



HAL
open science

Effects of Cyclosporine A in Ex Vivo Reperfused Pig Lungs

Stéphane Gennai, Redha Souilamas, Maxime Maignan, Angélique Brouta, Christophe Pison, Eric Fontaine, Raphael Briot

► **To cite this version:**

Stéphane Gennai, Redha Souilamas, Maxime Maignan, Angélique Brouta, Christophe Pison, et al.. Effects of Cyclosporine A in Ex Vivo Reperfused Pig Lungs. *Microcirculation*, 2014, 21 (1), pp.84-92. 10.1111/micc.12082 . hal-01962227

HAL Id: hal-01962227

<https://hal.univ-grenoble-alpes.fr/hal-01962227v1>

Submitted on 8 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Effects of Cyclosporine A in *Ex Vivo* Reperfused Pig Lungs

STÉPHANE GENNAI,^{*,†} REDHA SOUILAMAS,[‡] MAXIME MAIGNAN,^{*,†} ANGÉLIQUE BROUTA,[†] CHRISTOPHE PISON,[§] ERIC FONTAINE,[§] AND RAPHAËL BRIOT^{*,†}

^{*}Emergency Department, Grenoble University Hospital, Grenoble Cedex, France; [†]TIMC IMAG Laboratory, UMR 5525, La Tronche Cedex, France; [‡]Thoracic Surgery Department, European Georges Pompidou Hospital, Paris, France; [§]Laboratory of Fundamental and Applied Bioenergetics, INSERM U1055, Joseph Fourier University, Grenoble Cedex, France

Address for correspondence: Stéphane Gennai, Centre Hospitalier Universitaire de Grenoble, CS 10217, 38043 Grenoble Cedex 09, France.

E-mail: sfgennai@gmail.com

Received 16 January 2013; accepted 10 August 2013.

ABSTRACT

Objective: Several works highlight the role of CsA in the prevention of IRI, but none focus on isolated lungs. Our objective was to evaluate the effects of CsA on IRI on *ex vivo* reperfused pig lungs.

Methods: Thirty-two pairs of pig lungs were collected and stored for 30 minutes at 4°C. The study was performed in four groups. First, a control group and then three groups receiving different concentrations of CsA (1, 10, and 30 μ M) at two different times: once at the moment of lung procurement and another during the reperfusion procedure. The *ex vivo* lung preparation was set up using an extracorporeal perfusion circuit. Gas exchange parameters, pulmonary hemodynamics, and biological markers of lung injury were collected for the evaluation.

Results: CsA improved the PaO₂/FiO₂ ratio, but it also increased PAP, Pcap, and pulmonary vascular resistances with dose-dependent effects. Lungs treated with high doses of CsA displayed higher capillary-alveolar permeability to proteins, lower AFC capacities, and elevated concentrations of pro-inflammatory cytokines.

Conclusions: These data suggest a possible deleterious imbalance between the beneficial cell properties of CsA in IRI and its hemodynamic effects on microvascularization.

KEY WORDS: ischemia/reperfusion injury, lung physiology, pulmonary function, lung transplantation

Abbreviation used: AFC, alveolar fluid clearance; ANOVA, analyse of variances; ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; CsA, cyclosporine A; E_TCO₂, end tidal carbon dioxide pressure; EVLP, *ex vivo* lung perfusion; *f*, frequency; FiO₂, inspired oxygen fraction; FITC-D70, fluorescein isothiocyanate labeled dextran; IL-1 β , interleukin-1 beta; IRI, ischemia reperfusion injuries; *K*, FITC-D70 transport rate coefficient; MPTP, mitochondrial permeability transition pore; NHBD, non-heart-beating donors; PaCO₂, arterial carbon dioxide pressure; PaO₂/FiO₂, arterial oxygen pressure/inspired oxygen fraction; PAP, pulmonary arterial pressure; Pcap, capillary pressure; PEEP, positive end-expiratory pressure; *P*, perfusate; PVRa and PVRv, arteriolar and venular pulmonary vascular resistances; RAGE, receptor for advanced glycation end-products; SEM, standard error of the mean; TLR, toll-like receptor; TNF α , tumor necrosis factor alpha; V_T, tidal volume; W/D, wet to dry weight.

Please cite this paper as: Gennai S, Souilamas R, Maignan M, Brouta A, Pison C, Fontaine E, Briot R. Effects of cyclosporine A in *ex vivo* reperfused pig lungs. *Microcirculation* 21: 84–92, 2014.

INTRODUCTION

Lung transplantation is now commonly used for the treatment of chronic pulmonary diseases. The number of patients registered for the waiting list increases each year; thus, new ways need to be discovered on how to enlarge the pool of lung donors [21]. To reach this goal, utilization of lungs from marginal donors or NHBD should be considered. Techniques of EVLP have shown to be a promising solution [12,43], and the prevention of IRI has become a major challenge [13].

In the past two decades, several publications have highlighted the role of CsA in the prevention of IRI when

administered during pre-conditioning (before ischemia) and post-conditioning (during ischemia and before reperfusion) of organ transplantations in several animal species [15,19,20,25,30,45,50]. Besides its graft anti-rejection activity, CsA inhibits MPTP opening. Many studies focus on the prevention of IRI in the myocardial tissue [19,20,33].

The study on the effects of CsA in the prevention of IRI on lungs has been focused more on isolated cells and rodents, but not on large mammals [15,25,30]. We aimed change that stigma and evaluate the effects of CsA in EVLP on pig lungs.

MATERIALS AND METHODS

Animal Preparation

Animal care and procedures were made according to the Helsinki convention for the use and care of animals. Experiments were performed on 32 pigs weighing 19.9 ± 1.6 kg. The pigs were anesthetized with an inhalation of 5% Isoflurane (Belamont, Cournon d'Auvergne, France), and mechanically ventilated with 2% Isoflurane. Tidal volume (V_T) was 7 mL/kg and respiratory frequency (f) was twelve breaths per minute. A five-centimeter H₂O PEEP was maintained and 10,000 U of Heparin i.v. (Sanofi-Aventis, Ploërmel, France) was administered. The pigs were killed using pentobarbital i.v. (Chemische Fabrik, Berg, Germany) (25 mg/kg) and potassium chloride i.v. (5 g). Pneumoplegia was performed by infusing 1 L of the preservation fluid Perfadex[®] (Vitrolife AB, Gothenburg, Sweden) at 4°C in the right ventricle. Perfadex[®] was buffered with Trometamol (Addex-THAM, Kabi, Sweden). Finally, the lungs were extracted and stored in a cold room at 4°C for 30 minutes.

Ex Vivo Preparation

The usefulness of EVLP is well known and described in literature [7,12,17,43]. Many parameters of our *ex vivo* preparation was performed in a "state of the art" EVLP setting and published by research teams that are experts in the field [36]. In our experiments, our purpose was not to demonstrate or suggest an evolution of the EVLP technique, but rather to use such experimental preparations to evaluate the benefit of the CsA to reduce IRI.

The *ex vivo* lung function assessment system was primed with 2.8 L of Perfadex[®] added with 5% of bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA), and 2 mg/L of Trinitrine (Sanofi-Aventis). The pulmonary artery was cannulated with a 20-F cannula (Turemo, Ann Arbor, MI, USA) connected to the extracorporeal circuit. A pressure probe (Baxter, Uden, Holland) was first placed into the pulmonary artery, then a temperature probe (Sorin Group, Arvada, CO, USA) was connected to the membrane oxygenator; finally, a second temperature probe (Integral Process, Conflans Sainte Honorine, France) was placed at the pulmonary vein exit. During the rewarming phase, 2 L/min of oxygen and 2 L/min of nitrogen (93%) mixed with carbon dioxide (7%) were carried to the membrane oxygenator. Isotonic trometamol was used to obtain a physiologic pH in the mixed solution.

Rewarming Phase

The rewarming of the lung preparations was initiated by a slow infusion (100 mL/min) at 25°C. The peristaltic pump flow was gradually increased along with the temperature of the perfusion fluid. At 32°C, ventilation was started

($V_T = 50$ mL, $f = 12$ /min, PEEP = 5 cmH₂O, $FiO_2 = 50\%$) and then gradually increased by increments of 20 mL up to a maximal V_T of 7 mL/kg. During this rewarming phase, the pump flow was progressively increased up to 1.3 L/min (normal cardiac output for a 20 kg pig). However, PAP was never allowed to exceed 25 mmHg. The pump flow was fixed with a lower pressure less than 25 mmHg in order to preserve the integrity of the capillary-alveolar membrane. The rewarming phase was considered complete when the temperature of the solution from the pulmonary veins reached 36°C, while full cardiac output and ventilation were also obtained. Oxygen flow to the gas mixer was then stopped and only the nitrogen and carbon dioxide supply to the reperfusion circuit was maintained. This steady state was maintained for 45 minutes before starting the evaluation phase. The mean time of hot ischemia was 18 ± 4 minutes and the mean time of cold ischemia was 117 ± 20 minutes.

Evaluation Phase

During the evaluation phase, gas exchange parameters (PaO_2/FiO_2 , $PaCO_2$, E_TCO_2), pulmonary hemodynamics, and several markers of lung injury were measured.

Pulmonary hemodynamics. PAP was continually monitored through a computer-integrated data acquisition system (Biopac, Santa Barbara, CA, USA). To estimate Pcap, the peristaltic pump was paused for a few seconds. The Pcap was then calculated using a model developed in our laboratory by Baconnier *et al.* [3]. In this model, pulmonary vasculature is considered three serial compliant compartments (arterial, capillary, and venous) separated by two resistances (arterial and venous). The Pcap was then estimated using zero time extrapolation of the slow component of the arterial occlusion profile. The respective PVRa and PVRv were then derived from this Pcap evaluation.

Markers of lung injury. Concentrations from two pro-inflammatory cytokines, TNF α and IL-1 β , were measured in perfusion fluid and in BAL fluid. We found that the ischemia-reperfusion of solid organs was responsible for the quick release of pro-inflammatory cytokines [13,14,18,27,39]. These pro-inflammatory cytokines were mainly secreted by the alveolar and parenchymal macrophages and secondarily secreted by the alveolar epithelial cells, which were in direct response to the oxidative stress [30]. This phenomenon explains why we can find the cytokines in both the alveolar space and the perfusate.

The concentrations of RAGE were also measured in perfusion and BAL fluid. The marker RAGE is relatively specific to the alveolar epithelial cell injury [7]. RAGE is predominantly produced by alveolar type I cells which covers 95% of the pulmonary alveolar surface. During an alveolar epithelial injury, RAGE is released in both the alveolar space

and in the vascular compartment [46]. Some recent studies have shown that an increase in the concentration of RAGE in BAL was directly correlated with the severity of the lesion [7,9,17]. RAGE concentration in the vascular compartment was also of interest in order to evaluate lung injury. If plasmatic RAGE was elevated in the ARDS [22], it could result in early mortality, ventilator free days, and the length of stay in an intensive care unit after lung transplantation [46].

We then calculated the rate of AFC, which estimates fluid reabsorption capacities and functional status of the alveolar epithelium. AFC was then measured as previously described [7,17]. At the end of the experiment, a catheter (PE 240 tubing; BD, Le Pont de Claix, France) was passed through a side port in the endobronchial tube into the lung and advanced until gentle resistance was encountered. Then 100 mL of warm (36°C) normal saline containing 5% bovine serum albumin was instilled through the catheter into the airspaces of the lung. After five minutes ($T = 0$) and 35 minutes ($T = 30$ minutes), samples were removed through the catheter by gentle aspirations. The change in protein concentration at $T = 30$ minutes was used to determine the volume of fluid cleared from the airspaces by the following equation:

$$\text{Distal alveolar fluid clearance (\%/hour)} = 2(1 - C_i/C_f),$$

where C_i is the protein concentration at $T = 0$ minute and C_f is the protein concentration at $T = 30$ minutes.

The BAL procedure performed at the end of the experiment also served as an estimate for the lung capillary-alveolar permeability to the macromolecules measured by a technique previously described by our team [5]. FITC-D70 (Sigma, St. Quentin-Fallavier, France), which is a fluorescent macromolecular indicator (same size as an albumin), was added into the perfusion fluid 30 minutes before BAL procedure (time for equilibration between perfusate and alveoli). At the same time, FITC-D70 concentrations were measured (fluorescence spectrophotometer NanoDrop ND-3300; Labtech, Palaiseau, France) in both the perfusate and in the alveolar fluid, which was sampled just after the initial instillation of BAL fluid. The permeability of the capillary-alveolar membrane was expressed as the transport rate coefficient (K) of FITC-D70 from the perfusion fluid to alveoli. The following formula was used to calculate this permeability coefficient:

$$K(\text{minute}^{-1}) = \frac{([\text{FITC-D70}] \text{ in BAL} / [\text{FITC-D70}] \text{ in perfusion fluid})}{30}$$

Cyclosporine Administration

The study was performed in four separate groups with eight animals each. First, a control group, and then three groups receiving different concentrations of CsA (Novartis, Stein,

Switzerland): 1, 10, and 30 μM (CsA1, CsA10, CsA30). CsA was administered during the lung procurement surgery (CsA added to the pneumoplegia solution) and during the EVLP procedure (CsA added to the reperfusion solution).

Statistics

Values are given as median and 25th and 75th centiles. Due to the data having abnormal distribution, non-parametric methods had to be used. We used the Spearman correlation coefficients to test the correlation between cyclosporine levels and other continuous variables. The Mann-Whitney rank-sum test was also used for two-group comparisons. The value $p < 0.05$ was considered to be statistically significant.

RESULTS

Gas Exchange Function

The $\text{PaO}_2/\text{FiO}_2$ ratio was significantly improved by an increased dose of CsA (Figure 1A), while the CO_2 gradient between perfusion fluid and exhaled air ($\text{PaCO}_2 - \text{E}_T\text{CO}_2$) decreased non-significantly in a CsA dose-dependent manner ($p = 0.0676$) (Figure 1B).

Hemodynamics

The PAP, the P_{cap} , and the PVR increased due to an administration of CsA with a dose-dependent effect (Figure 2A–C). The increase in PVR occurred predominantly on the venular part of the pulmonary vascular bed and for high doses of CsA (30 μM) (Table 1).

Markers of Lung Injury

Low (1 μM) and moderate (10 μM) doses of CsA showed tendencies to prevent the alveolar epithelial lesion, even if statistically insignificant, which was estimated by the rate of AFC and the alveolar concentration of RAGE (Table 1). Conversely, lungs treated with a high dose of CsA (30 μM) had a worse permeability coefficient K and displayed higher concentrations of pro-inflammatory cytokines (IL-1 β and TNF α) compared to the other groups (Figure 3A–D).

DISCUSSION

We performed the first evaluation of the effects of CsA on isolated pig lungs. The effects had never been studied yet on a lung model for large mammals. Our data showed dose-dependent effects of CsA on gas exchanges, but also on pulmonary hemodynamics, and possibly an aggravation of the IRI due to high doses of CsA. These results constitute an important step toward the use of CsA on humans to reduce lung IRI and consequently, primary graft dysfunction.

Within a few years, the EVLP technique has become a reference for the evaluation of lung grafts. Its interest has been demonstrated on animal lung preparations, especially

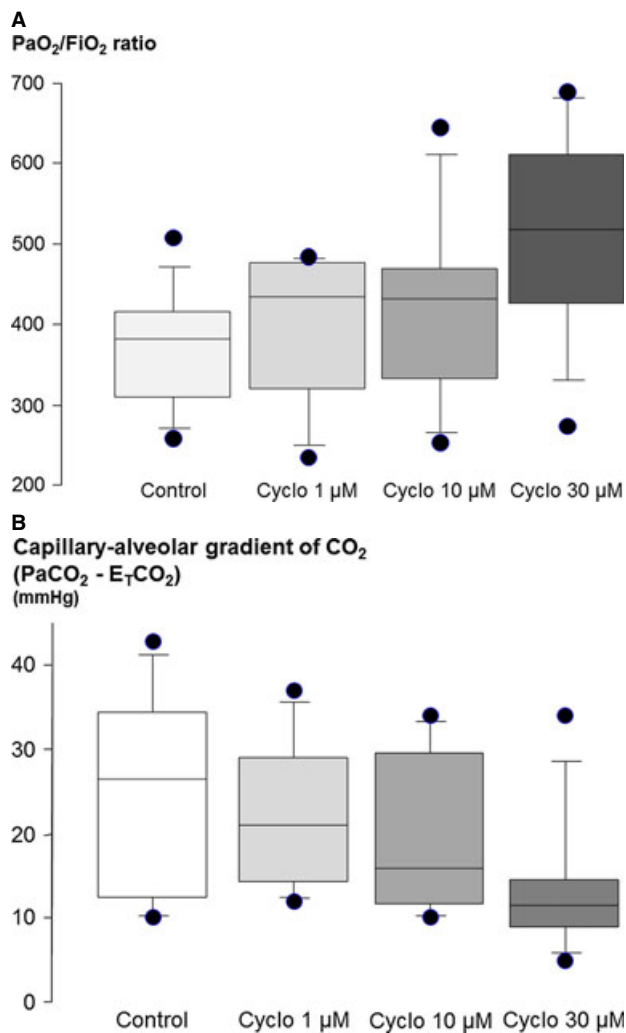


Figure 1. Gas exchange parameters. The horizontal line within the box shows the median. The values between the lower and upper quartiles (25th–75th centiles) are within the box. The whiskers represent the limits of the 10th–90th centile values. Extreme values are shown as filled circles. (A) PaO₂/FiO₂ ratio, $p < 0.01$ by Spearman correlation test. (B) Capillary-alveolar gradient of CO₂, $p = 0.0676$ by Spearman correlation test.

on pig [43] and human lungs [12]. This technique can be seen as bench test for lung function, allowing for the assessment of new therapies suppose to limit IRI. Gas exchange capacities and total pulmonary arterial resistance are more commonly studied physiopathological parameters. We also measured other hemodynamics (Pcap, longitudinal pulmonary resistance) and markers (AFC, RAGE, cytokines, lung permeability) that have showed their pertinence in the evaluation of lung IRI [5,7].

Cyclosporine Rationale

It has been hypothesized that IRI is mostly related to mitochondrial death as a consequence of MPTP opening.

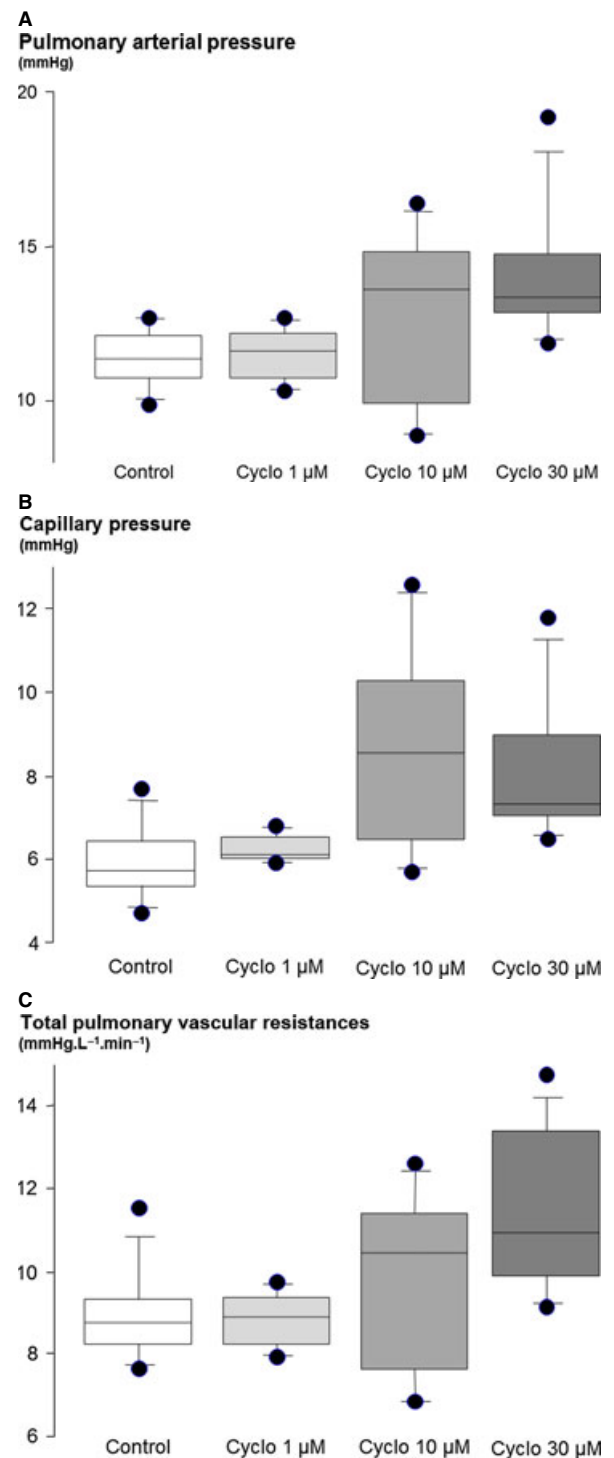


Figure 2. Hemodynamic parameters. The horizontal line within the box shows the median. The values between the lower and upper quartiles (25th–75th centiles) are within the box. The whiskers represent the limits of the 10th–90th centile values. Extreme values are shown as filled circles. (A) PAP, $p < 0.01$ by Spearman correlation test. (B) Pcap, $p < 0.01$ by Spearman correlation test. (C) Total pulmonary vascular resistances, $p < 0.01$ by Spearman correlation test.

Table 1. Lung function parameters after ischemia reperfusion

	Control (n = 8)	CsA 1 μ M (n = 8)	CsA 10 μ M (n = 8)	CsA 30 μ M (n = 8)	p-value
PVRa (mmHg/L/min)	4.3 [3.9; 4.6]	4.5 [3.8; 4.7]	3.3 [2.9; 4.3]	4.7 [4.3; 5.8]	NS
PVRv (mmHg/L/min)	4.4 [4.2; 4.8]	4.7 [4.6; 5.0]	6.6 [5.3; 7.7]	6.7 [5.6; 7.4]	0.0047 [†]
K (10^{-3} per minute)	5 [1; 7]	3 [1; 8]	3 [1; 5]	8 [3; 16]	0.0378 [‡]
AFC (% of the initial volume/hour)	9.4 [0.0; 44.3]	56.4 [16.1; 59.0]	59.2 [37.4; 70.0]	14.8 [0.0; 34.8]	NS
RAGE in LBA (pg/mL)	1.6 [1.4; 10.8]	1.9 [1.7; 2.1]	1.6 [1.5; 2.4]	1.6 [1.5; 2.3]	NS
RAGE in P (pg/mL)	0.0 [0.0; 1.3]	1.5 [0.7; 1.7]	1.4 [1.4; 1.5]	1.3 [0.0; 1.5]	NS

Values are median and interquartile range. p-values are calculated with nonparametric tests (two-group Mann–Whitney test or multi-group global Kruskal–Wallis comparison).

[†]Global comparison. [‡]CsA 30 μ M versus other three groups.

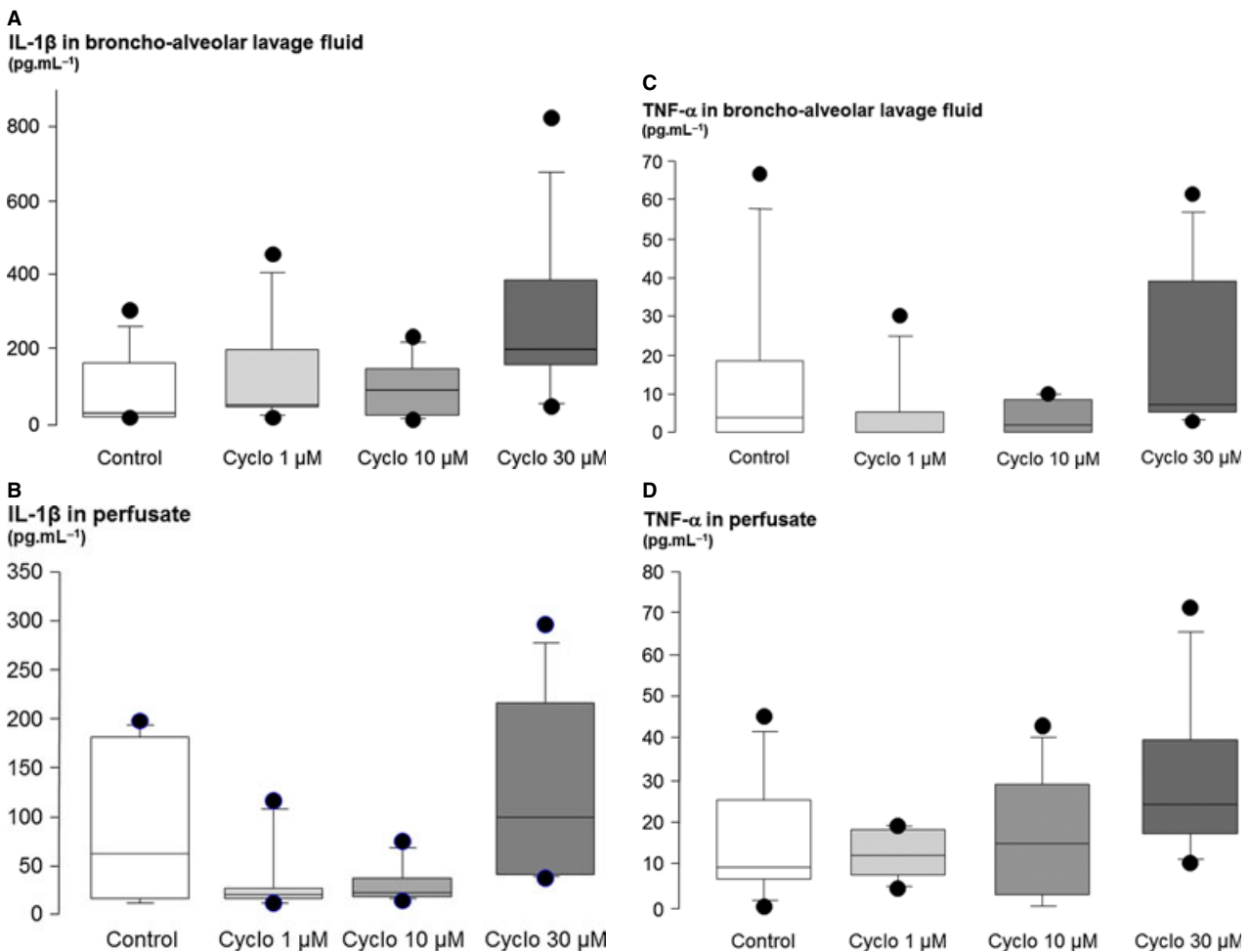


Figure 3. wPro-inflammatory cytokines in broncho-alveolar lavage and in perfusate. The horizontal line within the box shows the median. The values between the lower and upper quartiles (25th–75th centiles) are within the box. The whiskers represent the limits of the 10th–90th centile values. Extreme values are shown as filled circles. **(A)** Interleukin-1 β in broncho-alveolar lavage fluid, $p < 0.05$ for Cyclo 30 μ M versus all groups by Mann–Whitney test. **(B)** Interleukin-1 β in perfusate, $p < 0.05$ for Cyclo 30 μ M versus all groups by Mann–Whitney test. **(C)** TNF α in broncho-alveolar lavage fluid, $p < 0.05$ for Cyclo 30 μ M versus all groups by Mann–Whitney test. **(D)** TNF α in perfusate, $p < 0.05$ for Cyclo 30 μ M versus all groups by Mann–Whitney test.

Located in the inner mitochondrial membrane, the MPTP remains unremarkable under normal physiological conditions. Stress can lead to its opening, resulting in the swelling of the matrix due to osmotic forces. It then induces further failure of the mitochondrial outer membrane and the release of the cytosol pro-apoptotic factors [19]. The inhibition of the opening of MPTP is thought to be the main pathway for CsA action. Several *in vitro* and *in vivo* animal models showed CsA interests in pre and post-conditioning for the prevention of IRI on different organs such as heart, kidney, and liver [19,20,45,50]. In humans, CsA administered just before coronary reperfusion (post-conditioning) has been proven to be an efficient way to reduce the size of myocardial infarction [33]. However, few studies have been published on CsA effects on lung IRI. *In vitro* studies on post hypoxia-reoxygenation injuries showed that alveolar macrophages pretreated by CsA secreted less chemokines than controls [30]. Moreover, endothelial cells incubated with CsA selectively reduced pro-inflammatory mediator secretion of NF κ B and EGR-1 [15]. Nevertheless, some of the pathways involved in IRI can be activated by CsA, such as the metalloproteinase and the TLR [1,28,41]. Such insights can explain the increased levels of pro-inflammatory cytokines we measured in our experiments with high doses of CsA (30 μ M). In an *in vivo* ischemic lung model, Krishnasadan *et al.* showed that rats pre-conditioned with CsA displayed less tissue myeloperoxidase content, leukocyte accumulation, and vascular permeability [25]. Although these studies mainly focused on isolated cells and small animals, we chose to evaluate the role of CsA in the prevention of lung IRI in a pre-clinical study conducted on large animals, which allows for a more integrated evaluation of the lung function.

The different concentrations we chose to test were derived from previous publications on the subject. In *in vitro* studies, the average concentration of CsA leading to observable positive effects in cellular bath solution is 1 μ M [15,20,30]. Higher concentrations (10 and 30 μ M) were chosen from previous *in vivo* publications reporting blood concentrations of CsA between 1 and 5 μ M in humans [8,47], and up to 90 μ M in rats [26].

Microvascular Effects of IRI and CsA

In our data, CsA has shown to be deleterious on pressures and resistances, with a dose-dependent effect. Although daily administrations of CsA for three weeks seemed to prevent pulmonary hypertension induced by chronic hypoxia [24], several studies showed that CsA could be responsible for hypertension in humans after lung, heart, kidney, or liver transplantations [16,29,38,49]. Two stages were described, the first, which was acute hypertension during initiation of CsA treatment, and second, a chronic hypertension after long-term administration. CsA binds to Cyclophilin-A (an

immunophilins cytoplasmic receptor) in smooth vascular muscles and may directly affect blood pressure regulation by reducing the endothelial production of nitric oxide by NO synthase [37]. This mechanism could account for the increase in PAP, Pcap, and PVR we observed in our lungs treated with CsA, especially those receiving higher doses (10 and 30 μ M).

It has been studied that IRI induces a hypoxic mediator-induced active vasoconstriction, which results in a perivascular compression by edema, and an intravascular obstruction by thromboembolism or endothelial swelling [13]. The active reversible vasoconstriction accounts for approximately fifty percent of the hypoxic pulmonary hypertension. Endothelial cell exposure to CsA generates reactive oxygen and nitrogen species [35] that may enhance this pulmonary vasoconstriction. These early hemodynamic effects may be synergic with intrinsic cellular properties of CsA against IRI. However, beyond a certain level of CsA (over 10 μ M in our experiment), vasoconstriction and blood flow redistribution may aggravate the injury by an over-perfusion of mildly injured zones. Increasing blood flow and PAP to lesser damaged and equally injured zones can allow for major fluid filtration through the capillary-alveolar membrane as described by the Starling equation [42]. Over-perfusion could have re-opened non-flowing leaky capillaries in zone 1, called "blind capillaries" (i.e., open at their arterial end and obstructed at their venous end) and shifted the obstruction point downstream under zone 2 conditions toward the venous ends of the capillaries and veinules. These microvascular mechanisms have been described in other models of isolated lung injury [2,6], which were consistent with an increase of the post-capillary (i.e., venular) part of the PVR observed in our experiments with high doses of CsA. This may contribute to a possible aggravation of IRI with the highest dose of CsA as seen by the increased cytokines. Although blood gases temporarily improved due to an immediate blood flow redistribution, there is still a delayed capillary-alveolar fluid transfer and pulmonary edema formation.

CsA Effects in Gas Exchanges

CsA increased PaO₂/FiO₂ ratio and decreased CO₂ gradient in a dose-dependent manner. Such gas exchange improvements could be due to an enhancement of the hypoxic pulmonary vasoconstriction mediated by CsA. Furthermore, lung IRI observed during the primary graft dysfunction was similar to those found in the ARDS [11,40]. The heterogeneous lesions from the alveolar epithelial tissue and the pulmonary capillary bed features microvascular obstructions accompanied by cellular fragments and microthrombi. The heterogeneity of these types of lesions has been shown through histological analyses in ARDS [48], IRI [13], and also by clinical surveys showing various radiologic infiltrations in a patient's

pulmonary transplant [32]. IRI is a heterogeneous pulmonary vasoconstriction that leads to a redistribution of pulmonary blood flow from injured lung zones to normal lung areas. Many works highlight the importance of hypoxic vasoconstriction in maintaining oxygenation during acute lung injury [4,44]. This vascular reactivity limits the ventilation and perfusion mismatch, reduces the alveolar dead space, and consequently improves oxygenation. We assumed that a part of the gas exchange improvements observed earlier in our CsA treated lungs were related to such blood redistribution.

CsA Effects on Capillary-Alveolar Membrane

CsA could possibly restore the capillary-alveolar barrier function. Indeed, several publications on IRI lung models have shown that CsA was able to diminish the secretion of pro-inflammatory mediators [15,30] and decrease lung vascular permeability by more than 50% relative to the animals in the control group [25]. Such effects may have reduced edema formation and improved gas exchanges throughout the capillary-alveolar membrane. With this hypothesis, we consistently noted a trend in alveolar epithelial function improvement with low (1 μM) and moderate (10 μM) doses of CsA. In these groups, CsA seemed to increase the rate of AFC and decreased RAGE level in BAL fluid. These two parameters have been shown to reflect lung status after ischemia-reperfusion [7].

However, cytokine concentrations were evidently worsened in lungs treated with 30 μM of CsA, which was similar to their elevated lung vascular pressure and resistance, although the $\text{PaO}_2/\text{FiO}_2$ ratio and CO_2 gradient were high in those lungs.

We conclude from these observations that CsA has a preeminent vasoconstrictive effect on lung vasculature compared to its other actions. Low doses of CsA may have beneficial anti-inflammatory and anti-apoptotic effects, whereas high doses of CsA (30 μM) may display hemodynamic effects. Moreover, in our data, the venular resistances (i.e., post-capillary bed) were enhanced by CsA administration. This phenomenon may increase capillary leakage and edema formation and lead to a worsening of IRI. At the same time, the globally sustained hypoxic pulmonary vasoconstriction allows for a limit on the shunt effect and maintains gas exchanges. Such mechanisms may account for the alterations of capillary-alveolar function coexisting with normal blood gases that was observed in our lungs treated with 30 μM of CsA.

Limits of the Study

A possible limit encountered in our study might be the short ischemic time (135 ± 21 minutes) to which our lungs have been exposed. Indeed, a longer ischemia may provoke a more severe IRI and perhaps give the opportunity for the CsA to

emphasize its positive effects. Nevertheless, the duration of ischemia in our model was similar to several other studies performed with CsA [15,25,30].

A possible bias may also be related to the induction of anesthesia with Isoflurane in live animals. Indeed, several works show that halogen gases inhibit the MPTP [10,23,31,34], which could interfere with the CsA action in the prevention of IRI. This preventive action was expected for Sevoflurane [10,31], while Isoflurane showed contrasting results [23,34]. In our protocol, Isoflurane was only used for the induction of general anesthesia before euthanasia and lung procurement surgery. As observed in the exhaled gas analysis we assumed that there was almost no gas left in the alveoli at reperfusion time. Moreover, Isoflurane has been used in every group, thus limiting the effects of possible drug interference in the results analysis.

CONCLUSION

IRI prevention is a major challenge in lung transplantation. In our pig EVLP model, CsA showed a dose-dependent improvement in $\text{PaO}_2/\text{FiO}_2$ ratio that may be related to a parallel enhancement of hypoxic pulmonary vasoconstriction. Low doses of CsA showed a non-significant trend toward an improvement in capillary-alveolar membrane injury. Lungs treated with high doses of CsA (30 μM) presented an aggravation in lung permeability and cytokines concentrations, suggesting a deleterious imbalance between the possible beneficial properties of CsA on IRI cells and their hemodynamic effects in microvascularization. Further studies should focus more on lungs subjected to longer ischemia and treated with low or moderate doses of CsA.

PERSPECTIVE

We evaluated for the first time the effects of CsA on IRI in *ex vivo* reperfused pig lungs. Our data suggests a possible deleterious imbalance between the beneficial cell properties of CsA and its hemodynamic effects on microvascularization. For future experiments, it would be interesting to focus more on smaller doses of CsA which might limit hemodynamic drawbacks on lung microcirculation, while keeping their beneficial cellular effect on IRI. Unlike our experiment, in which the length of cold ischemia was limited, other experiments should test CsA in various cold ischemic time situations (i.e., broad spectrum of IRI severity) for highlighting the efficacy of CsA.

ACKNOWLEDGMENTS

This study was funded by the French Health Ministry and by the association "Vaincre la mucoviscidose." We thank Mr. Hervé Chaussard for help during surgery, Ms. Dorra

Guergour and Ms. Annie Foquin for help with biological analysis, and Ms. Adrienne Varela for help with editing the manuscript.

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

- Andrade CF, Kaneda H, Der S, Tsang M, Lodyga M, Chimisso Dos Santos C, Keshavjee S, Liu M. Toll-like receptor and cytokine gene expression in the early phase of human lung transplantation. *J Heart Lung Transplant* 25: 1317–1323, 2006.
- Anglade D, Corboz M, Menaouar A, Parker JC, Sanou S, Bayat S, Benchetrit G, Grimbert FA. Blood flow vs. venous pressure effects on filtration coefficient in oleic acid-injured lung. *J Appl Physiol* 84: 1011–1023, 1998.
- Baconnier PF, Eberhard A, Grimbert FA. Theoretical analysis of occlusion techniques for measuring pulmonary capillary pressure. *J Appl Physiol* 73: 1351–1359, 1992.
- Brimioulle S, Julien V, Gust R, Kozlowski JK, Naeije R, Schuster DP. Importance of hypoxic vasoconstriction in maintaining oxygenation during acute lung injury. *Crit Care Med* 30: 874–880, 2002.
- Briot R, Bayat S, Anglade D, Martiel JL, Grimbert F. Monitoring the capillary-alveolar leakage in an A.R.D.S. model using broncho-alveolar lavage. *Microcirculation* 15: 237–249, 2008.
- Briot R, Bayat S, Anglade D, Martiel JL, Grimbert F. Increased cardiac index due to terbutaline treatment aggravates capillary-alveolar macromolecular leakage in oleic acid lung injury in dogs. *Crit Care* 13: R166, 2009.
- Briot R, Frank JA, Uchida T, Lee JW, Calfee CS, Matthay MA. Elevated levels of the receptor for advanced glycation end products, a marker of alveolar epithelial type I cell injury, predict impaired alveolar fluid clearance in isolated perfused human lungs. *Chest* 135: 269–275, 2009.
- Burckart GJ, Venkataramanan R, Ptachcinski RJ, Starzl TE, Gartner JC, Zitelli BJ, Malatack JJ, Shaw BW, Iwatsuki S, Van Thiel DH. Cyclosporine absorption following orthotopic liver transplantation. *J Clin Pharmacol* 26: 647–651, 1986.
- Calfee CS, Ware LB, Eisner MD, Parsons PE, Thompson BT, Wickersham N, Matthay MA. Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. *Thorax* 63: 1083–1089, 2008.
- Chen HT, Yang CX, Li H, Zhang CJ, Wen XJ, Zhou J, Fan YL, Huang T, Zeng YM. Cardio-protection of sevoflurane postconditioning by activating extracellular signal-regulated kinase 1/2 in isolated rat hearts. *Acta Pharmacol Sin* 29: 931–941, 2008.
- Christie JD, Sager JS, Kimmel SE, Ahya VN, Gaughan C, Blumenthal NP, Kotloff RM. Impact of primary graft failure on outcomes following lung transplantation. *Chest* 127: 161–165, 2005.
- Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, Sato M, Laratta J, Azad S, Madonik M, Chow CW, Chaparro C, Hutcheon M, Singer LG, Slutsky AS, Yasufuku K, de Perrot M, Pierre AF, Waddell TK, Keshavjee S. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med* 364: 1431–1440, 2012.
- De Perrot M, Liu M, Waddell TK, Keshavjee S. State of the art. Ischemia reperfusion-induced-injury. *Am J Respir Crit Care Med* 167: 490–511, 2003.
- Den Hengst WA, Gielis JF, Lin JY, Van Schil PE, De Windt LJ, Moens AL. Lung ischemia-reperfusion injury: a molecular and clinical view on a complex pathophysiological process. *Am J Physiol Heart Circ Physiol* 299: H1283–H1299, 2010.
- Farivar AS, Mackinnon-Patterson BC, Barnes AD, McCourtie AS, Mulligan MS. Cyclosporine modulates the response to hypoxia-reoxygenation in pulmonary artery endothelial cells. *Ann Thorac Surg* 79: 1010–1016, 2005.
- First MR, Neylan JF, Rocher LL, Tejani A. Hypertension after renal transplantation. *J Am Soc Nephrol* 4: S30–S36, 1994.
- Frank JA, Briot R, Lee JW, Ishizaka A, Uchida T, Matthay MA. Physiological and biochemical markers of alveolar epithelial barrier dysfunction in perfused human lungs. *Am J Physiol Lung Cell Mol Physiol* 293: L52–L59, 2007.
- Gerlach J, Jorres A, Nohr R, Zeilinger K, Spatkowski G, Neuhaus P. Local liberation of cytokines during liver preservation. *Transpl Int* 12: 261–265, 1999.
- Gomez L, Thibault H, Gharib A, Dumont JM, Vuagniaux G, Scalfaro P, Derumeaux G, Ovize M. Inhibition of mitochondrial permeability transition improves functional recovery and reduces mortality following acute myocardial infarction in mice. *Am J Physiol Heart Circ Physiol* 293: 1654–1661, 2007.
- Griffiths EJ, Halestrap AP. Protection by Cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts. *J Mol Cell Cardiol* 25: 1461–1469, 1993.
- International Society for Heart and Lung Transplantation. Annual report. 2010.
- Jabaudon M, Futier E, Roszyk L, Chalus E, Guerin R, Petit A, Mrozek S, Perbet S, Cayot-Constantin S, Chartier C, Sapin V, Bazin JE, Constantin JM. Soluble form of the receptor for advanced glycation end products is a marker of acute lung injury but not of severe sepsis in critically ill patients. *Crit Care Med* 39: 480–488, 2011.
- Kanaya N, Kobayashi I, Nakayama M, Fujita S, Namiki A. ATP sparing effect of isoflurane during ischaemia and reperfusion of the canine heart. *Br J Anaesth* 74: 563–568, 1995.
- Koulmann N, Novel-Chate V, Peinnequin A, Chapot R, Serrurier B, Simler N, Richard H, Ventura-Clapier R, Bigard X. Cyclosporin A inhibits hypoxia-induced pulmonary hypertension and right ventricle hypertrophy. *Am J Respir Crit Care Med* 174: 699–705, 2006.
- Krishnadasan B, Naidu BV, Rosengart M, Farr AL, Barnes A, Verrier ED, Mulligan MS. Decreased lung ischemia-reperfusion injury in rats after preoperative administration of cyclosporine and tacrolimus. *J Thorac Cardiovasc Surg* 123: 756–767, 2002.
- Lee YH, Park KH, Ku YS. Pharmacokinetic changes of cyclosporine after intravenous and oral administration to rats with uranyl nitrate-induced acute renal failure. *Int J Pharm* 194: 221–227, 2000.
- Lemay S, Rabb H, Postler G, Singh AK. Prominent and sustained up-regulation of gp130-signaling cytokines and the chemokine MIP-2 in murine renal ischemia-reperfusion injury. *Transplantation* 69: 959–963, 2000.
- Lim SW, Li C, Ahn KO. Cyclosporine-induced renal injury induces toll-like receptor and maturation of dendritic cells. *Transplantation* 80: 691–699, 2005.
- Morrison RJ, Short HD, Noon GP, Frost AE. Hypertension after lung transplantation. *J Heart Lung Transplant* 12: 928–931, 1993.
- Naidu BV, Krishnadasan B, Byrne K, Farr AL, Rosengart M, Verrier ED, Mulligan MS. Regulation of chemokine expression by Cyclosporine A in alveolar macrophages exposed to hypoxia and reoxygenation. *Ann Thorac Surg* 74: 899–905, 2002.
- Obal D, Dettwiler S, Favocchia C, Scharbatke H, Preckel B, Schlack W. The influence of mitochondrial KATP-channels in the cardioprotection of preconditioning and post-conditioning by sevoflurane in the rat *in vivo*. *Anesth Analg* 101: 1252–1260, 2005.
- Oto T, Griffiths AP, Lewey BJ, Pilcher DV, Williams TJ, Snell GI. Definitions of primary graft dysfunction after lung transplantation:

- differences between bilateral and single lung transplantation. *J Thorac Cardiovasc Surg* 132: 140–147, 2006.
33. Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, Elbelghiti R, Cung TT, Bonnefoy E, Angoulvant D, Macia C, Raczka F, Sportouch C, Gahide G, Finet G, André-Fouët X, Revel D, Kirkorian G, Monassier JP, Derumeaux G, Ovize M. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 359: 473–481, 2008.
 34. Preckel B, Schlack W, Comfère T, Obal D, Barthel H, Thämer V. Effects of enflurane, isoflurane, sevoflurane and desflurane on reperfusion injury after regional myocardial ischemia in the rabbit heart in vivo. *Br J Anesth* 81: 905–912, 1998.
 35. Redondo-Horcajo M, Romero N, Martínez-Acedo P, Martínez-Ruiz A, Quijano C, Lourenço CF, Movilla N, Enríquez JA, Rodríguez-Pascual F, Rial E, Radi R, Vázquez J, Lamas S. Cyclosporine A-induced nitration of tyrosine 34 MnSOD in endothelial cells: role of mitochondrial superoxide. *Cardiovasc Res* 87: 356–365, 2010.
 36. Sanchez PG, Bittle GJ, Burdorf L, Pierson RN, Griffith BP. State of art: clinical ex vivo lung perfusion: rationale, current status, and future directions. *J Heart Lung Transplant* 31: 339–348, 2012.
 37. Sander M, Lyson T, Thomas GD, Victor RG. Sympathetic neural mechanisms of cyclosporine-induced hypertension. *Am J Hypertens* 9: S121–S138, 1996.
 38. Scherrer U, Vissing SF, Morgan BJ, Rollins JA, Tindall RS, Ring S, Hanson P, Mohanty PK, Victor RG. Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. *N Engl J Med* 323: 693–699, 1990.
 39. Serrick C, Adoumie R, Giaid A, Shennib H. The early release of interleukin-2, tumor necrosis factor-alpha and interferon-gamma after ischemia reperfusion injury in the lung allograft. *Transplantation* 58: 1158–1162, 1994.
 40. Shargall Y, Guenther G, Ahya VN, Ardehali A, Singhal A, Keshavjee S; ISHLT Working Group on Primary Lung Graft Dysfunction. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part VI: treatment. *J Heart Lung Transplant* 24: 1489–1500, 2005.
 41. Soccia PM, Gasche Y, Miniati DN, Hoyt G, Berry GJ, Doyle RL, Theodore J, Robbins RC. Matrix metalloproteinase inhibition decreases ischemia-reperfusion injury after lung transplantation. *Am J Transplant* 4: 41–50, 2004.
 42. Starling EH. On the absorption of fluids from the connective tissue spaces. *J Physiol* 19: 312–326, 1896.
 43. Steen S, Liao Q, Wierup PN, Bolys R, Pierre L, Sjöberg T. Transplantation of lungs from non-heart-beating donors after functional assessment ex vivo. *Ann Thorac Surg* 76: 244–252, 2003.
 44. Theissen IL, Meissner A. Hypoxic pulmonary vasoconstriction. *Anaesthetist* 45: 643–652, 1996.
 45. Travis DL, Fabia R, Netto GG, Husberg BS, Goldstein RM, Klintmalm GB, Levy MF. Protection by Cyclosporine A against normothermic liver ischemia-reperfusion in pigs. *J Surg Res* 75: 116–126, 1998.
 46. Uchida T, Shirasawa M, Ware LB, Kojima K, Hata Y, Makita K, Mednick G, Matthay ZA, Matthay MA. Receptor for advanced glycation end-products is a marker of type I cell injury in acute lung injury. *Am J Respir Crit Care Med* 173: 1008–1015, 2006.
 47. Van Assche G, D'Haens G, Noman M, Vermeire S, Hiele M, Asnong K, Arts J, D'Hoore A, Penninckx F, Rutgeerts P. Randomized, double-blind comparison of 4 mg/kg versus 2 mg/kg intravenous cyclosporine in severe ulcerative colitis. *Gastroenterology* 125: 1025–1031, 2003.
 48. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 342: 1334–1349, 2000.
 49. Winkler M, Brinkmann C, Jost U, Oldhafer K, Ringe B, Pichlmayr R. Long-term side effects of cyclosporine-based immunosuppression in patients after liver transplantation. *Transplant Proc* 26: 2679–2682, 1994.
 50. Yang CW, Ahn HJ, Han HJ, Kim WY, Li C, Shin MJ, Kim SK, Park JH, Kim YS, Moon IS, Bang BK. Pharmacological preconditioning with low-dose cyclosporine or FK506 reduces subsequent ischemia/reperfusion injury in rat kidney. *Transplantation* 72: 1753–1759, 2001.