Early Administration of FTY720 Prevents Chronic Airway as Well as Vascular Destruction in Experimental Rat Lung Transplantation

S.W. Hirt, M. von Suesskind-Schwendi, T. Puehler, C. Schmid, and K. Lehle

ABSTRACT

Background. Chronic rejection (CR) in terms of bronchiolitis obliterans (BO) and vascular sclerosis (VS) still represents the major obstacle for pulmonary graft survival in the medium and long term course after lung transplantation (LTX). Aside from nonspecific stimuli, early acute rejection (AR) seems to be causative especially in cases of a late diagnosis or inadequate treatment. This study investigated the effects of FTY720, a new immunosuppressant that promotes lymphocyte sequestration into lymph nodes and Peyer’s patches, on the development of CR after experimental LTX.

Methods. A total of 50 rats underwent allogenic (F344-to-WKY) and syngenic (WKY-to-WKY) left LTX. Group 1 animals had no treatment. Group 2 animals were administered FTY720 (3 mg/kg body weight per day) at the maximum time of AR (day 14) and continued up to day 100 after LTX. Group 3 animals were treated with the same dosage of FTY720 from day 0 to 100. The grades of AR and CR were classified according to the criteria of the International Society for Heart and Lung Transplantation.

Results. Within 14 days after allogenic LTX, all nontreated rats developed early AR followed by severe CR with VS and BO. Similar data were observed for FTY720 treatment of existing AR (group 2). Only early administration of FTY720 (at the time of LTX) significantly reduced the proportion of animals with severe acute vascular rejection ($P < 0.001$). However, all of these allografts showed high-grade acute airway inflammation. After long-term application, the chronic inflammatory response was absent; none of the allografts developed BO and VS.

Conclusion. Only application of FTY720 immediately after LTX prevented lymphocyte recirculation and lung injury.
transplants were sacrificed at POD 100. Rat lungs were harvested on POD 20 (early phase) or 100 (late phase).\textsuperscript{3} Lung sections were stained with hematoxylin-eosin and Masson Goldner Trichrome to grade acute rejection (AR) and CR according to the classification of the International Society for Heart and Lung Transplantation (ISHLT): POD, postoperative day. Statistics included the difference in the proportion of animals with high-grade rejecting allografts (ISHLT-A 3.5–4; ISHLT-B2R) (vs group 1). n.s., non-significant.

\p{p}^*\textless.05.

<table>
<thead>
<tr>
<th>Group #</th>
<th>ISHLT-A</th>
<th>P Value</th>
<th>ISHLT-B</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9x A4</td>
<td></td>
<td>9x B2R</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A 3.5-4x A4</td>
<td>n.s.</td>
<td>5x B2R</td>
<td>n.s.</td>
</tr>
<tr>
<td>3</td>
<td>A 2.5; 2.5; 3; 3; 3; 3.5</td>
<td>*</td>
<td>B 1R; 1R-2R; 2R; 2R; 2R</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

ISHLT-grade, classification of acute vascular (ISHLT-A) and airway rejection (ISHLT-B) according to the actual working formulation of the International Society of Heart and Lung Transplantation (ISHLT); POD, postoperative day. The definition of BO included both histological variants “constructive bronchiolitis” and “organizing pneumonia.”\textsuperscript{5}

Histological scoring was performed three times in blinded fashion. We tested the hypothesis of a difference among populations as the proportion of animals with high-grade rejected allografts

Fig 1. Representative histopathology of allografts from postoperative day (POD) 20 (a–c) and POD 100 (d–f) (magnification, 50x). Inserts (magnification, 400x) presented details from a representative terminal bronchiolus wall. Allografts from group 1 (a) and group 2 (b) were diagnosed with International Society of Heart and Lung Transplantation (ISHLT)-A4/B2R and presented first signs of perivascular and fibrointimal fibrosis. (a) Destruction of the smooth muscle cell layer and a subepithelial infiltration of mononuclear cells. (b) Infiltrated fibrocytes in the airway lumen. Allografts from group 3 (c) show ISHLT-A3/B1R-B2R without chronic alterations. The insert demonstrates peribronchial mononuclear cell infiltration. On POD 100, group 1 (d) and group 2 (e) commonly demonstrate bronchiolitis obliterans (BO)-like lesions in form of “constrictive bronchiolitis” (as described by Jonigk et al\textsuperscript{5}). In addition, vasculopathy is also common in these allografts. Allografts from group 3 (f) were free of BO and chronic vascular rejection. tB, terminal bronchiolus; V, vessel.
(ISHLT-A3.5-4; ISHLT-B2R; C1; D1) within study groups. 95% confidence intervals for the difference between group proportions were used to approximate statistical significance ($P < .05$).

RESULTS

FTY720 Did Not Affect the State of Health

Allogeneic LTX resulted in early weight loss (7%–13% compared to initial weight; $P < .001$), which was regained within 7 (4/11) days (groups 1 and 2). Only early treatment (group 3) retarded weight gain (15 [12/18] days; $P = .009$). On POD 100, median weight from animals in groups 1 (379 [375/391] g) and 2 (397 [390/413] g) were comparable ($P = .095$), while group 3 rats were significantly smaller (334 [307/342] g; $P = .002$). However, the general state of health was not affected.

FTY720 Reduced the Extent of Acute Vascular Rejection

On POD 20, allografts from groups 1 and 2 showed severe acute vascular rejection and high-grade lymphocytic bronchiolitis (Table 1, Fig 1a, 1b). Only early application of FTY720 (group 3) attenuated the extent of AR. Only 1/6 allografts developed high-grade AR (ISHLT-A3.5/B2R). The remaining allografts showed ISHLT-B2R. However, the proportion of animals with severe acute vascular rejection was significantly reduced (Table 1). Nevertheless, these allografts were diagnosed with moderate acute vascular rejection (Fig 1c). At the same time, most allografts from groups 1 and 2 developed vasculopathy and first signs of chronic bronchiolar alteration (Fig 2). Two-thirds of the allografts showed initial signs of intraluminal polyps of granulation tissue in more than one terminal bronchiol or loose subepithelial fibrin structures around terminal bronchiol. Only a single allograft showed BO. The development of these early chronic alterations was only inhibited in group 3 (Fig 2). Syngeneic grafts and nontransplanted right lungs demonstrated no conspicuous changes.

Early Administration of FTY720 Prevented CR in the Long-Term Follow-up (POD 100)

Allografts from groups 1 and 2 developed pronounced chronic airway rejection with BO (C1) and VS (D1) (Fig 1d, 1e, 2). Sixty percent of these lungs were completely scarred. Early application of FTY720 prevented BO and VS (Fig 2). There was no airway inflammation. Only minimal to mild acute vascular rejection persisted.

DISCUSSION

Our data clearly showed FTY720 monotherapy to delay BO and VS after rat LTX when the treatment started immediately posttransplantation. In contrast, allografts with severe AR and high-grade lymphocytic bronchiolitis at the time of drug initiation did not benefit from FTY720 treatment. Despite the critical discussion about the validity of the F344-to-WKY rat LTX model to verify the development of CR after LTX,6 we and others have presented histological evidence of BO-like lesions in this model.5,7,8

In the present study, early treatment of FTY720 at the time of LTX reduced lymphocytic infiltration in the early phase after LTX that mitigated inflammation over long-term application. Perioperative administration of FTY720 in another LTX model also resulted in a reduction in lymphocyte function but the lung allografts did not achieve indefinite survival. Transfection of adenoviral vectors containing the CTLA4Ig gene (AdCTLA4Ig) in combination with FTY720 increased graft survival.9 Various mechanisms could improve the effectiveness of gene therapy to prolong graft survival.10 In contrast to the lung, early application of FTY720 after kidney transplantation not only reduced lymphocyte function but also prolonged graft survival.10
The underlying mechanism of FTY720 treatment could be induction of lymphocyte apoptosis,11 sequestration of circulating lymphocytes, or inhibition of T-cell function.12 Suppression of acute allograft rejection and resolution of chronic inflammation after early application of FTY720 might have been responsible for freedom from BO and VS in our rat LTX model.

Comparable data have been shown by Delbridge et al.13 Early treatment with FTY720 in a rat ischemia-reperfusion model after kidney transplantation reduced renal fibrosis. In contrast, treatment of allografts with existing AR has only been successful in a rat heterotopic cardiac transplantation model:14 Oral administration of FTY720 to treat existing AR prolonged the mean survival time,14 as also observed after subcutaneous injection of FTY720 after canine renal transplantation.15 Despite these promising experimental data in the treatment of CR after organ transplantation the drug failed in a clinical study.16 Initial efficacy findings in a renal transplant program were encouraging; however, later phase III development, did not show a therapeutic advantage of FTY720 over currently available therapies.16

Early administration of FTY720 after allogeneic LTX may be a promising additional therapeutic tool to prevent chronic allograft rejection.

REFERENCES