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_a) 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_a was non-toxic and was associated with significant improvement and long-term stabilization in pulmonary function. Patients received CSA_a for 6 and 18 months, respectively which allowed both patients to be liberated from additional immunosuppression. CSA_a 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 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_a) 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_a in two pediatric patients with BOS refractory to aggressive systemic immunosuppression. In both cases CSA_a 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_a 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 clls for GVHD prophylaxis. 56×10^6 CD34+ cells were infused in the graft that had $< 2 \times 10^6$ 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 ($\sim$ 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. CSA_a was begun as in case #1 using 75 mg once daily x 1month, 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 CSA_a 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_a. 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_a 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_a was discontinued.

To improve tolerance to CSA_a 30 minutes to 1 hour before CSA_a, 2 cc of lidocaine 4% (Roxane labs, topical solution) is placed in the nebulizer cup. CSA_a was instructed to be taken with regular deep inhalations/exhalations. CSA_a 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_a in BOS after allo-HSCT has not previously been reported. Based on preclinical and clinical studies of inhaled cyclosporine, we hypothesized that CSA_a 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_a 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_a 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_a has proven efficacy in the treatment of acute and chronic lung rejection.[6,7,8,9] CSA_a 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_a, 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_a 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_a. In both of our cases CSA_a 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_a are unknown use up to several months appears to be a safe, effective and steroid-sparing for chronic lung GVHD. Recently photophoresis has emerged as a potential alternative to stabilize declining PFT's for BOS in allo-HCST.[17] However, photophoresis is costly, time-

consuming, and not readily available. Thus, we suggest that a controlled trial with CSA$_a$ be considered for the at risk BOS allo-HSCT population.

Table

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

Time before/after CSA$_a$e	FVC	FEV$_1$	FEV$_{25-75}$	Prednisone (mg/d)	Tacrolimus (mg/d)	Mycophenolate mofetil (mg BID)
-5 month	57	54	34	30	3.0	400
CSA$_a$ begun	48	48	20	10	1.5	400
+ 1 month	63	61	26	5	1.5	400
+ 2 month	84	62	30	2.5	0.75	320
+ 4 month	94	88	46	0.5	1.5	320
+ 2.7 yr	87	83	62	none	1.5	320

Values % predicted VC = Vital Capacity, FVC = forced vital capacity, FEV$_1$ = forced expiratory volume in 1 second, FEF$_{25-75}$ = forced expiratory flow 25-75% VC.
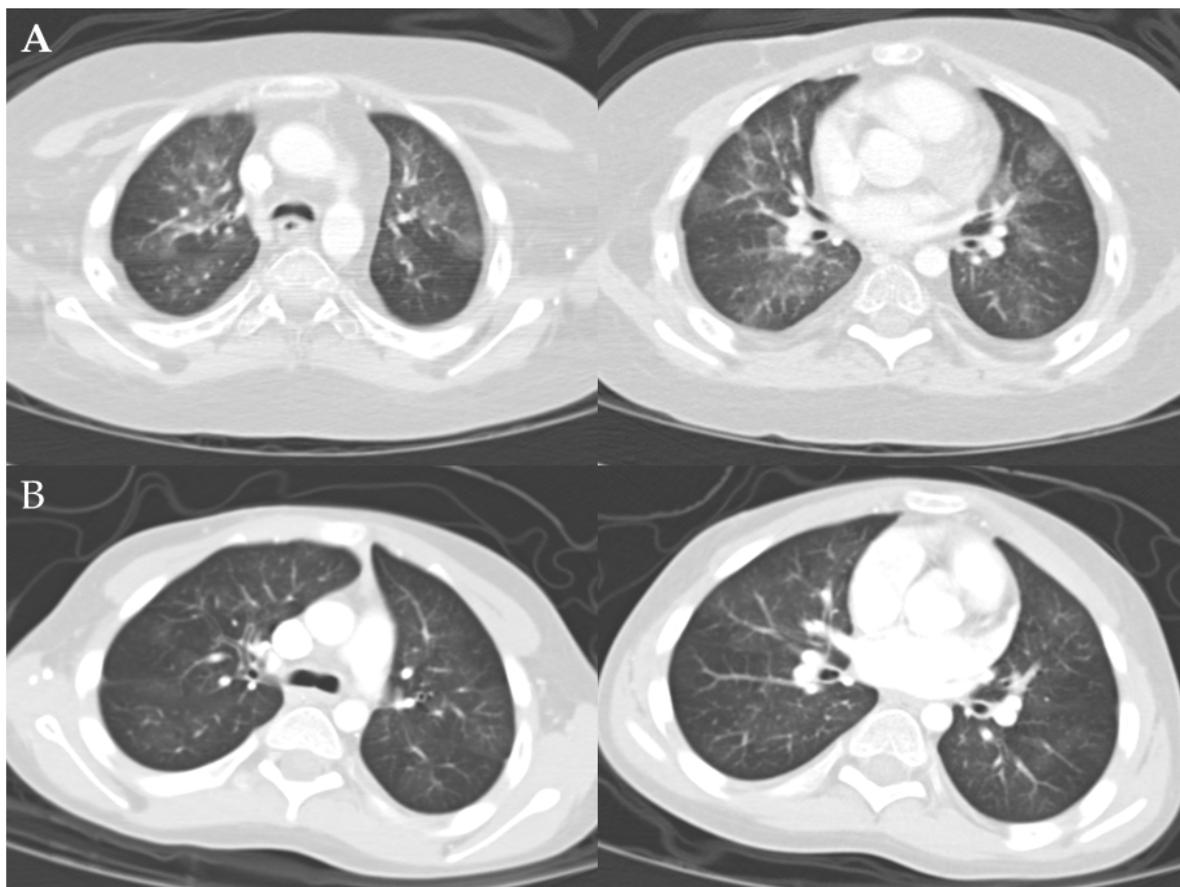
Figure

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

1. Chien JW, Martin PJ, Gooley TA, Flowers ME, Heckbert SR, Nichols WG, et al. Airflow obstruction after myeloablative allogeneic hematopoietic stem cell transplantation. *American journal of respiratory and critical care medicine* 2003;**168**(2):208-14.

2. Cooke KR, Yanik G. Acute lung injury after allogeneic stem cell transplantation: is the lung a target of acute graft-versus-host disease? *Bone Marrow Transplantation* 2004;**34**(9):753-65.

3. Markopoulo KD, Cool CD, Elliot TL, Lync DA, Newell JD, Jr., Hale VA, et al. Obliterative bronchiolitis: varying presentations and clinicopathological correlation. *European Respiratory Journal* 2002;**19**(1):20-30.

4. Corcoran TE. Inhaled delivery of aerosolized cyclosporine. *Advanced Drug Delivery Reviews* 2006;**58**(9-10):1119-27.

5. O'Riordan TG, Iacono A, Keenan RJ, Duncan SR, Burckart GJ, Griffith BP, et al. Delivery and distribution of aerosolized cyclosporine in lung allograft recipients. *American Journal of Respiratory & Critical Care Medicine* 1995;**151**(2 Pt 1):516-21.

6. Keenan RJ, Zeevi A, Iacono AT, Spichty KJ, Cai JZ, Yousem SA, et al. Efficacy of inhaled cyclosporine in lung transplant recipients with refractory rejection: correlation of intragraft cytokine gene expression with pulmonary function and histologic characteristics. *Surgery* 1995;**118**(2):385-91.

7. Iacono AT, Keenan RJ, Duncan SR, Smaldone GC, Dauber JH, Paradis IL, et al. Aerosolized cyclosporine in lung recipients with refractory chronic rejection. *American Journal of Respiratory & Critical Care Medicine* 1996;**153**(4 Pt 1):1451-5.

8. Keenan RJ, Iacono A, Dauber JH, Zeevi A, Yousem SA, Ohori NP, et al. Treatment of refractory acute allograft rejection with aerosolized cyclosporine in lung transplant recipients. *Journal of Thoracic & Cardiovascular Surgery* 1997;**113**(2):335-40; discussion 40-1.

9. Iacono AT, Johnson BA, Grgurich WF, Youssef JG, Corcoran TE, Seiler DA, et al. A randomized trial of inhaled cyclosporine in lung-transplant recipients.[see comment]. *New England Journal of Medicine* 2006;**354**(2):141-50.

10. Griese M, Rampf U, Hofmann D, Fuhrer M, Reinhardt D, Bender-Gotze C. Pulmonary complications after bone marrow transplantation in children: twenty-four years of experience in a single pediatric center. *Pediatric Pulmonology* 2000;**30**(5):393-401.

11. Hasegawa S, Ockner DM, Ritter JH, Patterson GA, Trulock EP, Cooper JD, et al. Expression of class II major histocompatibility complex antigens (HLA-DR) and lymphocyte subset immunotyping in chronic pulmonary transplant rejection. *Arch Pathol Lab Med* 1995;**119**(5):432-9.

12. Dowling RD, Zenati M, Burckart GJ, Yousem SA, Schaper M, Simmons RL, et al. Aerosolized cyclosporine as single-agent immunotherapy in canine lung allografts. *Surgery* 1990;**108**(2):198-204; discussion -5.

13. Keenan RJ, Duncan AJ, Yousem SA, Zenati M, Schaper M, Dowling RD, et al. Improved immunosuppression with aerosolized cyclosporine in experimental pulmonary transplantation. *Transplantation* 1992;**53**(1):20-5.

14. Letsou GV, Safi HJ, Reardon MJ, Ergenoglu M, Li Z, Klonaris CN, et al. Pharmacokinetics of liposomal aerosolized cyclosporine A for pulmonary immunosuppression. *Annals of Thoracic Surgery* 1999;**68**(6):2044-8.

15. Mitruka SN, Won A, McCurry KR, Zeevi A, McKaveney T, Venkataramanan R, et al. In the lung aerosol cyclosporine provides a regional concentration advantage over intramuscular cyclosporine. *The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation* 2000 Oct;**19**(10):969-75.

16. Hayes-Jordan A, Benaim E, Richardson S, Joglar J, Srivastava DK, Bowman L, et al. Open lung biopsy in pediatric bone marrow transplant patients. *Journal of Pediatric Surgery* 2002;**37**(3):446-52.

17. Sengsayadeth SM, Srivastava S, Jagasia M, Savani BN. Time to explore preventive and novel therapies for bronchiolitis obliterans syndrome after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2012;**18**(10):1479-87.

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