), and alveolar fibrin deposition score ($P = .04$), were reduced in the DN group. Caspase-cleaved cytokeratin 18 in plasma (a marker of epithelial apoptosis) was significantly reduced in the DN group ($P = .013$). Furthermore, gene-expression levels of proinflammatory cytokines in the transplanted graft, including interleukin-6 ($P = .04$) and macrophage inflammatory protein 2 ($P = .03$) were significantly decreased in the DN group.

Conclusions: A homodimer of recombinant annexin V reduced ischemia reperfusion injury in a lung transplant animal model, by reducing cell death and tissue inflammation. (J Thorac Cardiovasc Surg 2015;■:1-9)



Functional improvement of the rat transplanted lung by diannexin treatment.

Central Message

Diannexin reduces ischemia reperfusion injury in a rat lung transplant model by reducing cell death, inflammation, and alveolar injury.

Perspective

Primary graft dysfunction is the clinical manifestation of ischemia reperfusion injury after lung transplantation, and it limits successful outcomes. We have demonstrated that diannexin reduces ischemia reperfusion injury in a rat lung transplant model by reducing cell death, inflammation, and alveolar injury. This novel therapy potentially can be used to prevent primary graft dysfunction.

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Primary graft dysfunction (PGD) is the clinical manifestation of ischemia reperfusion injury (IRI) in the acute phase after lung transplantation, characterized by significant deterioration of gas exchange and chest radiograph infiltration.[1] PGD is the leading cause of early morbidity and mortality after lung transplantation.[2] In addition, PGD is associated with decreased long-term survival,[2] impaired physiologic function[3] and the development of chronic lung allograft dysfunction.[4] No therapies have been established to treat this condition.

In the initial stage in the pathogenesis of IRI, phosphatidylserine translocation in the anoxic or early apoptotic endothelium stimulates leukocyte and activated platelet attachment, resulting in impaired blood flow and the

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Abbreviations and Acronyms

CK18-M30 = caspase-cleaved cytokeratin 18
DN group = Diannexin group
IRI = ischemia reperfusion injury
PAI-1 = plasminogen activator inhibitor-1
PGD = primary graft dysfunction
mRNA = messenger ribonucleic acid
TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling

development of a local proinflammatory and procoagulant microenvironment.[5,6] The multidrug resistance gene 1 product, P (permeability)-glycoprotein, transports phosphatidylserine to the outer leaflet of the plasma membrane bilayer.[7] Mice deficient in P-glycoprotein have been found to be protected against IRI in the kidney.[8]

Diannexin, a homodimer of recombinant human annexin V, is designed to shield phosphatidylserine, to prevent cell adhesion, improve blood flow and diminish subsequent tissue injury.[9] In fact, in vivo microscopy in a liver ischemia reperfusion model showed that administration of this drug leads to significant improvement in microcirculatory flow and to reduced tissue injury,[10] and it has been proven to diminish tissue injury in several other IRI models. These include models of heart, using coronary artery occlusion; muscle flap; liver transplant; kidney; and islet transplant.[11-15] The theoretical benefit of this agent, compared with other agents, may be that it targets early IRI, rather than individual downstream pathways. However, the drug has not been examined in the context of lung IRI.

The disruption of the microcirculation with extremely reduced red blood cell velocities in the transplanted lung has been well described, with use of intravital microscopy in a pig model.[16] Additionally, significant numbers of apoptotic cells have been documented in human and animal transplanted lungs in the early reperfusion phase.[17-19] Apoptotic cells externalize phosphatidylserine on the membrane[20] and therefore can be targeted by this treatment. We hypothesized that the shielding of phosphatidylserine by the annexin V homodimer can be protective against IRI in lung transplantation.

METHODS

A rat syngeneic orthotopic single left-lung transplant model was used. Male, inbred Lewis rats (250-300 g; Charles River Laboratories, Inc, Senneville, Quebec, Canada) were used. The rat lung transplant technique has been described.[18,19] The investigational protocol was approved by the Toronto General Hospital Research Institute/University Health Network Animal Care committees. All animals received humane care in compliance with guidelines from the Canadian Council on Animal Care.

Experimental Design

The recombinant homodimer of human annexin V (Diannexin/ASP8597) was gifted by Astellas Pharma, Inc (Tokyo, Japan). This agent is 73 kDa in size and has a half-life of 7 hours in rat.[9] Rats were allocated to 1 of 2 study groups (n = 10 per group) in a blinded and randomized fashion. In the control group, normal saline (vehicle control) was administered to the donor lung and the recipient. In the Diannexin group (DN group), the drug was administered to both the donor lung and the recipient.

Donor rats were anesthetized, tracheally intubated, and ventilated. Heparin sodium (300 Units) (Heparin Sodium Injection USP; Sandoz Canada Inc, Boucherville, Quebec, Canada) was administered through the inferior vena cava. The heart lung block was retrieved after being flushed with 20 mL of pulmonary preservation solution (Perfadex; Vitrolife, Uppsala, Sweden) containing the following: 6 μL of 3.3 mmol/mL tromethamine injection (THAM, Vitrolife), 1.2 μg of calcium chloride (Omega Laboratories Ltd, Montreal, Quebec, Canada), 10 μg of prostaglandin E_1 (Pfizer Canada, Inc, Kirkland, Quebec, Canada), and Diannexin (1000 μg/kg) or vehicle control. The inflated lung was preserved in 40 mL of Perfadex, comprised of the same components as the flushing solution, but without diannexin, for 12 hours at 4°C.

For the recipient rats, the right jugular vein was cannulated for fluid injection. Recipients were ventilated with 100% oxygen and isoflurane at a tidal volume of 10 mL/kg throughout the procedure. Methylprednisolone sodium succinate (5 mg; SOLU-MEDROL; Pfizer Canada, Inc) was systemically administered. A left thoracotomy was performed, and the left native lung was dissected free. The donor left lung graft was anastomosed using a cuff technique.[21] Implantation time or warm ischemic time was approximately 20 minutes. Five minutes after reperfusion, 1000 μg/kg of diannexin or normal saline was administered via a jugular vein catheter.

A separate ventilation technique[22] was used, wherein the native right lung and the transplanted left lung were independently ventilated with tidal volumes of 6 mL/kg (Harvard Apparatus Canada, Saint-Laurent, Quebec, Canada) and 4 mL/kg (flexiVent FX Module 3, SCIREQ, Montreal, Quebec, Canada), respectively. This technique facilitates the accurate measurement of isolated physiologic function in the transplanted graft. Blood gas analysis was performed via pulmonary vein of the graft at 2 hours after reperfusion (Figure 1).

Sample Processing

Blood was sampled in heparinized syringes, and centrifuged for 5 minutes at 6000 rpm to generate plasma. Plasma samples were snap-frozen and stored at −80°C. The lung graft samples were separated into 3 components: The upper portion was used for wet-to-dry lung weight ratio analysis; the middle portion was assessed histologically; and the lowest portion was snap-frozen immediately, to be used for mRNA (messenger ribonucleic acid) and protein studies. For wet-to-dry ratio analysis, lung tissue was weighed and placed in an oven at 80°C for 72 hours. The portion was reweighed, and the ratio of the lung weight from before and after drying was calculated.

Histologic Graft Assessment and Acute Lung Injury Score

Lung samples were fixed in 10% formalin and embedded in paraffin, sectioned (4-μm thickness), and stained with standard hematoxylin and eosin or Martius scarlet blue. The sections stained with hematoxylin and eosin were assessed and scored by a pulmonary pathologist in a blinded fashion. The scoring criteria included: alveolar hemorrhage (presence of red blood cells in air space); vascular congestion (>75% of alveolar septum occupied with red blood cells); alveolar fibrin; and leukocyte infiltration. These criteria were graded on a 4-level scale of abnormalities: normal appearance (0%); mild (<10%); moderate (10%-50%); and severe (>50%), scored from 0 to 3, respectively.

Immunofluorescence Staining

Diannexin localization. Deparaffinized slides were processed with antigen retrieval in boiling 0.01 M citrate buffer (pH = 6) for 20 minutes.