BRONCHODILATOR INHALATION DURING EVLP IMPROVES POST-TRANSPLANT GRAFT FUNCTION FOLLOWING WARM ISCHEMIA

β 2 AGONIST INHALATION DURING EVLP FOLLOWING Tx

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4326 words

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Abstract

Background: We hypothesized that an injured lung graft from donation after cardiac death donors could be reconditioned prior to transplantation using an *ex vivo* lung perfusion (EVLP) system and ventilation with high-dose short-acting beta 2 adrenergic receptor agonists.

Methods: Cardiac arrest was induced in a canine model by intravenous potassium chloride injection. Lungs were randomly assigned to two groups after 150 min of warm ischemia: inhalation of 1400μg of procaterol (BETA group, n = 5) or solvent (CON group, n = 5) during EVLP and left lungs were transplanted after 120 min of EVLP. Functional parameters, tissue adenosine triphosphate levels, and tissue cyclic adenosine monophosphate levels were measured 240 min after transplantation.

Results: Physiological pulmonary function was similar at the end of EVLP in both groups. However, compared with the CON group, significantly better graft oxygenation, dynamic pulmonary compliance, and reduced pulmonary vascular resistance were observed in the BETA group 240 min following transplantation. No severe adverse effects were observed after lung transplantation in the BETA group. Lung tissue adenosine triphosphate levels and cyclic adenosine monophosphate levels were significantly higher in the BETA group than in the CON group at the end of EVLP and 240 min following transplantation.

Conclusions: High-dose nebulized procaterol during EVLP ameliorated lung graft dysfunction at the early post transplantation period without severe adverse effects. These data suggest that lung reconditioning with procaterol ventilation during EVLP improves lung graft function after transplantation.

236 words

Introduction

The shortage of donor organs is a long-standing issue in transplantation medicine[1-4]. Therefore, there is hope that utilization of new lung repair technologies, such as *ex vivo* lung perfusion (EVLP), would increase the number of lungs recovered and ultimately transplanted. To date, several groups have reported the use of the EVLP system as a device for direct pharmacological graft intervention through inhalation or perfusion in large animal models[5-10], and human lungs[11-13]. However, only a few studies have assessed transplantation outcomes[7,9,11] and there is little data on the clinical efficacy of the EVLP system as a means of pharmacological administration. Previously, we demonstrated that the lung injury in case of uncontrolled donation after cardiac death (DCD) is ameliorated by administration of procaterol[14], a short acting bronchodilator, or dibutyryl cyclic adenosine monophosphate in a canine model of EVLP[15].

Ischemia results in not only hypoxia but also cessation of anaerobic glycolysis and leads to reduced intracellular generation of adenosine triphosphate (ATP). Loss of ATP impairs many energy-dependent systems, such as ion pumps, leading to swelling of cells, influx of Ca^{2+}, depletion of glycogen stores, accumulation of lactic acid, and reduction of protein synthesis. Prolonged ischemia makes such cell injury irreversible and results in necrosis. If blood flow is

restored before cells are injured irreversibly, the cells can recover from injury. However, under certain circumstances, ischemia-reperfusion injury occurs and results in cell death[16]

Beta 2 adrenergic receptor is widely distributed in the lungs in the airway smooth muscle, vascular smooth muscle, epithelial cells, endothelial cells, and mast cells. The stimulation of beta 2 adrenergic receptors activates adenylyl cyclase and consequently increases intracellular cyclic adenosine monophosphate (cAMP) levels, subsequently leading to the relaxation of bronchial and pulmonary vascular smooth muscles[17] and enhancement of endothelial barrier[18]. In the endothelium, the stimulation of beta 2 adrenergic receptors activates nitric oxide synthase leading to vasodilation[19]. Furthermore, stimulation of beta 2 receptors in the alveolar epithelium leads to amelioration of pulmonary edema[20].

In the present study, we hypothesized that injured lung grafts from DCD donors could be reconditioned with high-dose nebulized procaterol during an EVLP system prior to transplantation in a canine lung transplantation model.

Methods

Animal preparation

Beagle dogs weighing 10–12.5 kg (Kitayama Labes Co., Hongo Farm, Yamaguchi, Japan) were used for all experiments. All animals received humane care in accordance with the Guide for the

Care and Use of Laboratory Animals (National Institutes of Health publication 85-23, revised 1985). The study protocol was approved by the local animal study committee.

Animals were premedicated with an intramuscular injection of midazolam (0.5 mg/kg), xylazine (0.25 mg/kg), and atropine sulfate (0.05 mg/kg). Animals were intubated and ventilated with an inspired oxygen fraction (FiO2) of 0.5, tidal volume of 25 ml/kg, a respiratory rate of 15 breaths/min, and a positive end-expiratory pressure (PEEP) of 5 cmH_2O, following an intravenous injection of vecuronium bromide (0.3 mg/kg). Anesthesia was maintained with 0.6%–1.5% sevoflurane.

Induction of lung injury

Cardiac arrest was induced by an intravenous injection of potassium chloride (0.5 mEq/kg) without heparinization. The right chest was opened before cardiac arrest to allow biopsy of lung tissue. Following cardiac arrest, the tracheal tube was disconnected from the ventilator and left open to the air. Animals were then kept in a styrofoam box for the 150-min warm ischemic period. Mechanical ventilation was then restarted and donor lungs were retrieved after pulmonary flushing. Retrieved lungs were reconditioned and assessed using an EVLP system prior to transplantation.

Experimental design

Figure 1 shows the experimental design. Lungs were randomly divided into two groups (n = 5 per group). In the group reconditioned with beta 2 adrenergic agonist inhalation (BETA group), donor lungs were reconditioned during acellular EVLP with intermittent inhalation of a total of 1400 μg of the beta 2 adrenergic receptor agonist, procaterol hydrochloride hydrate [0.01% procaterol inhalation solution (14 ml)]. Procaterol inhalation was conducted a total of four times during the EVLP period by dividing the total duration equally. Specifically, 350 μg of procaterol was given by a nebulizer (Aeroneb Professional Nebulizer System, Aerogen, Ireland) every 30 min during the 120 min of EVLP. The control group (CON) received an equal volume of control solvent during EVLP. Left lungs were then transplanted to the recipient dogs and observed for 4 h.

Lung procurement

Both pleura and pericardium were opened following median sternotomy. The main pulmonary artery was cannulated through the right ventricular outflow tract and the superior and inferior vena cava were ligated. After the incision of left atrial appendage, 900–1000 ml of cold ET-Kyoto solution (Otsuka Pharmaceutical Factory, Tokushima, Japan) at a height of 30-cm

above the heart was perfused antegradely and retrogradely. Ventilation was continued until lung retrieval. Pulmonary arteries were cannulated with specialized cannulae (Vitrolife, Denver, CO).

Ex vivo lung perfusion

Acellular EVLP was performed as described by Cypel *et al.*,[21] except that the left atrium was drained openly. In the circuit, lungs were perfused with 1500 ml of STEEN™ solution (Vitrolife, Sweden) enriched with cefazolin (500 mg), heparin (10,000 IU), and methylprednisolone (500 mg). After both donor lungs were transferred to the XVIVO™ chamber (Vitrolife, Denver, CO), the trachea was cannulated and connected to the ventilator. The pulmonary artery cannula was connected to the perfusion circuit and antegrade flow was started at 10% of the full flow rate at room temperature. The flow was gradually increased during the first 30 min of perfusion up to the full flow rate, calculated as the estimated cardiac output (40 ml/kg of body weight). The temperature of the perfusate was gradually increased to 37°C with flow rate. Mechanical ventilation was started at 20 min, when the outflow temperature reached 32°C with a FiO2 of 21%, tidal volume 10 ml/kg, 10 breaths/min, and a PEEP of 5 cmH_2O. At the same time, gas flow (86% nitrogen, 8% carbon dioxide, and 6% oxygen) to the oxygenator was started to deoxygenate the perfusate. EVLP was run at the full flow rate for 90 min following the 30 min step-up procedure. Functional assessments, including

pulmonary artery pressure, peak airway pressure, and partial pressure of oxygen in pre- and post-lung perfusate, were performed following 30 min and 90 min of full flow EVLP after 10-min exposure to an FiO2 of 1.0.

Transplantation procedure

Recipient dogs were ventilated with an FiO2 of 1.0, tidal volume of 25 ml/kg, a respiratory rate of 15 breath/min, and a PEEP of 5 cmH_2O. A Swan–Ganz catheter was placed in the main pulmonary artery and arterial lines were inserted via the right femoral artery into the abdominal aorta. After EVLP, the left lung was transplanted, as previously described[15,22,23] and reperfused for 4 h to evaluate post-transplant lung function. The right pulmonary artery was occluded with a tourniquet to assess graft function 45 min after reperfusion.

Lung function following transplantation

Baseline hemodynamic measurements (heart rate, arterial blood pressure, pulmonary arterial pressure, central venous pressure, and cardiac output), blood gas analysis of arterial and mixed venous blood, and peak airway pressures were recorded. During the post-transplant period, all parameters were recorded at 30, 75, 135, and 195 min following occlusion of the right pulmonary artery. At the end of the experiment, partial resection of the left lower lobe was

performed to assess the wet-to-dry lung weight ratio (WDR), a representative index of pulmonary edema. Wet samples were weighed and heated at 100°C for 36 h and then reweighed to calculate the WDR.

Lung tissue energy levels

Graft tissue samples were collected from the right lower lobe following EVLP and from the left lower lobe following 4 h of transplantation. ATP levels were determined by high-performance liquid chromatography using a Shin-pack CLC-ODS column (15 cm × 6.0 mm; Shimadzu, Japan) and 100 mmol/l sodium phosphate buffer (pH 6.0) at a wavelength of 260 nm, as previously described[24].

Lung tissue cAMP levels

Graft tissue samples were collected from the right lower lobe following EVLP and from the left lower lobe following 4 h of transplantation. cAMP levels were determined with a cAMP radioimmunoassay kit (Yamasa, Chiba, Japan), as previously described[24]. Protein levels were determined at the same time in accordance with the method reported by Lowry *et al.*,[25].

Statistical analysis

All data are presented as mean ± standard deviation. We performed the Mann-Whitney *U*-test and Student's *t*-test for comparisons between the two groups. Analysis of variance for repeated measures was used to evaluate statistical difference within each group. A P value ≤ 0.05 was considered significant.

Results

There were no significant differences in total body weight between the BETA and CON groups (donor weight: 11.9 ± 0.4 kg vs. 11.9 ± 0.4 kg, $P = 0.881$; and recipient weight: 10.7 ± 0.5 kg vs. 10.8 ± 0.3 kg, $P = 0.886$, respectively).

Lung function during EVLP

Oxygenation (BETA group: 492.8 ± 63.6 mmHg; CON group: 560.0 ± 61.6 mmHg; $P = 0.064$), pulmonary vascular resistance (PVR) (BETA group: 1425.8 ± 319.4 dyn·s·cm^{-5}; CON group: 1442.1 ± 415.5 dyn·s·cm^{-5}; $P = 0.473$), and dynamic lung compliance (BETA group: 23.1 ± 3.2 ml/cmH_2O; CON group: 24.8 ± 5.9 ml/cmH_2O; $P = 0.291$) were comparable between the BETA and CON groups at the end of EVLP (Figure 2).

Lung function and pulmonary hemodynamics after transplantation

All five recipients in both groups survived the 4 h observation period following transplantation.

All animals in the BETA group had stable lung function after occlusion of right pulmonary artery, whereas lung function in the CON group deteriorated over time (Figure 3). Compared with the CON group, higher PaO2 (BETA group: 602.4 ± 71.6 mmHg; CON group: 95.6 ± 68.4 mmHg; $P < 0.001$), higher dynamic lung compliance (BETA group: 25.9 ± 3.1 ml/cmH_2O; CON group: 12.1 ± 1.4 ml/cmH_2O; $P < 0.001$), and lower mean PVR (BETA group: 553.4 ± 219.9 dyn·s·cm^{-5}; CON group: 884.6 ± 146.6 dyn·s·cm^{-5}; $P = 0.049$) was observed in the BETA group 4 h following transplantation. The heart rate was significantly higher in the CON group than in BETA group during the post-transplant period (BETA group: 95.6 ± 5.3 /min; CON group: 119.8 ± 3.9 /min; $P = 0.006$) (Figure 3).

Wet-to-dry lung weight ratio

The WDR in the BETA group was significantly lower than that in the CON group at 4 h following reperfusion (BETA group: 6.4 ± 0.5; CON group: 10.2 ± 1.6; $P < 0.001$).

Macroscopic findings and histological findings 4 h following transplantation

The CON group had more severe macroscopic pulmonary edema (Figure 4). Histological findings revealed more severe injury, including perivascular edema, infiltration of inflammatory cells, hemorrhage, and hyaline formation within alveolar space in the CON group compared

with the BETA group (Figure 4).

Lung tissue cAMP and ATP levels

Lung tissue cAMP levels were significantly lower in the CON group than in the BETA group at the end of EVLP (BETA group: 2.1 ± 1.3 pmol/protein mg; CON group: 0.7 ± 0.1 pmol/protein mg; $P = 0.018$) and at 4 h following transplantation (BETA group: 3.7 ± 3.2 pmol/protein mg; CON group: 0.8 ± 0.2 pmol/protein mg; $P = 0.043$) (Figure 5A). Lung tissue ATP levels were significantly lower in the CON group than in the BETA group at the end of EVLP (BETA group: 2.0 ± 0.7 nmol/dry weight mg; CON group: 1.3 ± 0.3 nmol/dry weight mg; $P = 0.049$) and at 4 h following transplantation (BETA group: 2.3 ± 0.8 nmol/dry weight mg; CON group: 1.0 ± 0.4 nmol/dry weight mg; $P = 0.005$). In addition, compared with pre-transplantation levels (immediately after EVLP), ATP levels in the CON group were significantly decreased at 4 h post-transplantation ($P = 0.036$), whereas ATP levels in the BETA group did not deteriorate following transplantation (Figure 5B).

Comment

This study demonstrated that high dose of nebulized procaterol during EVLP prevented deterioration of lung function after warm ischemia in the early post transplantation period in a

canine model. In addition, the recipients were free from severe adverse reactions despite high-dose administration of procaterol.

The primary mechanism we found by which beta 2 adrenergic receptor activation protects the ischemic lungs during EVLP is increased tissue levels of cAMP. Enhancement of cellular cAMP has been suggested to be protective on reperfusion after ischemic insult[26-29]. Beta 2 adrenergic receptors are present not only in the air way smooth muscle but also in the vascular smooth muscle and endothelium causing an increase in cAMP levels and leading to vasodilation and endothelial barrier enhancement. Though there was no significant difference between the CON group and the BETA group during EVLP, PVR was lower and lung edema was milder in the BETA group after transplantation.

In terms of dynamic compliance, there was no significant difference between the groups during EVLP, probably because of too deteriorated lung injury. As the pulmonary vascular permeability increased because of the severe warm ischemic injury, the lungs became edematous in both the CON and BETA groups after EVLP was initiated and some of the airways were submerged with fluid particularly in the dorsal part, making it difficult to measure the exact airway pressure during EVLP. However, the dynamic compliance was higher in the

BETA group than in the CON group after transplantation. One possible reason for this was that the ischemia reperfusion injury in the post transplantation period may be different from that during acellular EVLP. Infiltrating leukocytes are a major factor in ischemia reperfusion injury. Our group had previously indicated that elevating intracellular cAMP levels through a beta 2 agonist administration inhibited leukocyte infiltration in a rat model[24]. In the recent study, we re-perfused the lung grafts with acellular perfusate during EVLP; however, the lung grafts were re-perfused with blood after transplantation. The difference in injury between the CON and BETA groups seemed to be more apparent after transplantation than during acellular EVLP because of the existence of leukocytes in the perfusate. In addition, this result indicated the sufficient effect of an inhalation delivery of procaterol, although the grafts were severely edematous during drug inhalation via EVLP.

In the present study, we demonstrated that the inhalation of a beta 2 adrenergic receptor agonist during EVLP caused increased lung tissue ATP and cAMP levels compared with that in the control group not only at the end of EVLP but also over the 4 h of transplantation. In addition, ATP tissue levels in solvent-treated grafts deteriorated over the 4 h of transplantation leading to severe ischemia-reperfusion injury. Time-dependent decreases in ATP levels of nonventilated and ventilated lungs following death have been previously reported[23,24]. A strong positive

correlation between ATP levels and the viability of lung parenchymal cells and strong negative correlation between ATP levels and endothelial permeability have been demonstrated in a rat model[30]. This is in agreement with our findings of inferior physiological functions and deteriorated lung edema of the CON group after transplantation. We believe that prompt initiation of intermittent inhalation of a beta 2 agonist during EVLP led to more efficient restoration of aerobic metabolism following warm ischemic injury. As a result, increased production of cAMP from ATP led to vasodilation and reduced lung edema. Thus, increased drug delivery and ventilation and effective perfusion of lung grafts resulted from use of the EVLP system. This sequence of events may also reduce ischemia-reperfusion injury from EVLP.

In the present study, we ventilated lung grafts with high-dose procaterol during EVLP. The dose of procaterol (1400 μg) was more than 100 times the single dose used in human per body weight. Although tachycardia and arrhythmia are frequently observed side effects of procaterol, lung graft recipients in the BETA group had normal heart rates with stable hemodynamics following transplantation. In contrast, recipients in the CON group had tachycardia and arrhythmia with unstable hemodynamics due to deterioration in lung function. Ware *et al.*, reported a randomized, blinded, placebo-controlled trial of high-dose nebulized albuterol versus

placebo during the period of active donor management in brain dead organ donors concluding that high-dose albuterol did not lead to improved donor oxygenation or increased donor organ utilization but did result in tachycardia[31]. We believe the reasons for the differences between their report and our study is that we used EVLP system to deliver drug. Short acting beta 2 agonist has higher solubility in water than long acting beta 2 agonist, therefore inhaled procaterol diffused from airways to circulation system. As a result, inhaled procaterol acted on not only airways but also vessels in EVLP system. After EVLP, the perfusate with diffused procaterol was discarded which consequently led to less side effects the recipient experienced, even though high-dose procaterol was administered to the graft. Different from inhalation, infusion delivery is greatly influenced by thrombi[32] in DCD lungs. Water-soluble drug delivery using inhalers during EVLP seems to have a major advantage of preventing systemic toxicity to the recipient by confining the drug to the target organ.

This study has two limitations. The first is the development of a DCD model. There was no agonal period, which is present in almost all clinical cases of DCD donors, as we introduced cardiac arrest by intravenous injection of potassium chloride. The other limitation is the heterogeneity of a long warm ischemic injury. In this study, there were always some thrombi in the pulmonary arteries when we recovered the lungs from the donors, partly because we did not

administer heparin. The thrombi may have prevented homogeneous perfusion of the grafts, affecting PVR.

In conclusion, we demonstrated that inhalation of procaterol during EVLP ameliorates warm ischemic injury in the injured lung grafts, leading to stable outcomes early after transplantation. Further, we demonstrated that high-dose inhalation of short-acting beta 2 agonists during EVLP does not have serious adverse effect on recipients. This result indicates the possibility of EVLP as a means of pharmacological administration. We are planning further studies to apply our results to a clinical setting. Next step might be related to a research using high dose beta 2 agonists in experimental human donor lungs or in clinical EVLP, if situations are allowed.

Disclosure statement

The authors have no conflicts of interest to disclose.

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Figure Legends

Figure 1

Experimental design. EVLP, *ex vivo* lung perfusion; Tx, transplantation.

Figure 2

Physiological lung measurements during 90 min of EVLP at the full flow rate in the beta 2 adrenergic agonist group (BETA; squares) and the solvent group (CON; open circles). EVLP, *ex vivo* lung perfusion; PVR, pulmonary vascular resistance.

Figure 3

Physiological lung measurements following transplantation in the beta 2 adrenergic agonist group (BETA; squares) and the solvent group (CON; open circles). *$P < 0.05$, **$P < 0.01$.

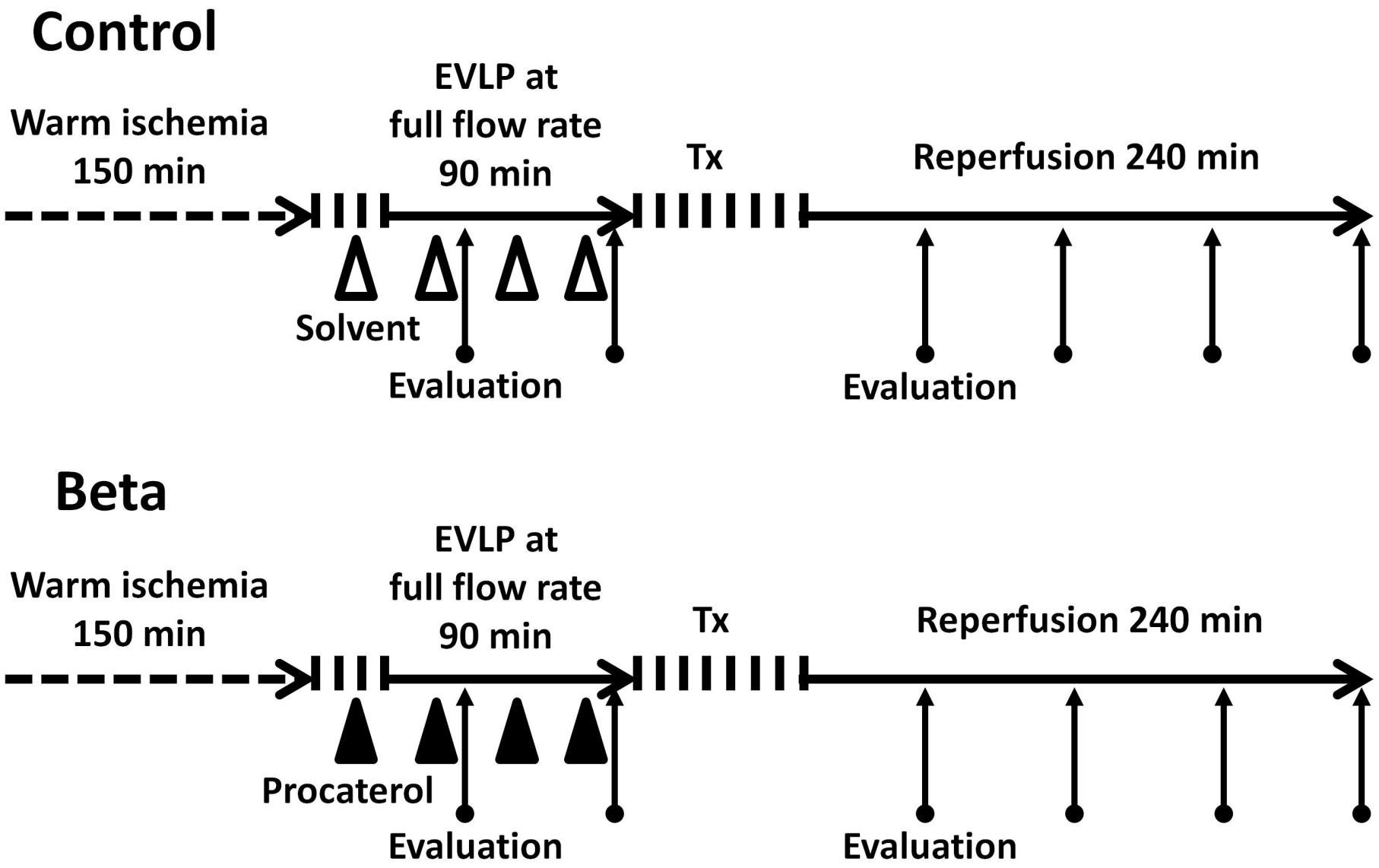
Figure 4

Macroscopic and histological findings 4 h following transplantation in the beta 2 adrenergic agonist group (BETA) and the solvent group (CON).

Figure 5

Lung tissue cAMP levels after EVLP and 4 h following transplantation in the beta 2 adrenergic agonist group (BETA) and the solvent group (CON) (A). Lung tissue ATP levels after EVLP and 4 h following transplantation in the beta 2 adrenergic agonist group (BETA) and the solvent group (CON) (B). ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; EVLP, *ex vivo* lung perfusion; Tx, transplantation. *$P < 0.05$.

Figure 1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Warm ischemia
150 min
EVLP at
full flow rate
90 min
Tx
Reperfusion 240 min
Solvent
Evaluation
Evaluation
Beta
Warm ischemia
150 min
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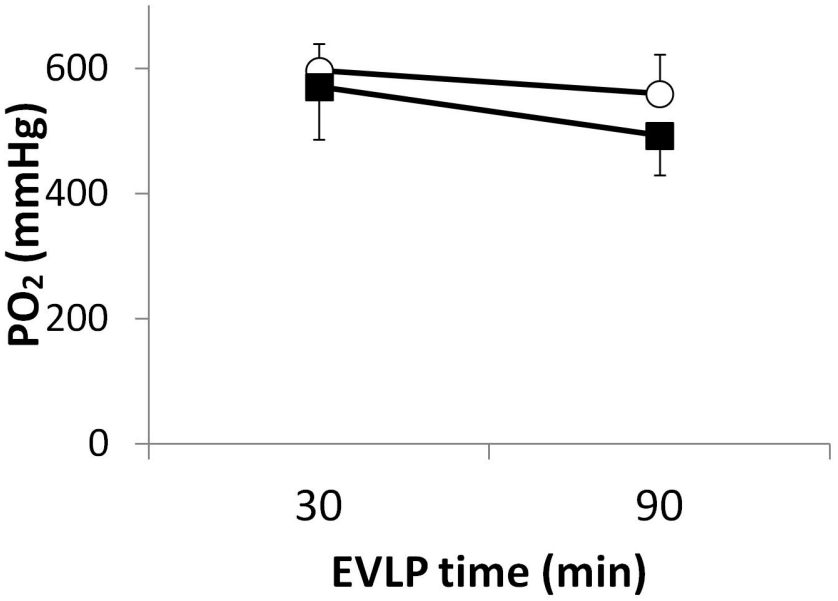
full flow rate
90 min
Tx
Reperfusion 240 min
Procaterol
Evaluation
Evaluation

Figure 2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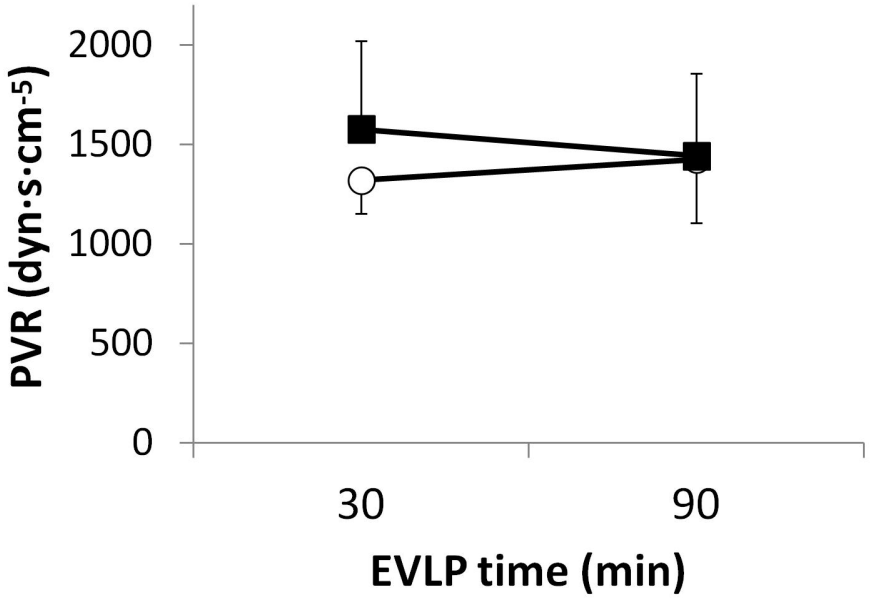
EVLP time (min)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PVR (dyn·s·cm-5)
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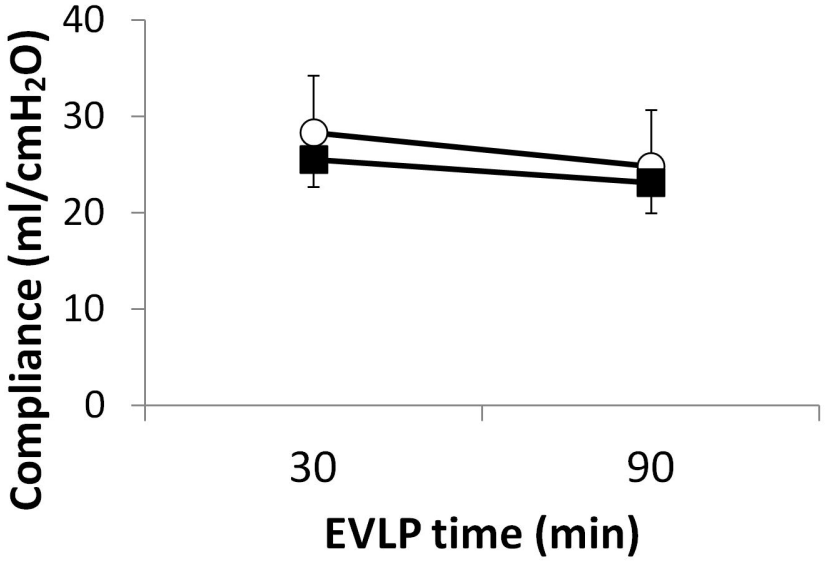
EVLP time (min)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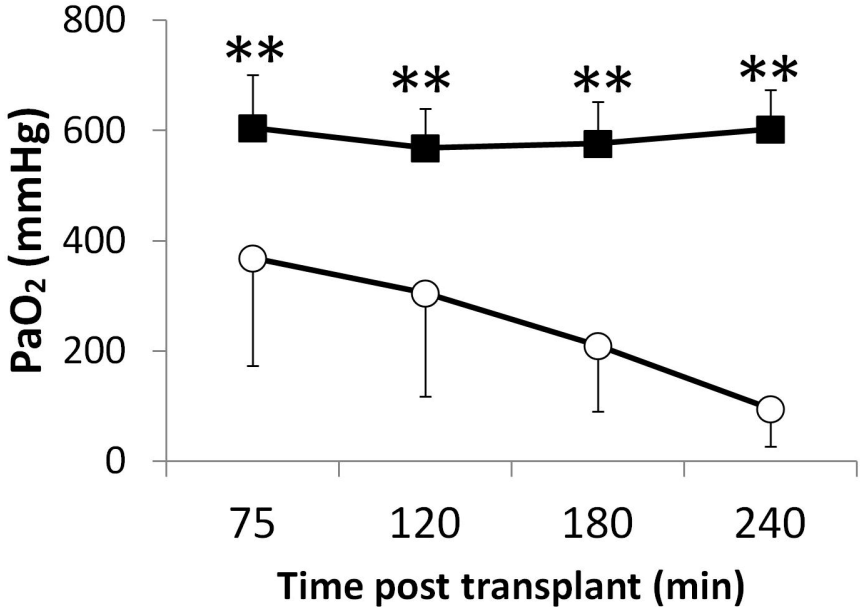
Compliance (ml/cmH2O)
30
90
EVLP time (min)


CON
BETA

Figure 3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600
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PaO2 (mmHg)
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75
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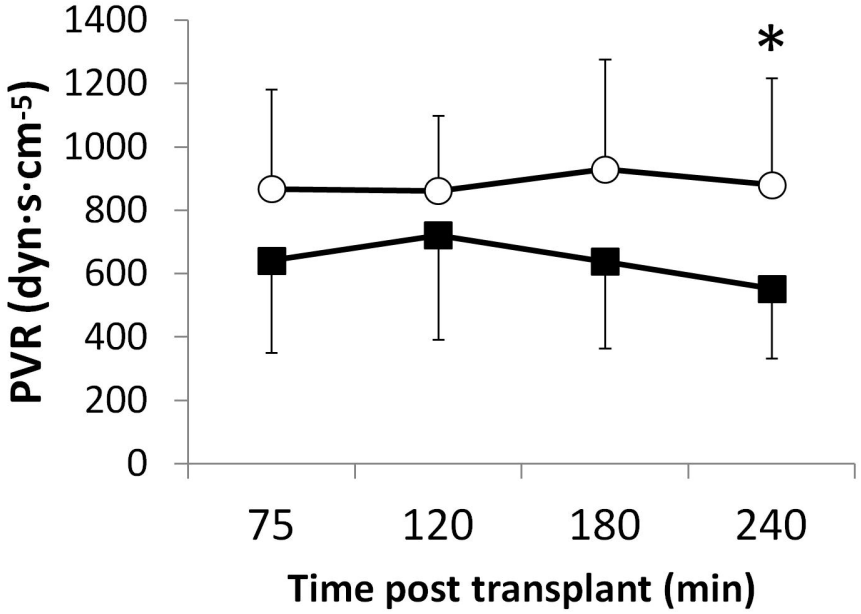
180
240
Time post transplant (min)


1400
1200
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PVR (dyn·s·cm-5)
*
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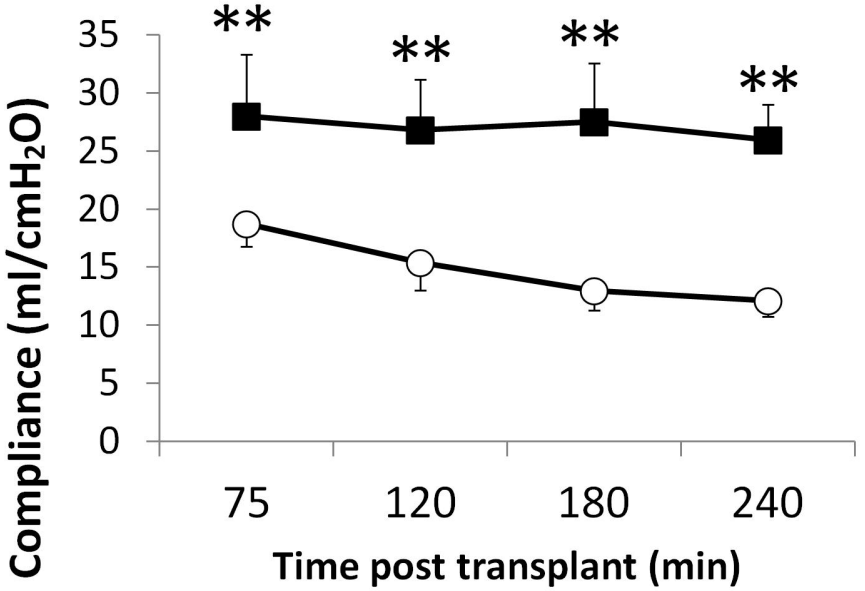
240
Time post transplant (min)


35
30
25
20
15
10
5
0
Compliance (ml/cmH2O)
**
**
**
**
75
120
180
240
Time post transplant (min)

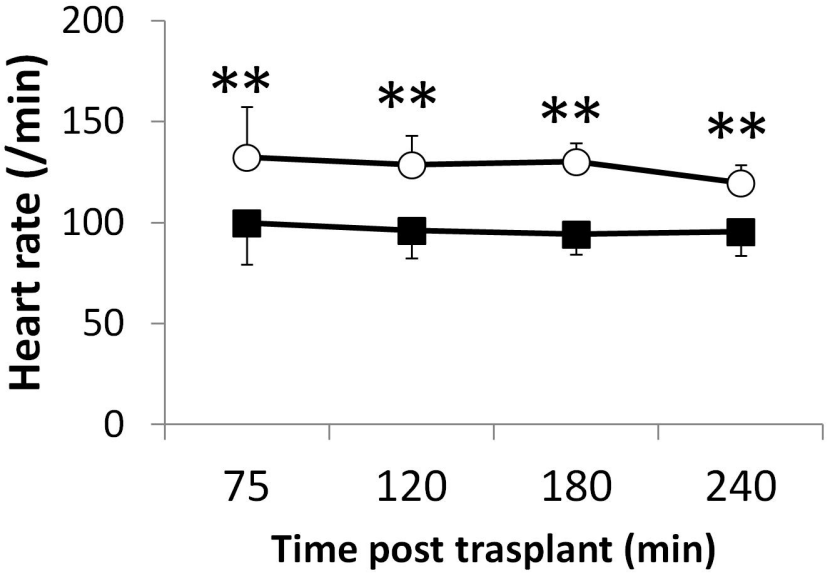
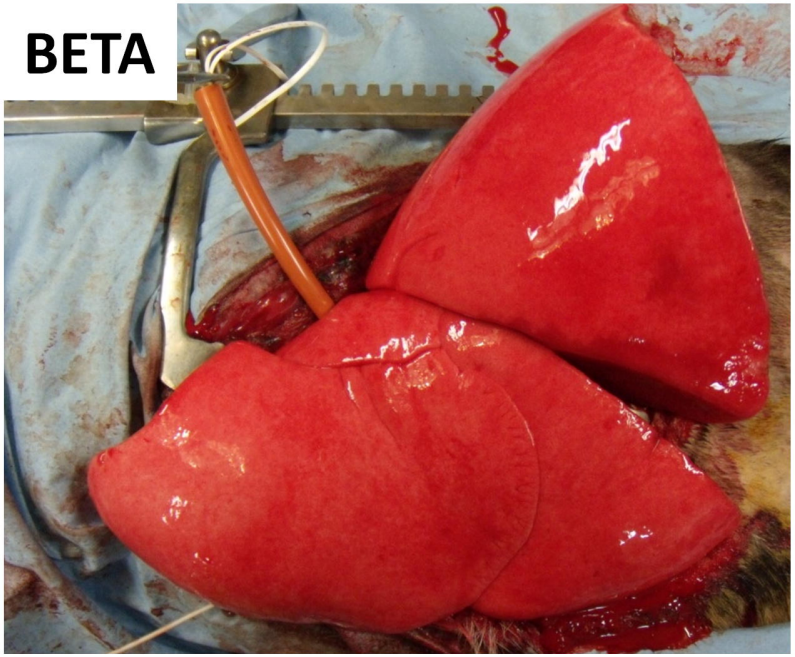
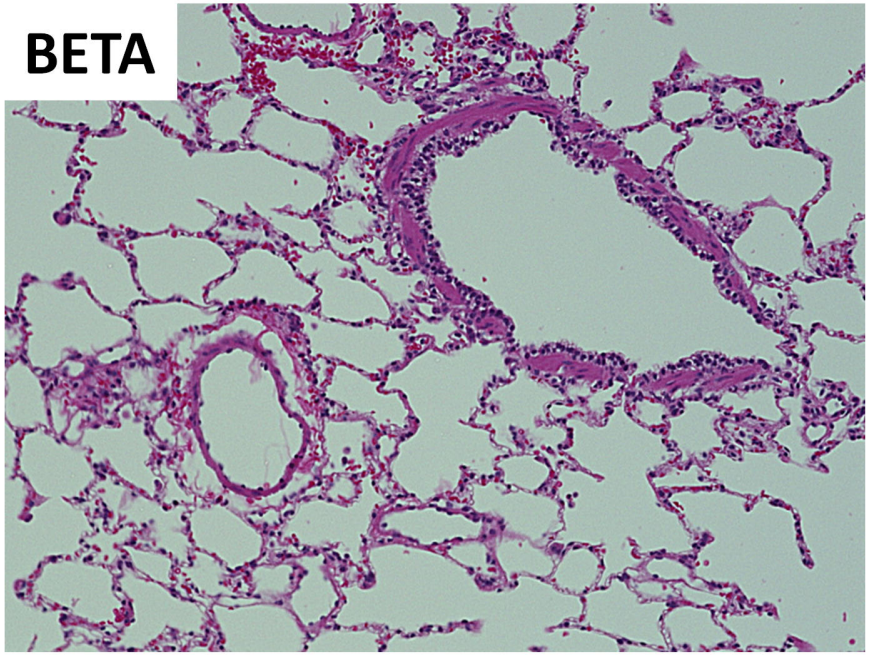
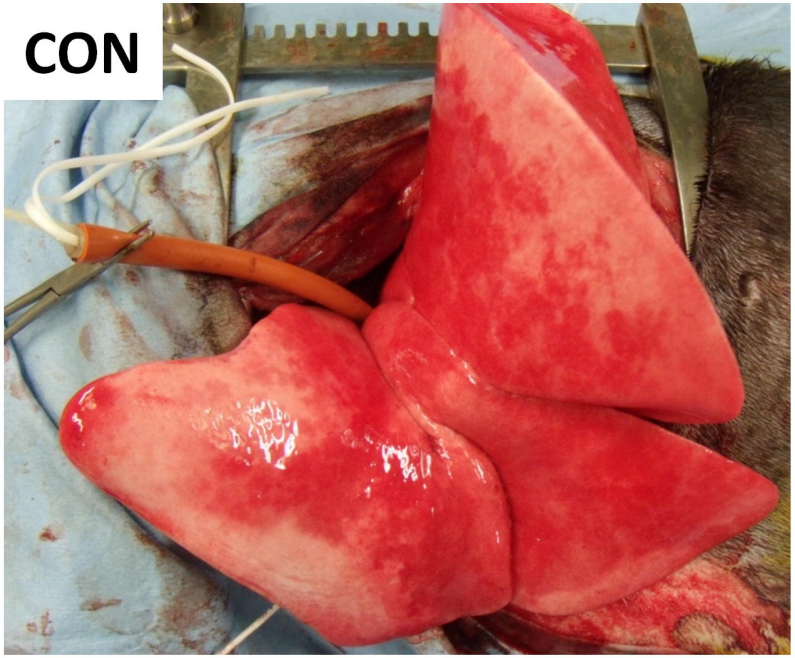

200
150
100
50
0
Heart rate (/min)
**
**
**
**
75
120
180
240
Time post trasplant (min)

Figure 4







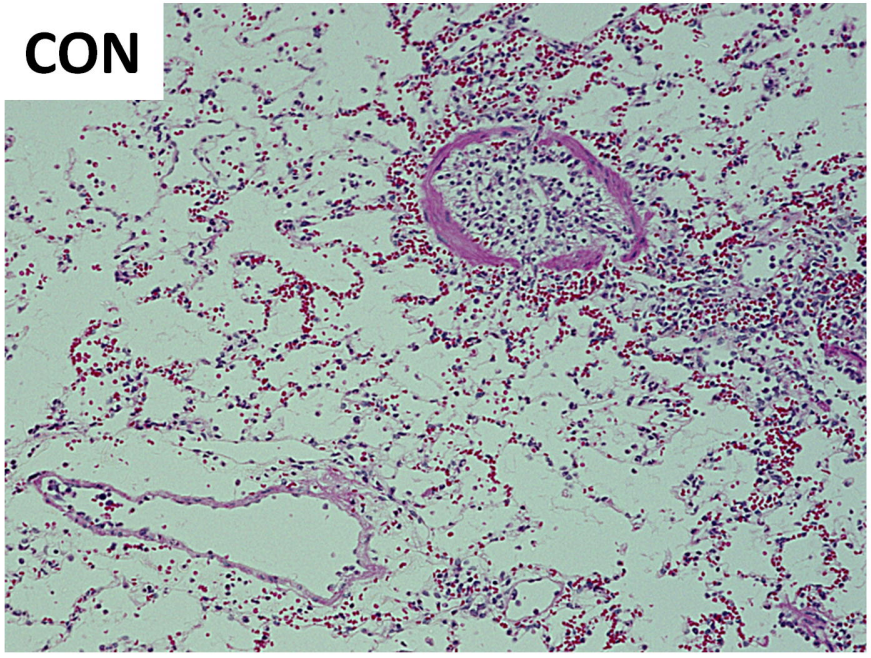
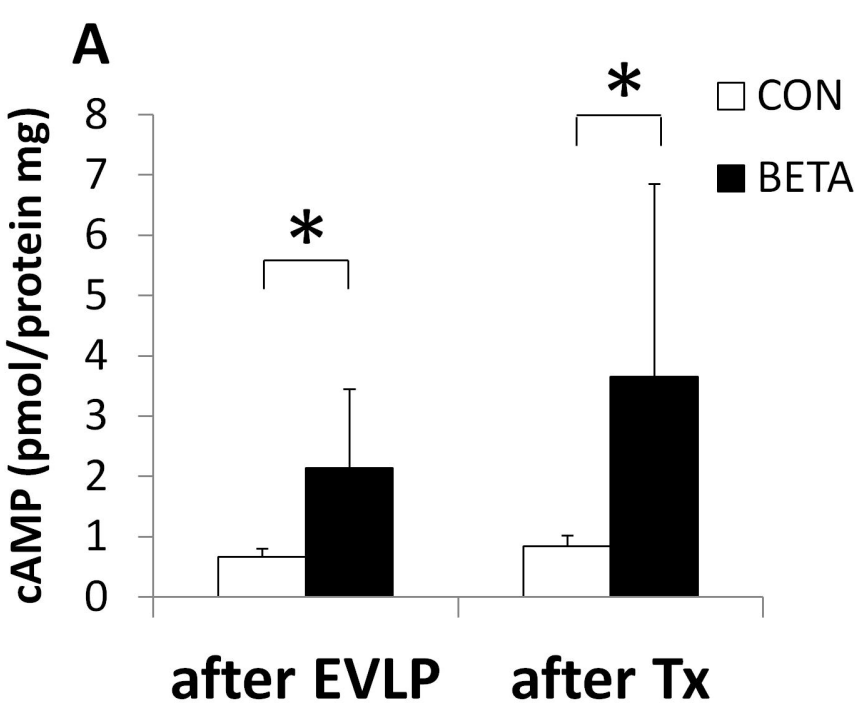
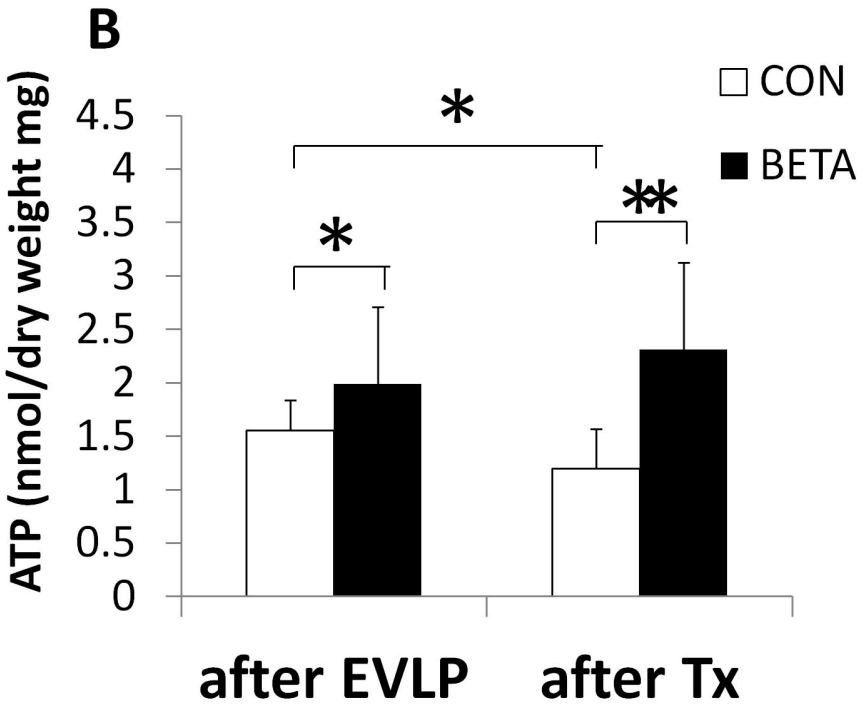


Figure 5


A
cAMP (pmol/protein mg)
8
7
6
5
4
3
2
1
0
*
*
CON
BETA
after EVLP
after Tx


B
ATP (nmol/dry weight mg)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
**
CON
BETA
after EVLP
after Tx