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Normal gas exchange after 30-h ischemia and treatment with phosphodiesterase inhibitor PDI747☆

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Abstract

Objective: Phosphodiesterases (PDEs) negatively regulate the concentrations of cAMP and/or cGMP, which act as downstream second messengers to the prostaglandins. PDE type-4 (PDE4) is selective for cAMP and is found in high concentrations in endothelial, epithelial, and different blood cells. The aim of this study was to evaluate if PDI747, a novel selective inhibitor of PDE4, can restore pretransplant cAMP levels and thereby posttransplant organ function after prolonged cold ischemia. **Methods**: Left lung transplantation was performed in pigs (25–31 kg). Donor lungs were flushed with low potassium dextran glucose (LPDG) solution only (control, $n = 5$)or, in addition with 1 μmol of PDI747 (PDI747, $n = 5$) and stored for 30 h at 1 °C. PDI747 animals further received a bolus of PDI747 (0.3 mg/kg) 15 min prior to reperfusion and a continuous infusion (0.3 mg/kg per hour) during the 5 h after reperfusion. After occlusion of the right pulmonary arteries and the right main bronchus, hemodynamic and gas exchange parameters and extravascular lung water (EVLW) levels of the transplanted lung were assessed. **Results**: Two control animals died of severe lung edema leading to heart failure (control, $n = 3$). One animal in the treatment group was excluded due to a patent ductus arteriosus (PDI747, $n = 4$). Gas exchange at the end of the experiment was restored to normal levels in the PDI747 group (Pa, O_2 47.6 ± 11.2 kPa, Pa,CO_2 6.4 ± 1.8 kPa) but not in the control group (Pa, O_2 7.7 ± 2.9 kPa, Pa, CO_2 11.9 ± 3.0 kPa, $P_{PaO2} < 0.0001$, $P_{Pa, CO2} = 0.06$). Extravascular lung water (EVLW) was normal in the PDI747 group (8.5 ± 1.1 ml/kg) and clearly elevated in the control group (16.2 ± 5.6 ml/kg, $P = 0.007$). Airway pressure in the PDI747 group was significantly lower than in the control group (7.8 ± 0.5 cm H_2O vs. 11.3 ± 0.6 cm H_2O, respectively, $P < 0.0001$). The free radical mediated tissue injury measured by lipid peroxidation (TBARS) was significantly reduced ($P = 0.001$) in the PDI747 group. **Conclusions**: With the inhibition of PDE4 with PDI747 we achieved normal gas exchange, no posttransplant lung edema, normal airway pressures, and a reduced free radical injury after 30 h of cold ischemia.

Keywords: Lung preservation; Ischemia/reperfusion-injury; Phosphodiesterase-inhibitor; cAMP

1. Introduction

After lung transplantation, severe life-threatening graft dysfunction occurs in 10–20% of patients and is mainly caused by ischemia/reperfusion (I/R) injury, leading to increased pulmonary vascular permeability, increased airway pressures, and alveolar epithelial cell damage. Edema, inflammation, neutrophil infiltration, intraparenchymal hemorrhage, necrosis, and apoptosis are some of the histo-pathological markers of this injury. Despite intensive research not all mechanisms are fully understood but endothelial dysfunction has been shown to play an important role. Past studies have also indicated that some of the key factors and underlying mechanisms resulting in graft dysfunction include oxygen free radicals [1], activation and migration of neutrophil granulocytes [2], and activation of the complement system [3,11].

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In the early 1960s, inhibitors of the cyclic nucleotides cAMP and cGMP, the cyclic nucleotide phosphodiesterases (PDEs), were discovered [4]. PDEs are the critical enzymes responsible for the degradation of cAMP and/or cGMP. Thereby the synthesis of prostaglandins and nitric oxide (NO) is modulated.

Nine different types of PDE (types 1–9) and several subgroups (A, B, C, etc.) have been identified, but only the properties of types 6–9 are well understood. Also, PDE4 has been shown to be a selective inhibitor of cAMP. The concentrations of PDEs vary with different tissues. For example, in human airway epithelial cells (hAEC) there are high amounts of PDE types 1, 4, 5 and 7, and very little of types 2 and 3. In addition to this, high activity of PDE4 was found in airway smooth muscle cells [6]. In porcine airway epithelial cells (pAEC) on the other hand, PDE types 4 (PDE4) and 5 have the highest activity [7]. Several groups have investigated the role of cAMP in the development of endothelial dysfunction and have shown in vitro that inhibition of cAMP-consuming reactions or stimulation of cAMP production results in decreased endothelial leakage [5].

PDI747 is a new, selective, and potent inhibitor of PDE4. Therefore, it reduces the decline in cAMP concentrations during ischemia and possibly during reperfusion. Since cAMP is a second messenger in the prostaglandin synthesis pathway, the resulting increase in cAMP levels upon PDI747 treatment will lead to a decrease in cellular inflammatory responses and reduce the onset of lung edema, while causing bronchodilatation [9]. Thus, we hypothesize that the selective inhibition of PDE4 will reduce ischemia/reperfusion injury after prolonged cold ischemia.

2. Materials and methods

Ten weight-matched pairs of pigs (26–36 kg, aged 3–4 months) served as donors and recipients, with the heavier pig used as the donor. Two groups were formed: the control group ($n = 5$) and the group treated with PDI747 ($n = 5$), and were studied in an alternating fashion. Harvest and transplant of lungs were performed under non-sterile conditions by the same surgeon.

2.1. Drug preparation and administration

PDI747 (4395 mg; Novartis Pharma AG, Switzerland) was dissolved in 10 ml of 5% glucose solution. One milliter of this solution was then added to 1000 ml Perfadex (LPDG, Medisan Pharmaceuticals AB, Uppsala Sweden), resulting in a concentration of 1 μmol/l. The recipient received a bolus of PDI747 (0.3 mg/kg) 10 min prior to reperfusion and was infused continuously (0.3 mg/kg) throughout the observation period of 5 h.

All animal procedures were approved by the ethics committee and were performed in accordance with the 'Guide for the Care and Use of Laboratory Animals' (National Institutes of Health publication 85-23, revised 1985).

2.2. Donor procedure—explantation

The Heart–Lung-Block was harvested through a median sternotomy from animals ventilated with 100% oxygen and 5 cm H_2O of positive end-expiratory pressure (PEEP). Heparin (300 IE/kg) was given via the aorta, and the common pulmonary artery was canulated (Sarns, Inc, Ann Arbor, MI). Two hundred and fifty micrograms of prostaglandin E1 (PGE1) (Prostin VR Pediatric, Pharmacia and Upjohn AG, Duebendorf) was injected into the common pulmonary artery in order to achieve maximal vasodilatation. The lungs were flushed with 1500 ml of cold (4 °C) low potassium dextran glucose (LPDG) (Perfadex Medisan Pharmaceuticals AB, Uppsala Sweden) with (PDI747 group) or without (control group) PDI747 at a pressure of 40 cm H_2O.

The Heart–Lung-Block was stored semi-inflated with 100% oxygen in Perfadex solution at 1 °C for 30 h.

2.3. Recipient procedure—transplantation

The recipient pig was intubated and ventilated with 100% oxygen (FIO_2: 1.0) and a PEEP of 5 during the whole procedure. Tidal volume was 500 ml and was not changed after occlusion of the right, native lung. Isoflurane concentration varied between 0.2 and 1.5% depending on the animals' need.

Prior to surgery, the Swan–Ganz catheter was placed in the right jugular vein and a fiber-optic thermistor-tipped catheter (System Cold Z-021, Pulsion, Munich, Germany) was placed via the carotid artery into the descending aorta.

A pneumonectomy on the left side was performed. Briefly, the hemizygos vein was first resected and the left pulmonary vein and the left artery were separated. The left main bronchus and the carina were then exposed in order to access the contralateral side. To assess graft function, only the right main bronchus and the right pulmonary arteries (right upper and main) were encircled from the left chest cavity.

The left lung was then separated from the Heart–Lung-Block. Prior to the implantation, the pig received heparin intravenously (100 IE/kg). The anastomoses were completed with an everting mattress suture for the left atrium (Prolene 5-0), a running suture for the pulmonary artery (Prolene 5-0), and a running suture for the bronchus (Prolene 4-0). A left atrial line (LA) was placed. A thoracic drainage was introduced and the chest was closed temporarily. One hour after reperfusion, the right lung was excluded from ventilation and perfusion by occlusion of the earlier encircled right main bronchus and the right pulmonary arteries (arteries and bronchus) were occluded to assess graft function only.

2.4. Assessment

During the assessment period, the animals were under general anesthesia. Blood pressure (systemic and pulmonary), central venous pressure, oxygen saturation and the expired CO_2 were measured continuously. Extravascular lung water (EVLW) levels, cardiac output, arterial and venous blood gas parameters were measured at the following times: prior to thoracotomy (T1), 10 min before (T2) and 10 min after (T3) occlusion of the right side, and then every hour (T4–T7).

2.5. Extravascular lung water (EVLW) levels

EVLW levels as a direct assessment of reperfusion edema was measured as previously described [22]. A fiber optic catheter (System Cold Z-021, Pulsion, Munich, Germany) was advanced via the external carotid artery into the descending aorta. The indicator bolus consisted of two components: indo-cyanine green served as an intravascular marker and ice cold 5% glucose as a thermal intra- and extravascular indicator. The bolus was injected via the external jugular vein with a temperature-controlled injector. The dilution curves for the dye and temperature were recorded simultaneously in the descending aorta with the thermistor-tipped fiber-optic catheter. Thoracic intra- and extravascular fluid volumes were determined based on the measurement of the mean transit times for thermal and dye indicators and of the decay time volumes calculated from the indicator dilution curves. The lung water computer (System Cold Z-021, Pulsion, Munich, Germany) determined the mean transit time for the thermal indicator and for the dye indicator and calculated the total thermal volume (ITTV), intrathoracic blood volume (ITBV), and extravascular thermal volume (ETV). The ETV is calculated as follows:

$$ETV = ITTV - ITBV.$$

All measurements were made in triplicate. The mean values were used for analysis.

Upon completion of the various analyses, the animals were euthanized and the anastamoses were controlled and samples of the transplanted lung were taken. From the snap frozen samples, myeloperoxidase (MPO) activity and thiobarbituric acid-reactive substance (TBARS) assays were done.

2.6. Myeloperoxidase (MPO) assay

Quantitative MPO activity was determined as previously described [12]. Enzyme activity is expressed as the change in optical density unit per milligram of tissue protein (delta OD/mg per min).

2.7. Thiobarbituric acid reactive substances (TBARS)

TBARS was measured in 10% wet wt. per volume homogenate to determine the extent of lipid peroxidation in the graft tissue. Aliquots (0.2 ml) of this homogenate were added to tubes containing 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid solution adjusted to 3.5 pH with NaOH, and 1.5 ml of 0.8% solution of thiobarbituric acid. The mixture was brought to a volume of 4 ml by addition of distilled water heated at 95 °C for 60 min and then cooled with tap water. One milliliter of distilled water and 5 ml of butanol/pyridine (15:1) were added (all chemical by Fluka AG, Switzerland). The solution was centrifuged at 4000 rpm for 10 min. The absorbance of the upper layer was measured at 532 nm with a spectrophotometer (Kadas 100, Dr. Lange AG Zurich, Switzerland). The TBARS levels were determined by reference to a standard curve of 1,1,3,3-tetramethoxypropane (Sigma Chemicals, Switzerland), and the results were expressed as nanomoles of malondialdehyde (MDA) per gram of wet lung.

2.8. Statistics

All data were expressed as mean values ± standard deviation. Comparison between the two groups was achieved using unpaired *t*-tests for the variables donor weight, recipient weight, total ischemic time, MPO activity, MDA (TBARS) and the hemodynamic variables at the end of the observation period (T7). To evaluate the statistical difference between the groups regarding EVLWI and the gas exchange over the period T3–T7, an analysis of variance for repeated measures was performed. In order to get approximately normally distributed observations, log-transformed data were used there. For all the analyses, *P*-values less than 0.05 were considered significant. Statistical analysis was made with SPSS for Windows.

3. Results

Two animals in the control group died shortly after occlusion of the right side, due to pulmonary edema and consecutive heart failure and thus were excluded from the statistical analysis. In the treated group, one animal was also excluded because of a wide-open ductus arteriosus, such that the final sample sizes were $n = 3$ for the control group and $n = 4$ for thePDI747 treated group.

No statistical differences were noted in donor weight (control group 30.0 ± 1.7 kg; PDI747 group 28.3 ± 2.8 kg; $P = 0.38$), recipient weight (control group 29.3 ± 3.1 kg; PDI747 group 27.3 ± 2.4 kg; $P = 0.35$) and total ischemic time (control group 1797 ± 5 min; PDI747 group 1800 ± 0 min; $P = 0.29$).

EVLW in the transplanted lung at the end of the assessment period (T7) was normal in the PDI747 group

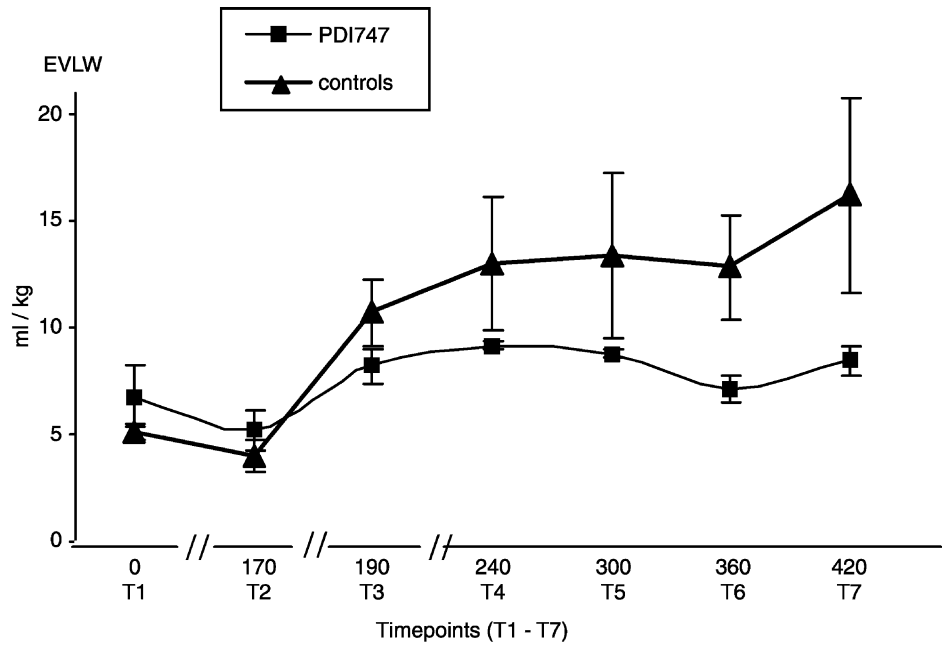

Fig. 1. Means and standard deviations of extravascular lung water (EVLWI) for both groups during the whole period of observation. Time points T1–T7: prior to thoracotomy (T1), 10 min before (T2) and 10 min after (T3) occlusion of the right side, and then every hour (T4–T7). In the control group the lungs deteriorated by the end of the experiment while the treated pigs were stable throughout the experiment. Using an ANOVA for repeated measurements on T3–T7 using log-transformed data, a significant difference was found between the groups ($P = 0.007$).

(8.5 ± 1.2 ml/kg) and severely elevated in the control group (16.2 ± 5.6 ml/kg). During the assessment period (T3–T7), the difference between the two groups was statistically significant ($P = 0.007$) (see Fig. 1).

At the end of the experiment, we found normal gas exchange in the PDI747 group (Pa_{O_2} 47.6 ± 11.2 kPa; Pa_{CO_2} 6.4 ± 1.8 kPa), whereas the control animals showed poor oxygenation (Pa_{O_2} 7.7 ± 2.9 kPa) and extensive hypercapnia (Pa_{CO_2} 11.9 ± 3.0 kPa). During the assessment period (T3–T7), the difference between the two groups was strongly significant for Pa_{O_2} ($P < 0.0001$) and borderline significant for Pa_{CO_2} ($P = 0.06$) (see Figs. 2 and 3).

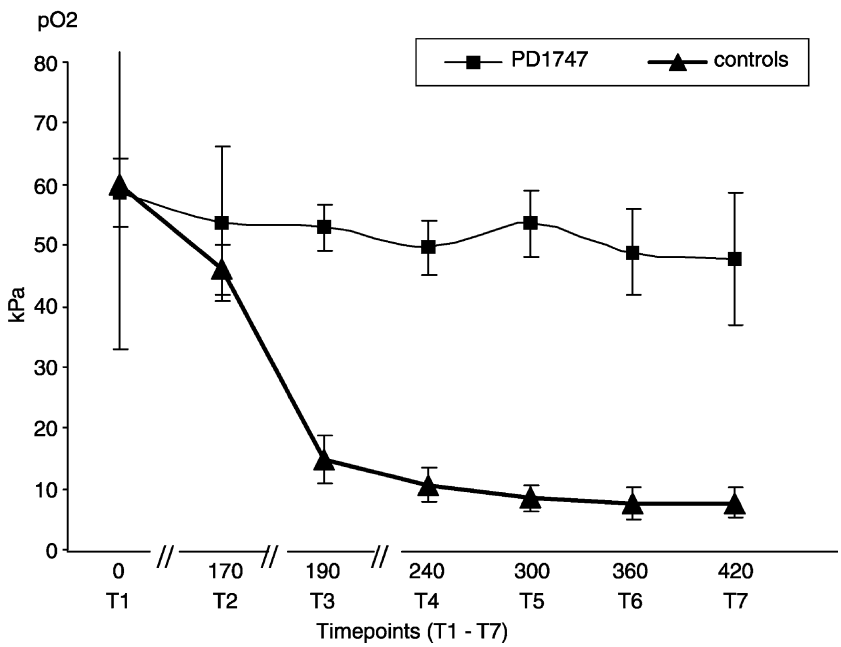

Fig. 2. Means and standard deviations of Pa,o_2 for both groups during the whole period of observation (T1–T7). Time points T1–T7: prior to thoracotomy (T1), 10 min before (T2) and 10 min after (T3) occlusion of the right side, and then every hour (T4–T7). The gas exchange was almost normal in the treated group and severely reduced in the control group. Using an ANOVA for repeated measurements on T3–T7 using log-transformed data, a significant difference was found between the groups ($P < 0.0001$).

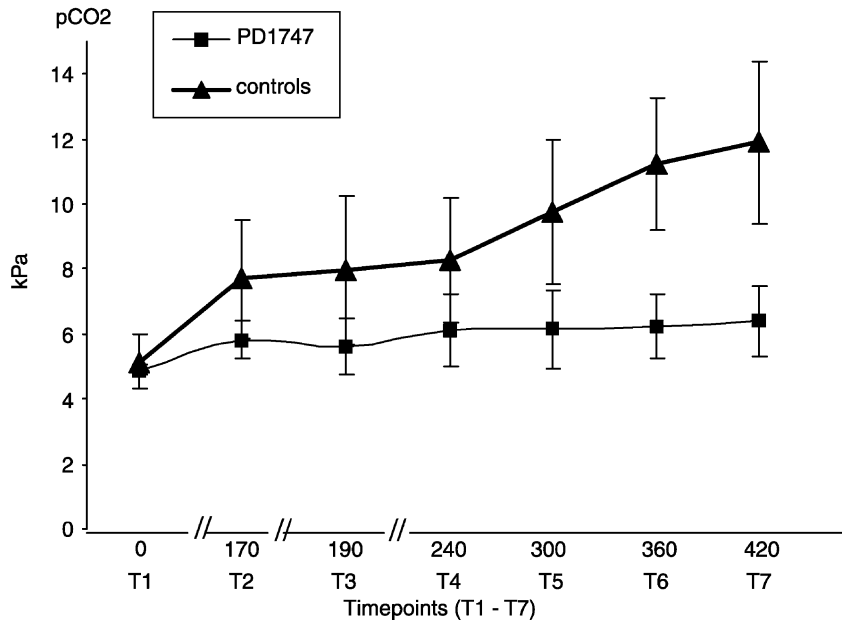

Fig. 3. Means and standard deviations of Pa,CO_2 for both groups during the whole period of observation (T1–T7). Time points T1–T7: prior to thoracotomy (T1), 10 min before (T2) and 10 min after (T3) occlusion of the right side, and then every hour (T4–T7). Pigs in the control group were close to Pa,CO_2-narcosis whereas the treated pigs had only a slight increase in Pa,CO_2. Using an ANOVA for repeated measurements on T3–T7 using log-transformed data, this difference between the groups was borderline significant ($P = 0.06$).

At the end of the experiment, MPO activity was 1.10 ± 0.09 in the control group and 1.06 ± 0.20 in the PDI747 group, and this difference was not significant ($P = 0.75$). A significant difference of MDA (TBARS) between the groups was found (control group 0.19 ± 0.03; PDI747 group 0.03 ± 0.02; $P = 0.001$). Among the other measured variables, we found significant differences between the groups only for heartrate (control group 139.0 ± 22.1; PDI747 group 180.5 ± 7.6; $P = 0.02$), P_{INSP} (control group 28.3 ± 1.5 cm H_2O; PDI747 group 18.5 ± 1.7 cm H_2O; $P = 0.001$) and P_{MEAN} (control group 11.3 ± 0.6 H_2O; PDI747 group 7.8 ± 0.5 H_2O; $P < 0.0001$).

4. Discussion

In the present study, using a pig single-lung transplant model, we demonstrated that donor and recipient treatment with PDI747 protected the transplanted lung from I/R injury induced by extended cold ischemia (30 h) and 5 h of reperfusion. Our results showed significantly better graft oxygenation, decreased edema, airway pressures, and lipid peroxidation in the PDI747 treated group compared to the control group.

The pathophysiology of I/R injury has been extensively studied over the past 25 years and various mechanisms have been described. The sequence of events following ischemic injury can briefly be summarized as follows: a deficit in O_2 supply results in decreased mitochondrial oxidative phosphorylation and thus a depletion of ATP; one of the consequences of decreased ATP is failure of ATP-dependent pumps causing an accumulation of Na^+ and H_2O, leading to the swelling of cells and culminating in edema of the graft. Although reperfusion and reoxygenation are essential for the ischemic tissue to regain function, it increases the damage caused by ischemia. This phenomenon is believed to involve the local generation of oxygen-derived free radicals and the activation of leukocytes interacting with endothelial cells. This interference finally leads to cell-death, infiltration of activated inflammatory cells, and development of a local inflammatory reaction causing further edema and graft destruction.

The cyclic nucleotides cAMP and cGMP are second messengers in the prostaglandin and NO synthesis pathways. During I/R, however, the production of both second messengers is attenuated. Comparing the relative importance of both the pathways, Bhabra et al. demonstrated in an ex-vivo model of lung preservation that the failure of the NO/cGMP pathway is more detrimental than the failure of the prostaglandin/cAMP pathway [10,14]. Past experimental work done by our group supports these findings. Schmid et al. showed that with the addition of tetrahydrobiopterin, a cofactor for NO synthase, posttransplant edema was reduced [15]. With the intravenous application of 8-Br-cGMP, an analogue of cGMP, Hillinger et al. showed that I/R injury was reduced with a decrease in edema and free radical injury [16]. Lugnier et al. demonstrated a cross talk between PDEs and NO leading to upregulated NO production upon PDE4 inhibition [24].

Nevertheless, there is much evidence that normal to high levels of intracellular cAMP are associated with a limited

response of inflammatory cells such as neutrophils, monocytes, macrophages, dendritic cells, and lymphocytes [8,25]. The exact mechanisms by which cAMP modulates cell function are not completely understood but appear to depend on the activation of protein kinase A and the subsequent phosphorylation of hydroxy-amino acid residues.

In transplanted organs I/R injury has also been shown to activate antigen-dependent reactions. Hestek et al. showed the anti-inflammatory effect of PDE4 inhibitor on a T cell and dendritic cell level [25]. Experimental and clinical data suggest that I/R injury increases graft immunogenicity by upregulating MHC class I and II antigens resulting in an increased incidence of acute rejection episodes and early graft loss [13]. Schade et al. tested several PDE4-inhibitors of a newer generation for their immunosuppressive properties [23], which all showed limited protection against epithelial disturbance, infiltration of immune cells, and luminal obliteration. Some of the tested inhibitors, such as Cilomilast (Ariflo) and Roflumilast, exhibited a strong antiproliferative effect on fibroblasts, suggesting a possible benefit in preventing against the development of obliterative bronchiolitis.

The different PDE types modulate cAMP and/or cGMP levels differently depending on cell types. PDE4 is selective for cAMP and several authors have shown high levels of PDE4 not only in inflammatory cells but also in epithelial and endothelial cells, in the matrix of the pulmonary arteries, and in the bronchi [9,17,18]. In an in-vitro study of perfused rat lung, Held et al. showed a partial reversibility of endothelin-1-induced vasoconstriction and bronchoconstriction with the PDE4 inhibitor, Rolipram [19].

In an orthotopic rat lung transplantation model, Pinsky and Stern found a dose-dependent improvement of arterial oxygenation, a reduction in pulmonary arterial resistance and a subsequent increase in arterial flow upon treatment with a PDE4 inhibitor. Rabe et al. demonstrated the effects of several different PDE inhibitors in causing relaxation of human pulmonary arteries [17]. At dosages up to 1 mg/kg i.v., only minor changes in heart rate, arterial blood pressure and cardiac output were seen. This, however, is in contrast to our findings. In our study, the animals were treated with 0.3 mg/kg per hour PDI747, which caused a significant increase in heart rate ($P = 0.02$) but no significant changes in blood pressure. The reduction of inspiratory airway pressure after treatment with a PDE4 inhibitor is well documented and reflects the effect on inflammatory cells as well as on airway smooth muscle cells [9].

Unfortunately, in rats the pharmacological side effects of arthritis and periarthritis exist, as have been shown for other PDE inhibitors such as Rolipram and Zaprinast [20,21]. For PDI747 Vogel et al. also observed segmental vasculitis after topical administration (non-published data from Novartis). These results caused disclosure of the research on PDI747.

In summary, in this experimental model where the donor lungs and recipients were treated with PDI747, we achieved reduced airway pressures, lung edema, and excellent gas exchange after prolonged cold ischemia.

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