Successful Treatment of Pulmonary Graft versus Host Disease with Aerosolized Cyclosporine

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Abstract
We report the successful use of aerosolized cyclosporine (CSA) therapy in two children who developed bronchiolitis obliterans (BOS) following a haploidentical peripheral blood stem cell transplant for refractory biphenotypic leukemia and HLA-matched sibling bone marrow transplant for relapsed acute lymphoblastic leukemia respectively. CSA was non-toxic and was associated with significant improvement and long-term stabilization in pulmonary function. Patients received CSA for 6 and 18 months, respectively which allowed both patients to be liberated from additional immunosuppression. CSA, appeared to be a safe, effective, and steroid-sparing therapy for chronic BOS following allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Keywords
Graft versus host disease, Cyclosporine, Inhaled, Aerosolized, Bronchiolitis obliterans syndrome

Introduction
Bronchiolitis obliterans syndrome (BOS) is a noninfectious small airway disorder that elicits obstructive pulmonary physiology as a consequence of graft versus host disease (GVHD), following allogeneic hematopoietic stem cell transplantation (allo-HSCT) or lung transplantation. After allogeneic bone marrow transplantation 14% of the patients develop the disease with 65% mortality within 3 years. [1] Therapeutic trials with augmented immune suppression have shown limited benefit. Allo-immunologic airway epithelial and endothelial cells injury mediate BOS when they respond to bone marrow derived mononuclear cells, as well as natural killer and dendritic cells.[2] Eventually, in allo-immunologic injury stimulated myofibroblasts secrete proteoglycans and collagen fibrils that create a permanent matrix during the failed repair process.[3]
In BOS drug delivery of conventional systemic immunosuppressive drugs to target airway cells may be limited.[4,5] Aerosolized/inhaled cyclosporin (CSA) had been used in acute and chronic lung transplant rejection refractory to conventional therapy.[6,7,8,9] Herein, we describe the use of CSA in two pediatric patients with BOS refractory to aggressive systemic immunosuppression. In both cases CSA was administered for 6 and 18 months, respectively, was well tolerated, with no apparent toxicity, and resulted in significant clinical improvement. Our results support the use of CSA as a potential adjunct treatment for pulmonary manifestations of GVHD.

Case Series

Case 1

A 6 year old Caucasian female with refractory biphenotypic leukemia received haploidentical stem cell transplant after achieving remission with a reinduction course of high-dose cytosine arabinoside and mitoxantrone. Preparative regimen consisted of total body irradiation 1200 cGy, thiotepa, anti-thymocyte globulin and cyclophosphamide. CD34+ cells were collected using the CliniMACS® protocol (IDE 8544) which passively depletes T cells for GVHD prophylaxis. 56 x 10^6 CD34+ cells were infused in the graft that had < 2 x 10^6 CD4+ cells. No additional GVHD prophylaxis was given.

Eleven days post-transplant rash and diarrhea led to skin and gastrointestinal biopsies consistent with acute GVHD. The rash improved following prednisone (2 mg/kg/day). However, gastrointestinal GVHD resolved only after combinations of Infliximab (5 mg/kg IV weekly) Daclizumab (1 mg/kg/dose days, total of 5 doses) and tacrolimus 0.5 mg orally twice/day (~ trough level 5-15 ng/ml). During year one after transplant she continued to have signs of skin GVHD managed with topical corticosteroids. Persistent non-productive cough, shortness of breath and diffuse wheeze were attributed to chronic lung GVHD. Chest CT showed bilateral patchy glass infiltrate, peribronchial thickening. Bronchoscopy revealed moderate mucoid secretions and bronchoalveolar lavage (BAL) noted 28% neutrophils, 66% macrophages, 5%, lymphocytes and 1% eosinophils. Cultures, smears and PCRs were negative for bacteria, virus, and mycobacterium. Despite monthly methylprednisolone (5mg/kg) lung function failed to stabilize and the patient developed compression fracture of L2. During continued treatment with Fluticasone (110-220 mcg per puff, four puffs three times a day), prednisone (10 mg orally three times a day), Mycophenolate mofetil (200 mg twice daily), and tacrolimus (1.5 mg twice daily) there was improvement in pulmonary function tests (PFTs, table 1). Eventually two and a half years following transplant respiratory failure necessitated ICU hospitalization and endotracheal intubation. Repeat BAL.
was negative for an infectious etiology. A steroid sparing therapy was sought due to significant steroid side-effects (growth delay, vertebral compression fracture, and myopathy). CSAa was begun (initial dose of 35 mg, followed by 50 mg on day 2 escalated to 75 mg once daily). 1 month after initial therapy PFTs showed significant improvement permitting corticosteroids to be discontinued.

CSAa was discontinued after 6 months with no observed side effects. Random blood CSA levels were undetectable. Currently 6 years after allogenic BMT PFTs have remained near normal, without evidence of pulmonary GVHD.

Case 2

A 12 year old boy with Down's syndrome underwent an HLA-matched allogeneic bone marrow transplant for relapsed B cell precursor acute lymphoblastic leukemia. Preparative regimen consisted of Busulfan and cyclophosphamide. GVHD prophylaxis consisted of tacrolimus and methotrexate. Acute GVHD of the skin (grade III) resolved with addition of prednisone (2 mg/kg/day) and all immune suppression was weaned off and discontinued 7 months following his bone marrow transplant.

Eighteen months later chronic GVHD of the skin (grade III) recurred. New respiratory symptoms of GVHD were treated with antibiotics and oral prednisone 2 mg/kg/day in his home country. Evaluation in U.S. revealed mild respiratory distress, with bilateral wheezes and crackles, and oxygen saturation at room air of 91%. PFTs could not be performed due to patient’s developmental delay. Chest CT showed diffuse patchy pulmonary infiltrates with peribronchial thickening (Fig 1A). Bronchoscopy revealed normal inspection of the airways. BAL cultures, smears and PCRs were negative for bacteria, virus, and mycobacterium.

Respiratory symptoms persisted despite tacrolimus and prednisone (1 mg/kg/day). Because of complications associated with prolonged steroid therapy (severe myopathy, avascular necrosis hip, osteoporosis) a steroid sparing regimen was explored. CSAa was begun as in case #1 using 75 mg once daily x 1 month, then three times weekly. Clinical symptoms improved within 2-3 weeks. Chest CTs showed improvement at 9 months (Fig 1B). Systemic corticosteroid was weaned over a period of 6 months while using CSAa for a total of 18 months. Chronic GVHD of the skin was treated with psoralen ultraviolet light therapy (PUVA). Presently, patient is 3 years post-allogeneic BMT, off immunosuppressants and without clinical or radiographic signs of active pulmonary GVHD.
CSA Preparation and Administration

25 mg of CSA powder (Gallipot Inc, St Paul, MN) and 1ml of propylene glycol (Ruger Chemical, Irvington, NJ) were mixed and allowed to dissolve with constant stirring. The solution was filtered (0.2 micron sterile filter, Millipore Sterivex filter SVGS01015). Filtration was facilitated by placing the solution into a 60 ml syringe mounted in a caulking gun and applying a steady pressure into pre-sterilized glass vials.

Aerosol generation used 2 separate Pari LC Plus nebulizer sets (Pari Respiratory Company, Richmond, VA. Part no. 22 F81 with tubing and part no. 2280 without tubing). A standard portable air compressor was used to generate the aerosol. Separate nebulization set-up kits are required for the nebulized lidocaine and the CSAa. Patients used a mouth piece. To decrease waste of the medication a compressor interrupter valve was attached between the nebulizer cup and compressor tubing. The target dose for our patients was 75 mg CSA. This dose was one-quarter of the target dose used by Iacono et al. in adults with lung transplantation.[9] We chose a lesser target dose due to reduced body size of children and concern for tolerance. In addition, the use of an interrupter valve during CSA delivery increases the dose inhaled by the patient and reduces the dose wasted to the environment. As clinical improvement was observed the frequency of administration was reduced to three times weekly, and, or CSA was discontinued.

To improve tolerance to CSA, 30 minutes to 1 hour before CSA, 2 cc of lidocaine 4% (Roxane labs, topical solution) is placed in the nebulizer cup. CSA was instructed to be taken with regular deep inhalations/exhalations. CSA treatments took about 45 minutes.

Discussion

The incidence of BOS in pediatric allo-HSCT patients is reported between 4.5% and 8.3% which is a similar incidence as reported in adult patients.[10] The diagnosis of BOS is based on pulmonary function abnormalities, radiographic findings or lung biopsy in the setting of symptoms of cough, dyspnea with or without exertion and/or wheezing. BOS is considered an airway specific lung injury. Accordingly, airflow obstruction on PFTs is a mainstay of diagnosis and PFTs routinely performed on all patients who are developmentally able to complete the studies. Patients without a pathologic diagnosis are classified as having BOS rather than bronchiolitis obliterans to acknowledge the uncertainty of any degree of irreversible airway fibrosis. In all cases lung injury from drugs or transfusion, and infectious diseases, particularly viral pulmonary illnesses, are included in the differential diagnosis and must be
excluded. However, any lung injury per se may perpetuate BOS as airway injury enhances epithelial HLA class II expression and hence may elicit GVHD-related BOS.[11]

The use of CSA in BOS after allo-HSCT has not previously been reported. Based on preclinical and clinical studies of inhaled cyclosporine, we hypothesized that CSA could achieve higher CSA concentrations in the lung with prolonged pulmonary retention compared to systemic administration and without the systemic toxicity.[6,7,8,12,13,14,15]. The time to effect of CSA in our patients was within 2 weeks in both cases. No other treatments were administered during this time to account for a reduction in BOS symptoms. To date studies of CSA in lung transplantation have used rate of histologic rejection as a measure of efficacy rather than reporting magnitude of benefit on PFTs.[9]

In lung transplant recipients CSA has proven efficacy in the treatment of acute and chronic lung rejection.[6,7,8,9] CSA results in significant improvement in histologic lung transplant rejection.[8] Iacono reported improved survival and extended chronic rejection-free survival in lung transplant patients with refractory acute and chronic rejection.[9] Pulmonary function improved compared to pretreatment measurement and those who responded showed significant decreases in BAL IL-6 expression and interferon-gamma messenger RNA levels.

Our first patient treated with CSA, had prolonged and refractory lung disease despite multiple immunosuppressive regimens. Though nonpulmonary GVHD (skin and gut) improved with an intensive systemic immunosuppressive regime, presumed lung GVHD persisted and the patient had symptomatic osteoporosis while lung function declined. The second patient developed clinical signs of BOS, though we could not measure pulmonary function tests due to patient’s age and developmental delay. His clinical improvement while using CSA correlated with improved lung imaging studies. Though our patients did not undergo open lung biopsy the clinical features of airflow limitation without infectious etiology are consistent with BOS.[16]

Hear in, we report clinical improvement in clinical lung GVHD in two allo-HSCT recipients following initiation of CSA. In both of our cases CSA was considered as salvage therapy for patients who had complications and failed to improve on conventional therapy for BOS. PFTs significantly improved in the patient tested and both clinical and radiographic signs of BOS dramatically resolved in both patients. Though the long term effects of CSA, are unknown use up to several months appears to be a safe, effective and steroid-sparing for chronic lung GVHD. Recently photopheresis has emerged as a potential alternative to stabilize declining PFT’s for BOS in allo-HCST.[17] However, photopheresis is costly, time-
consuming, and not readily available. Thus, we suggest that a controlled trial with CSA be considered for the at risk BOS allo-HSCT population.

**Table**

**Table 1:** Patient 1 Pulmonary Function Tests: Before and after CSAa

<table>
<thead>
<tr>
<th>Time before/after CSA</th>
<th>FVC %</th>
<th>FEV1</th>
<th>FEV 25-75</th>
<th>Prednisone (mg/d)</th>
<th>Tacrolimus (mg/d)</th>
<th>Mycophenolate mofetil (mg BID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5 month</td>
<td>57</td>
<td>54</td>
<td>34</td>
<td>30</td>
<td>3.0</td>
<td>400</td>
</tr>
<tr>
<td>CSA begun</td>
<td>48</td>
<td>48</td>
<td>20</td>
<td>10</td>
<td>1.5</td>
<td>400</td>
</tr>
<tr>
<td>+ 1 month</td>
<td>63</td>
<td>61</td>
<td>26</td>
<td>5</td>
<td>1.5</td>
<td>400</td>
</tr>
<tr>
<td>+ 2 month</td>
<td>84</td>
<td>62</td>
<td>30</td>
<td>2.5</td>
<td>0.75</td>
<td>320</td>
</tr>
<tr>
<td>+ 4 month</td>
<td>94</td>
<td>88</td>
<td>46</td>
<td>0.5</td>
<td>1.5</td>
<td>320</td>
</tr>
<tr>
<td>+ 2.7 yr</td>
<td>87</td>
<td>83</td>
<td>62</td>
<td>none</td>
<td>1.5</td>
<td>320</td>
</tr>
</tbody>
</table>

Values % predicted VC = Vital Capacity, FVC = forced vital capacity, FEV1 = forced expiratory volume in 1 second, FEV25-75 = forced expiratory flow 25-75% VC.

**Figure**

**Figure 1.** (A) Multiple multifocal areas of ground glass scattered throughout both lungs. There is mild to moderate peribronchial thickening. No evidence of bronchiectasis. (B) 9 months later pulmonary infiltrates are less confluent in the perihilar region than on the previous study (A). Notice less subcutaneous fat likely due to corticosteroid liberation.
References


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