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Differential Effects of FTY720, RAD, and CsA on Signs of Chronic Rejection in the Rat Tracheal Allograft Model

Gisbert Weckbecker, Valerie Caballero, Mireille Court, Catherine Cannet, and Paul-Georg Germann

Obliterative bronchiolitis (OB) is an unresolved issue in lung transplantation. The antirejection properties of FTY720, RAD, and cyclosporin A (CsA) were compared in the Brown-Norway to Lewis rat tracheal allograft model of OB. Using daily oral doses of 0.3, 2.5, and 7.5 mg/kg for FTY720, RAD, and CsA, respectively, the inhibition of tracheal graft rejection was studied by giving the drugs from day –3 prior to transplantation to day 3, –3 to 28, 0 to 28, 3 to 28, or 10 to 28. Grafts were removed on day 28 and processed for quantitative histology. The inhibitory effect of FTY720 on tracheal stenosis was most pronounced with the regimen day –3 to day 28 (obliteration 6.3% ± 4.1% vs 94.1% ± 3.4% in controls, mean ± SE). FTY720 (days –3 to day 28) completely prevented inflammatory cell infiltration of the graft and markedly (> 70%) decreased blood lymphocyte counts. RAD exerted strong to intermediate inhibitory action on the development of tracheal obliteration with all the treatment regimens tested (obliteration ranging from 3.2% ± 0.7% for days 0 to 28 to 57% ± 13% for days –3 to 3). CsA treatment was efficacious with the regimens –3 to 28 and 0 to 28. In conclusion, single agent regimens of the new immunosuppressants FTY720 and RAD have been identified that can prevent or inhibit signs of chronic rejection in the rat tracheal transplantation model.

Introduction

Obliterative bronchiolitis (OB) is the most common pulmonary complication among lung transplant recipients. OB involves injury of the bronchiolar epithelium, submucosal inflammatory cell infiltration, and growth of granulation tissue that eventually occludes the entire airway lumen.1 The absence of effective therapeutic interventions for OB has led to a search for new treatments. Here we evaluated the schedule-dependence of the efficacy of the new immunosuppressants FTY720 and RAD in comparison with cyclosporin A (CsA) (Neoral®) in the Brown-Norway (BN) to Lewis tracheal transplantation model. This allotransplantation model is widely used to study certain aspects of chronic lung rejection because its histological signs of chronic rejection are similar to those of human OB, apparently a result of an alloimmune response to the graft.2

The new immunosuppressants FTY720 and RAD differ in their mode of action. FTY720 strongly affects the migration of lymphocytes and inhibits the rejection of solid organ grafts without compromising the antiinfectious state of the immune system.3-5 RAD (everolimus) is a macrolide immunosuppressant with potent antiproliferative properties currently being evaluated in Phase III clinical transplantation studies.6,7 Recent preclinical investigations have focused on therapeutic effects of RAD in experimental lung transplantation.8

Materials and Methods

Transplantation Procedure and Graft Assessment

The tracheal allotransplantation from BN to Lewis rats was performed as described by Yonan and others.1 The key step involves transplantation...
TRACHEAL GRAFT OBLITERATION
Loss of tracheal lumen.

OBLITERATIVE BRONCHITIS
A pulmonary complication involving injury of the bronchial epithelium, submucosal inflammatory cell infiltration, and growth of granulation tissue that eventually occludes the entire airway lumen.

TRACHEAL GRAFT OBLITERATION
Loss of tracheal lumen.

of a BN trachea segment (12 rings) into the omental site of the recipient. The rats were allocated to the following treatment groups (4-10 rats per group):

• treatment daily from day –3 to day 3;
• treatment daily from day –3 to day 28;
• treatment daily from day 0 to day 28;
• treatment daily from day 3 to day 28;
• treatment daily from day 10 to day 28.

For the group day –3 to day 3, the treatment was stopped on day 3, and the animals were sacrificed on day 28. All drugs were administered daily by gavage. FTY720 was given at a dose of 0.3 mg/kg, Neoral® (CsA) at 7.5 mg/kg and RAD at 2.5 mg/kg. On day 28, tracheal graft segments were excised and processed for quantitative histology (blind evaluation).

Results

Time Dependency of the Antirejection Properties

The antirejection properties of FTY720, RAD, and CsA were compared in the BN to Lewis tracheal allograft model. As shown in Figure 1, the effect of FTY720 on the development of tracheal graft stenosis was most pronounced (obliteration 6.3% ± 4.1%) with the regimen day –3 to day 28. However, when the treatment started 1 h before transplantation (day 0 to day 28 regimen), the therapeutic efficacy was markedly decreased. CsA treatment was efficacious with the day –3 to day 28 regimen as well as the day 0 to day 28 regimen. RAD showed the least schedule dependence in that it exerted strong to intermediate inhibitory action on the development of tracheal obliteration with all the treatment regimens tested (Fig 1). In control animals treated with the drug vehicle daily, there was an almost complete loss of the airway lumen (94.1 ± 3.4, mean ± SE, see solid line in Fig 1).

Damage of airway epithelium and infiltration of inflammatory cells are further hallmarks of tracheal graft rejection. The best overall graft protection, that is, inhibition of tracheal graft obliteration in combination with a largely preserved epithelium (normal or cuboidal epithelial cells present in 3 of 4 grafts), and the complete absence of graft infiltration, was achieved with the FTY720 regimen day –3 to day 28. The FTY regimen 0 to 28 resulted in partial epithelial protection, whereas the other FTY regimens failed to protect the epithelium. CsA regimens –3 to 28, 0 to 28, and 3 to 28 also largely preserved the epithelial lining with cuboidal or normal epithelial cells. RAD treatment was associated with loss of epithelium (regimens –3 to 3, –3 to 28, 10 to 28) or the formation of cuboidal epithelial cells (regimens 3 to 28 and 0 to 28). CsA and RAD apparently did not affect the degree of lymphocyte infiltration of the graft.

Hematological Monitoring

Figure 2 shows the rapid and reversible decrease in blood lymphocyte counts under treatment with FTY720 as exemplified by the regimen day –3 to day 3. When given as long-term treatment, FTY720 induced a long-lasting drop in blood lymphocyte counts without signs of escape during treatment as exemplified by the day 0 to day 28 regimen (Fig 3a). RAD led to an increase in blood neutrophil counts (Fig 3b).

Discussion

Current immunosuppressive treatments for lung transplant patients are less than optimal, since half of all single-lung, double-lung, or heart-lung transplants develop OB. It is therefore important to evaluate new drugs such as FTY720 and RAD for their potential utility in the prevention or treatment of OB. In the current study, FTY720 and RAD, in comparison with CsA, were tested in the rat tracheal allograft model using various regimens. The perioperative treatment covers the critical period of the initial graft-host contact, whereas the delayed treatment may be relevant for assessing the therapeutic, rather than the preventive, potential of these drugs.

In line with studies by Yonan and others and King and others, and various CsA regimens potentially inhibited tracheal stenosis (Fig 1). However, delaying CsA treatment by 10 days relative to transplantation abrogated its inhibitory effect. At this time point, obliteration in untreated controls amounts to 8.1 ± 2.3 (mean ± SE, n = 5 rats; data not shown). When FTY720 was given from day –3 to day 28, a very potent inhibition of tracheal stenosis (as well as of other chronic rejection signs,
Figure 1. Effect of various treatment regimens of FTY720, cyclosporin A (CsA) (Neoral®), and RAD on the development of tracheal obliteration in the Brown-Norway to Lewis rat tracheal allograft model. The degree of obliteration in the control group (indicated with the black line; 3 rats each treated with vehicle RAD or vehicle CsA from days –3 to 28) amounted to 94.1% ± 3.4% (SE). Bars represent means ± SE, n = 4 to 10.

Figure 2. Effect of oral administration of FTY720 from day –3 to day 3 relative to transplantation on circulating blood cells. Treatment started on day –3, that is, shortly after blood was sampled for measuring baseline cell counts (mean of 5 rats ± SE).
such as infiltration and epithelial damage) was obtained, indicating a preventive potential of FTY720. FTY720 led to a rapid and persistent lowering of peripheral lymphocyte counts (Fig 2 and Fig 3), which is in line with the suppression of inflammatory cell infiltration. This observation points to the potential use of lymphocyte counts as a pharmacodynamic readout for FTY720. The single agent activities of these compounds is the basis for future studies on the utility of drug combinations. The combination treatment with CsA/FTY720 and CsA/RAD can exert synergistic antirejection effects in transplantation models.

REFERENCES


Figure 3. (A) Effect of oral administration of FTY720, Neoral®, or RAD from day 0 to day 28 (start 1 h prior to transplantation) on circulating lymphocytes (mean of 5 rats ± SE). (B) Effect of these treatments on neutrophil counts.