PDE-5 inhibitor donor intravenous preconditioning is superior to supplementation in standard preservation solution in experimental lung transplantation

Nikolaus Pizanis*, Vitaly Milekhin, Konstantinos Tsagakis, Ivan Aleksic, Markus Kamler, Heinz Jakob

Department of Thoracic and Cardiovascular Surgery, West German Heart Center Essen, University Hospital of Essen, Germany

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Abstract

Objective: Improvement of preservation is still a major research objective in lung transplantation. The effects of phosphodiesterase-5 (PDE-5) inhibitors during procurement are still not clear. It was the aim of this study to investigate the effect of sildenafil on post-transplanted lung function in a porcine model using different application procedures.

Methods: In control group lungs were flushed with buffered low-potassium dextran (LPD) solution (I) and compared to LPD solution with supplementation of 0.15 mg/kg body weight (BW) sildenafil (II), whereas in a third group 0.15 mg/kg BW sildenafil was administered intravenously 20 min prior to LPD flushing (III). All grafts were stored for 24 h at 4—6 °C. Hemodynamics and blood gases were monitored until 6 h after reperfusion. Lung tissue was taken for wet/dry ratio assessment.

Results: All animals of groups I and III survived the entire observation period in contrast to four animals of group II which died within 4 h after reperfusion due to severe reperfusion injury. Group II showed a lower mean PAP and a reduced pulmonary vascular resistance (PVR) throughout the observation period, but did not reach significance due to low number of surviving animals. Group III achieved significantly improved PO2/FiO2 fraction at all timepoints and a significant reduced PVR [434 ± 98 vs 594 ± 184 dyn s cm⁻⁵, II vs I; mean ± SD, p < 0.01] at 6 h. Wet/dry ratio was significantly higher in group II throughout the experiment.

Conclusions: Sildenafil allows for a better graft function after 24 h ischemia when given prior to standard flushing and preservation. This effect can be explained by a complete/homogenous preservation achieved by selective pulmonal vasodilatation. However, this effect seems to persist when sildenafil remains in the storage solution, leading to severe pulmonary edema.

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1. Introduction

Optimizing preservation in lung transplantation is still a major directive in research when considering the high occurrence of ischemia/reperfusion injury, following reperfusion edema in up to 50—60% in the literature [1] and with a mortality of up to 20% [2].

Insufficient perfusion and, thus, protection due to a reactive vasoconstriction in the graft during application of preservation solution may be an underestimated reason of graft dysfunction which has been examined only sparingly in the literature [3,4].

The selective pulmonary vasodilator sildenafil is a phosphodiesterase-5 (PDE-5) inhibitor, with widespread use for erectile dysfunction, and is increasingly used in the treatment of pulmonary hypertension with low systemic effects [5—8,15—18]. Previous studies using the same lung transplantation model performed in our center showed positive effects of sildenafil in lung transplantation, including an improved microcirculation as well as an increased oxygenation capacity after transplantation.

As standard preservation solution we used buffered low-potassium dextran (LPD) solution which is used clinically with superior results when compared to other preservation solutions [1,9,10].

In a preliminary study, we investigated the direct effect of intravenous sildenafil on the systemic and pulmonary hemodynamic parameters. Five fully instrumented pigs were given a 0.15 mg/kg body weight (BW) bolus into the truncus pulmonalis and observed over a period of 30 min. Interestingly, arterial pressure, pulmonary arterial pressure, and pulmonary vascular resistance decreased significantly but...
recovered over the time of 20 min, except the pulmonary vascular resistance, which still showed significant reduction after 30 min (Figs. 1 and 2). Therefore, in this study, the excellent selective vasodilatative effects of sildenafil lead to its use in order to counteract the reactive vasoconstriction and achieve a better organ protection.

The results lead us to the following considerations: the preservation solution should be enriched with sildenafil in order to achieve a homogenous and complete perfusion, or the preservation solution should be given 20 min after an intravenous application of 0.15 mg/kg BW sildenafil directly into the main pulmonary artery in group III. Retrieved grafts were stored for 24 h at 4–6 °C and transplanted as single left lung in the following.

2. Materials and methods

The experimental protocol was reviewed and approved by the committee on Research Animal Care, Essen Medical School. All animals received humane care in compliance with the 'Principles of Laboratory and Animal Care' and the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Resources published by the National Institutes of Health (NIH publication no. 85-23, revised 1985).

2.1. Experimental group and animals

Forty-two male pigs, German landrace (30–35 kg), were included in this study, 21 serving as donor animals and 21 as recipients. Donor animals were divided into three groups (n = 7) and received antegrade flush perfusion (0.5 l/min) with TRIS-buffered low-potassium dextran solution (Vitrolife, Göteborg, Sweden): (1) only in group I, (2) with addition of 0.15 mg/kg BW sildenafil in group II, and (3) after intravenous application of 0.15 mg/kg BW sildenafil directly into the main pulmonary artery in group III. Retrieved grafts were stored for 24 h at 4–6 °C and transplanted as single left lung in the following.

2.2. Donor operation

Anesthesia was induced with a combination of ketamine (30 mg/kg BW), stressnil (2 mg/kg BW), and atropine (0.05 mg/kg BW) i.m. After intubation analgesia was maintained with disoprian (10 mg/h), midazolam (1 μg/kg/h), and fentanyl (5 μg/kg/h) continuously administered intravenously. Mechanical ventilation (Fabius GS, Dräger, Germany) was performed standardized in a pressure-controlled mode with a ventilation rate of 15 breaths/min and a maximum inspiratory pressure of 25 mmHg at an inspiratory oxygen-tension (FiO2) of 0.5. The positive end-expiratory pressure was adjusted to 5 mmHg. Ventilation was not changed during the entire procedure. ECG was connected and a catheter was placed in the femoral artery for hemodynamic monitoring and later blood gas analysis.

After median sternotomy the pericardium and both pleural cavities were opened, and the inferior and superior vena cava, the aorta, and the pulmonary artery were isolated. Systemic anticoagulation was performed with 3 mg/kg BW heparin. An arterial blood gas analysis was performed. Afterwards all animals received 250 mg methylprednisolon intravenously.

The recipients from group III were administered an injection of 0.15 mg/kg BW sildenafil directly into the pulmonary artery via butterfly cannula 20 min before start of perfusion.

In an analogous manner groups I and II received equal amounts of saline solution 20 min prior to explantation. Afterwards a flushing tube was inserted into the main pulmonary artery. After complete inflow occlusion, the auricle of the left atrium was incised, the aorta and the pulmonary artery were clamped, and flush perfusion with 2 l of 6–8 °C cold buffered LPD solution was induced in groups I and III, respectively, and with 2 l of sildenafil supplemented (0.15 mg/kg BW) cold buffered LPD solution in group II. Ventilation of lungs was maintained throughout the entire procedure. The lungs were excised en bloc after cardiotomy, leaving a generous atrial cuff on the left pulmonary veins for the later anastomosis. Main bronchus was stapled
twice, keeping the lungs mildly inflated. Left lung was isolated back-table, stapling the right bronchus off and keeping a good length of the pulmonary artery. Finally, the left lung graft was stored mildly inflated in a refrigerator for 24 h in 4—6 °C cold LPD solution.

2.3. Transplant procedure

Anesthesia was induced in the same manner as described before. Mechanical ventilation (Fabius GS, Dräger, Germany) was standardized and performed in a pressure-controlled mode with a ventilation rate of 15 breaths/min and a maximum inspiratory pressure of 25 mmHg at an inspiratory oxygen-tension (FiO2) of 1.0. The positive end-expiratory pressure was adjusted to 5 mmHg. ECG was connected and a catheter was placed in the femoral artery for hemodynamic monitoring and later blood gas analysis. A Swan-Ganz Catheter (Baxter, Healthcare Corp., Irvine, CA) was placed through a jugular vein introducer sheath for monitoring central venous pressure and pulmonal arterial pressure.

After left-sided lateral thoracotomy in the fifth intercostal space, the pericardium was opened, and left pulmonary artery, tracheal bifurcation, and the left pulmonary veins were dissected. Umbilical tapes were applied to the right and left pulmonary arteries and the right main bronchus. Heparin (3 mg/kg BW) was administered intravenously. After clamping of the left main bronchus and the left pulmonary artery, the main stems of left pulmonary veins were ligated. Pneumectomy was performed. The left atrium was clamped. The ligated left pulmonary veins were incised opening an atrial cuff. The donor lung was implanted starting with an end-to-end anastomosis of the bronchus using a 4.0 Prolene running suture (Ethicon, Inc., Somerville, NJ). The pulmonary artery followed by the atrial cuff was anastomosed with 5.0 Prolene running sutures. Before reperfusion the graft vasculature was deaired by retrograde perfusion, the pulmonary artery was declamped, and the graft was ventilated. After a reperfusion period of 30 min the contralateral pulmonary artery and bronchus were clamped in order to evaluate only the function of the transplanted lung. If necessary, hemodynamic support was applied by administration of adrenaline or noradrenaline.

The use of catecholamines as well as diuretics was controlled and only performed after transplantation when following criteria were fulfilled:
- administration of 5 mg furosemide when urine production decreased under 50 ml/h,
- adrenaline/noradrenaline was started via perfusor when mean arterial pressure dropped below 50 mmHg.

Adrenaline was used primarily except when heart frequency exceeded 140 bpm or when systemic vascular resistance dropped below 500 dyn s⁻¹ cm⁻¹, in which case noradrenaline was favored. Dosage was adjusted observing hemodynamics, allowing a maximum of 0.4 μg/kg BW/min after which no further increase was allowed. These criteria were identical to all animals after transplantation.

Each experiment was terminated after an observation period of 6 h by an intracardial tetracainchloride (T61) injection. Lung probes were taken and weighted before

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![Fig. 3. In vivo microscopic recording of pulmonary edema in group II at timepoint 30 min (a), 1 h (b), 3 h (c), and with complete atelectasis (d). Progressing pulmonary edema can be observed with increasing of intra-alveolar space, reduction of alveolar structures up until atelectasis.](https://academic.oup.com/ejcts/article-abstract/32/1/42/451987)
and after baking at 65°C for 72 h to assess the wet/dry ratio.

2.4. Monitoring and measurements

Hemodynamic data were monitored continuously (Siemens SC9000 and Swan-Ganz Catheter, Baxter). Arterial blood gas analysis (Radiometer Copenhagen, Germany) and circulatory parameters (cardiac output (CO), pulmonary vascular resistance (PVR), and systemic vascular resistance (SVR)) were performed at each timepoint. The system was calibrated by injection of cold saline solution via the Swan-Ganz Catheter and parameters were calculated by the monitor system.

2.5. Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM) when not specified otherwise. Data were analyzed by repeated measure analysis of variance (ANOVA) and paired t-test. For data without repeated measurements a Mann–Whitney U-test was performed. p-values less then 0.05 were considered significant. All data were analyzed using SPSS-science (sigma stat and sigma plot, V3.0, V8.0 USA).

3. Results

3.1. Animal survival

All animals of groups I and III (n = 7) survived the experiment. In contrast, four animals of group II died within 4 h due to severe reperfusion edema, one at timepoint 2 h, two at timepoint 3 h, and one at timepoint 4 h after reperfusion.

The transplanted lungs of group II developed progressing edema even though basic therapy such as diuretics, bronchoscopic fluid aspiration, and catecholamine support up to 0.4 μg/kg body weight when necessary was applied (Fig. 3a–d).

3.2. Hemodynamic data

Pulmonary arterial pressure was significantly lower in group II at timepoint 1 h after beginning of reperfusion when compared to control. The values stayed lower throughout the observation time of 6 h, but did not reach relevance because of the number of deceased animals. Group III showed a significant lower value only at timepoint 6 h when compared to control (Fig. 4).

Pulmonary vascular resistance was significantly lower for group II at timepoint 1 h and showed reduced values over the observation time. No further significance was achieved again because of the low number of surviving animals (n = 3). Group III only reached significant lower values at timepoint 6 h when compared to control and presented with higher mean values than surviving animals of group II throughout the observation period (Fig. 5).

The oxygenation capacity of the transplanted lung was significantly higher in group III at all times after 1 h of reperfusion as evidenced by the arterial partial oxygen fraction to inspiratory oxygen ratio. Group II showed no overall difference to the control group (Fig. 6).

Fig. 4. Mean pulmonary pressure, all values are mean ± SEM (n = 7); (*) significant vs control (I) (p < 0.05).

Fig. 5. Mean pulmonary vascular resistance, all values are mean ± SEM (n = 7); (*) significant vs control (I) (p < 0.05).

Fig. 6. Mean arterial partial oxygen fraction to inspiratory oxygen ratio, all values are mean ± SEM (n = 7); (*) significant vs control (I) (p < 0.05).

Fig. 7. Wet/dry ratio in the different groups, all values are mean ± SD (n = 7); (*) significant vs control (I) (p < 0.05).
3.3. Lung water content

Wet/dry ratio values were significantly higher in group II. Probes were taken at the timepoint of death in animals which did not survive throughout the experiment. Group III did not show any statistical difference to control. However, a trend of elevated values in this group as indicator for a higher water content in the lung was noted (Fig. 7).

4. Discussion

In the present study we demonstrated (1) a high occurrence of reperfusion edema with following high mortality when sildenafil was given as an additive to standard preservation solution and (2) an excellent oxygenation with overall lower pulmonary pressure and vascular resistance with a sildenafil preconditioning 20 min before standard flush preservation. The results where compared with a control group which was preserved with standard solution only.

In a previous study we already observed a positive effect of sildenafil on post-transplanted lung function using the same model (yet unpublished results, 2005/2006). Sildenafil leads to an accumulation of cGMP by inhibiting the conversion to GMP, inducing a vasodilatation, especially in lung tissue because of its high occurrence of the phosphodiesterase-5 subtype. The second messenger cGMP has also been linked with an improvement of post-transplant lung graft function, working as a substitute to the NO pathway [11,13,14]. The phosphodiesterase-5 inhibitor sildenafil has been also attributed protective effects in a similar 24-h ischemia lung transplantation model, allowing for survival, whereas PGE1-treated animals died within 2 h of reperfusion [12]. However, the explanation for the protective effect of sildenafil by Korom et al. was speculative and a limitation of the study described was the mortality of all control animals within 2 h of the transplantation.

The high occurrence of reperfusion edema in the sildenafil supplement group led to a high mortality in this study with an improvement of post-transplant lung graft function, working as a substitute to the NO pathway [11,13,14]. Hemodynamic parameters up until 30 min after beginning of reperfusion were similar in all groups, leaving impressive edema to be the cause of premature death before the end of the observation time, despite concomitant therapy. This unusual high reperfusion edema could be the result of two different causes: (1) due to the vasodilatative effects of the supplemented sildenafil auxiliary lung vessels and capillaries were opened, creating massive shunting and a higher fluid volume capacity of the lung, leading to a relative shortage of preservation solution. The reperfusion edema would be a result of insufficient preservation and, thus, protection. On the other hand, (2) the sildenafil supplement remained in the stored lung until beginning of reperfusion after 24 h, leading to a prolonged and maybe even a permanent vasodilatation, thus leading to extravasation and edema. This hypothesis is supported by the trend of low pulmonary pressure and vascular resistance observed when compared to both other groups (Figs. 4 and 5).

The mean pulmonary pressure increased during the observation period as expected after beginning of reperfusion in the control as well as the sildenafil preconditioned group. However, at timepoint 6 h the sildenafil preconditioned group showed a significant lower value than control. In contrast, the sildenafil supplement group showed no or only slight increase, with significant lower value at 1 h after reperfusion, and a trend of continuing lower values over the observation time (Fig. 4).

The pulmonary resistance showed a similar course, with increasing values in groups I and III, yet with a less pronounced course in group III with significant lower value 6 h after reperfusion. Group II again showed no or only slight increase with significant lower resistance at timepoint 1 h and a distinct trend of lower values over the observation time (Fig. 5).

The effects of low mean arterial pressure and pulmonary vascular resistance observed in group II shortly after the beginning of reperfusion seem to relate to the sildenafil supplement in the preservation solution, causing a prolonged vasodilatation and thereby leading to increased lung edema formation. The sildenafil preconditioning shows a protective effect after 6 h of reperfusion, clearly having a less intensive or long-lasting effect when administered 20 min before preservation, allowing a distribution in the whole circulatory system. However, this remains a matter of speculation at this time.

The oxygenation capacity showed a decrease in the control as well as in the sildenafil-supplemented group after reperfusion, recovering slowly with time. This phenomenon is known in lung transplantation and is a consequence of the overall ischemia/reperfusion injury with increased lung water content. The preconditioning with sildenafil, however, led to a preserved oxygenation capacity, significantly higher when compared to control from timepoint 1 h after reperfusion (Fig. 6).

The lung water content displayed by the wet/dry ratio was significantly higher in the sildenafil-supplemented group, as was anticipated when considering the observed progressive pulmonary edema. The sildenafil preconditioned group also showed a trend to higher values when compared to control, without reaching statistical significance (Fig. 7).

The effect of superior oxygenation could be contributed to an optimal preservation or to a remaining opening of lung capillaries by sildenafil, explaining the slightly increased lung water content observed in group III (or a combination of both). An increase of the functional capillary density of the post-transplanted lung as a surrogate to functional microcirculation could already be demonstrated in vivo by our group in the same model (yet unpublished results, 2005/2006). The continued vasodilatation of the pulmonary vessels leads to the opening of shunted capillaries, which leads to a higher blood circulation in the lung on one side and to a higher oxygenation capacity one the other side. However, this could lead to a potential reperfusion edema if sildenafil dosage is inadequately high.

In conclusion, sildenafil preconditioning is superior to application as an additive in the preservation solution as evidenced by the survival of all animals, the lower pulmonary pressure and vascular resistance after 6 h of reperfusion, and a superior oxygenation capacity. The simplicity of its use and the lack of significant hemodynamic side effects, potentially compromising the harvest of other organs in usual explantation procedures, make this procedure a realistic way to
establish novelty for preservation. However, the detailed mechanisms for the observed results as well as for the discrepancy due to different application forms are not yet known. Therefore, more studies using different dosage of this drug are necessary in order to find the optimal regime since high dosage leads to severe pulmonary edema. Clinical use should be deferred after these completing studies can be provided.

References


Appendix A. Conference discussion

Dr M. Strueber (Hannover, Germany): Congratulations on your investigations because I think this large animal model tells us best what’s going on with the lung preservation.

But if I follow you correctly, I think the conclusion saying that in your group II you have a long-lasting reduction of pulmonary vascular resistance may not be the answer. The answer could be that the preservation did not work in the first place. Because probably when the pulmonary vascular resistance is lowered at the time of preservation, the amount of flush perfusion you inject into the lung may not be sufficient to preserve the whole lung. It may just shunt through and preserve only a part of the lung.

Dr Pizanis: Well, I think this would be the case in the preconditioning because we could visualize the opening of the capillaries with the preconditioning of PDE-5. The group II was the bad group and there the sildenafil was only used as an additive in the flush perfusion. And I think this effect you described would have been seen in group III more than in group II. So I think even though we don’t understand it for sure, I must confess that the sildenafil remaining in the preservation solution seems to have more effect than the preconditioning on a normal circulating pig.

Dr Strueber: I disagree. Because if you apply it systemically, you will have a dilatation of all pulmonary vessels. And if you put it into the preservation solution, you only have selective dilatation of the vessels where your preservation fluid already is. So the danger of having a shunt seems to be very high.

Dr Pizanis: Good point. But that leads us to our next investigation where I think it’s very important to check out the different dosage of the sildenafil that we’re just performing, and I think the right dose is the key to good results.