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Role for primary immunosuppression with everolimus after pulmonary transplantation

J. Salman[a,c,*,1], K. Jansson[a,c,1], Th. Siemeni[a], W. Sommer[a,c], A.-K. Knoefel[a,c], L. Ahrens[a,c], T. Nakagiri[a], F. Ius[a], I. Tudorache[a], B. Kruse[a], S. Thissen[a], D. Jonigk[b,c], M. Strüber[a], A. Haverich[a,c], G. Warnecke[a,c,2], M. Avsar[a,2]

[a] Department of Cardiothoracic, Transplant and Vascular Surgery, Hannover Medical School, Hannover, Germany
[b] Institute for Pathology, Germany
[c] Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), German Center for Lung Research (DZL), Hannover, Germany

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ABSTRACT

Purpose: Everolimus is a proliferation signal inhibitor used for triple immunosuppressive therapy following solid organ transplantation. Its positive benefits, such as reduction of acute rejection episodes and reduced nephrotoxicity have been shown in kidney- as well as heart transplantation. However the role of everolimus is less well defined in lung transplantation. We thus wished to study the effect of primary immunosuppression with everolimus in a preclinical large animal lung transplantation model.

Methods: Left-sided single lung transplantation from MHC-mismatched donors was performed in 11 adult minipigs. Intravenous pharmacologic immunosuppression was maintained for 28 days with 1.5 mg/kg/d methylprednisolone, 1.0 mg/kg/d azathioprine and cyclosporine A (blood levels 300-500 ng/ml; CsA group; $n = 5$). A further group (CsA + Ev; $n = 6$) received methylprednisolone, CsA (200–300 ng/ml) and Everolimus (5–10 ng/ml). Immunosuppression was discontinued on postoperative day (POD) 28. Graft survival was monitored by sequential chest X-rays, bronchoscopies and transbronchial biopsy histology.

Results: All animals survived the 28 day course of immunosuppressive therapy and showed healthy grafts on POD 28. Median allograft survival in the CsA group was 55 ± 15 days. CsA + Ev grafts showed median survival of 49 ± 86 days ($p = 0.37$).

Conclusion: Whereas everolimus might be advantageous as maintenance immunosuppressive agent, this data does not support an important role for everolimus in the immunosuppressive induction phase following lung transplantation.

1. Introduction

Lung transplantation is an accepted therapeutic option for end-stage pulmonary disease with a 1-year survival rate of above 80% [1]. However, pharmacologic immunosuppression remains unsatisfactory with a high incidence of infections [2], frequent acute rejection episodes [3] and early onset of bronchiolitis obliterans [4,5]. During the past two decades, virtually no improvement of longterm allograft- and patient survival could be achieved [6]. The vast majority of lung transplant recipients receive a triple-drug maintenance regimen including a calcineurin inhibitor (CNI), a cell-cycle inhibitor and steroids [7].

As an alternative to azathioprine as cell-cycle inhibitor, everolimus, a macrocyclic lactone originally isolated from Streptomyces hygroscopicus that acts by blocking growth factor-driven cell proliferation [8], has been introduced. Whereas its bioavailability is greater than that of sirolimus, it has a shorter half-life, allowing more rapid achievement of a steady state [9]. Its benefits in terms of improved longterm graft survival after kidney transplantation and less allograft vasculopathy following heart transplantation could be shown in several clinical trials [10–14]. In combination with cyclosporine it allows marked reduction of cyclosporine without significant loss of efficacy. This combination provides early protection of renal function and leads to excellent renal graft survival and stable graft function at 3 years [15–19]. Nevertheless,

* Corresponding author at: Department of Cardiothoracic, Transplant and Vascular Surgery, Hannover Medical School, German Centre for Lung Research, Carl-Neuberg-Str.1, 30625 Hannover, Germany.
E-mail address: salman.jawad@mh-hannover.de (J. Salman).
[1] These authors share first authorship.
[2] These authors share senior authorship.

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Table 1
Animals.

Animal no.: recipient-donor	POD of death/survival	Clinical outcome	Histologic rejection grading	Chest X-ray score	No. of SLA-I-haplotype mismatches
CsA group (n = 5)					
80441 - 60179	89	Sac due to acute rejection	A4	4	1 or 2
60178 - 60148	36	100% Stenosis	A2	4	2
60642 - 61142	41	Sac due to acute rejection	A3/B2R/E2	3	2
60709 - 61158	84	Sac due to acute rejection	A2	3	2
62748 - 62997	70	Sac due to acute rejection	A4/B0/E1	3	1 or 2
CsA + Ev group (n = 6)					
88747 - 89129	55	Sac due to acute rejection	A2/B1R/E1	4	1 or 2
88946 - 70039	43	Sepsis	A0/B1R/E1	1	1 or 2
89341 - 69720	42	Sac due to acute rejection	A2/B1R/E1	4	1 or 2
89296 - 69742	43	Sac due to acute rejection	A3/B1R/E1	2	1 or 2
89339 - 89023	126	Sac due to acute rejection	A3-4	4	1 or 2
89297 - 70030	1295	LTS, elective sac	A0	0	1 or 2

Sac = sacrifice, LTS = long term surviving animal.

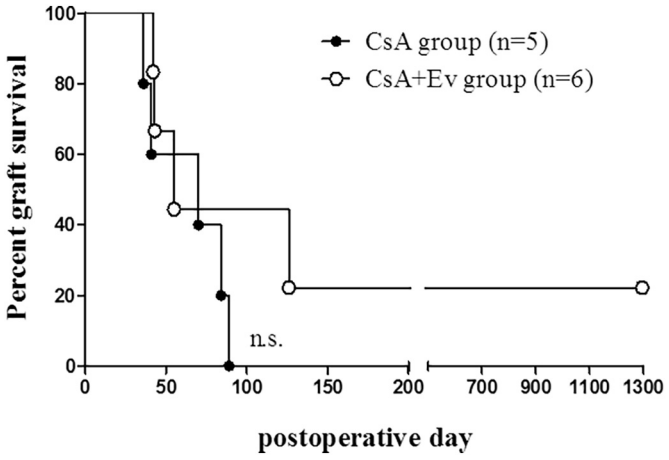

Fig. 1. Cumulative survival of lung allografts.

there is only little information about the use of everolimus in lung transplantation. Strueber el al reported the favorable outcome regarding less biopsy-proven acute rejection and lower respiratory tract infection of lung transplant patients treated with everolimus, compared to lung transplant patients treated with mycophenolate mofetil. Another multicenter study couldn't show a significant difference regarding freedom of BOS in lung transplant patients treated with everolimus compared to patients treated with mycophenolate [20,21].

In our model of pulmonary transplantation across major histocompatibility barriers in miniature pigs, we could show before, that high frequencies of putative regulatory T cells are associated with prolonged allograft survival [22–25]. However, the impact of immunosuppressive agents on this cell population is controversly discussed [26].

This study was designed to evaluate the effect of a primary 28-day course of Cyclosporine A in combination with Everolimus as compared to Cyclosporine A and Azathioprine on allograft survival and putative Treg frequencies.

2. Methods

2.1. Experimental animals

22 animals aged between 12 and 15 months were selected from an outbred, specific-pathogen-free herd consisting of 9 different breeding lines (Ellegaard, Dalmose, Denmark). Prospective lymphocytotoxic assay tissue typing for donor/recipient mismatching allowed for blinded randomization of animals. They were mismatched for the swine leukocyte antigen (SLA) class I DC45, W12 or FJ13, W9 haplotypes [27,28] and for staining with the mAb 74-11-10 SLA I (haplotype d).

All animals received humane care in compliance with the German animal protection legislation, approved by the local Institutional Animal Care and Research Advisory Committee and permitted by the Animal Welfare Service of the Lower Saxony State office for Consumer Protection and Food Safety.

2.2. Surgical technique

The surgical technique of left-sided single lung transplantation in pigs has been described elsewhere [29]. Briefly, lungs were harvested from donor animals (18–25 kg) after Euro-Collins cold flush perfusion. A permanent vascular access double-lumen 3.2 Quinton atrial catheter was inserted into the right jugular vein of recipient animals (15–25 kg). After thoracotomy in the fourth intercostal space, the left lung was transplanted using standard techniques. After extubation, the animals were put in boxes provided with heating lamps, underfloor heating, and drinking water.

2.3. Experimental groups and immunosuppression

Animals were assigned to the following treatment groups:

CsA group (n = 5): Cyclosporine A (Novartis Pharmaceuticals Co., Basel, Switzerland), adjusted to blood trough levels of 300–500 ng/ml, 1.5 mg/kg/d methylprednisolone (Urbason, Sanofi-Aventis, Höchst, Germany), 1.0 mg/kg/d azathioprine (Imurek, Aspen Germany GmbH, München, Germany). 2) Ev + CsA group (n = 6): CsA adjusted to blood trough levels of 200–300 ng/ml, everolimus (Novartis Pharma GmBH, Nürnberg, Germany) adjusted to blood trough levels of 5–10 ng/ml, 1.5 mg/kg/d methylprednisolone. Animals are summarized in Table 1.

Cyclosporine A and everolimus trough levels were monitored using standard radioimmunoassays. Empiric intravenous antibiotic therapy consisted of 2 mg/kg/d ciprofloxacin (Bayer, Leverkusen, Germany). All immunosuppressive medication and antibiotics were withdrawn on postoperative day (POD) 28 and the vascular access catheter was surgically removed.

2.4. Rejection monitoring

On POD 7, 28, 42, 56, 70, 84, and 98 and then in 4-weekly intervals, sequential chest radiographs were performed and a score from 0 (no pathological findings) to 4 (homogeneous infiltration of the left lung, normal right lung) was assigned. A full autopsy was performed on animals with radiologic grade 3 or 4 rejection; lung biopsy sections were stained with hematoxylin and eosin and reviewed by a blinded pathologist. Histological rejection was graded according to International Society for Heart and Lung Transplantation (ISHLT) guidelines [30].