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Epoprostenol-Associated Pneumonitis: Diagnostic Use of a T Cell Proliferation Assay

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Abstract

We describe a case of severe drug-induced interstitial pneumonitis in a woman with idiopathic pulmonary arterial hypertension on epoprostenol confirmed by a drug T cell proliferation assay. Proliferation assays were carried out in our patient and a healthy control. Isolated T cells were incubated with CD3-depleted peripheral blood mononuclear cells and then stimulated to proliferate with ³H-thymidine in the presence of epoprostenol, other prostanoid analogs, and controls. A significant ($P < 0.001$) T cell proliferation response occurred in our patient in the presence of epoprostenol alone. There was a trend towards an increased T cell response to treprostinil but this was statistically insignificant ($P=NS$). There was no significant T cell response either to the diluent alone, normal saline, iloprost or alprostadil. There was no significant proliferation to any drug in the healthy control. Hence, a drug T cell proliferation assay confirmed that epoprostenol can rarely incite a profound inflammatory response in the pulmonary interstitium.

Introduction

Epoprostenol was the first prostacyclin analog shown to enhance exercise tolerance, improve hemodynamics, and augment survival¹ in patients with idiopathic pulmonary arterial hypertension (IPAH). Although usually well tolerated, epoprostenol may induce

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systemic hypotension, flushing, headaches, nausea and diarrhea. Also, recent data have linked reversible thrombocytopenia to epoprostenol use² and in patients with post-capillary pulmonary hypertension, epoprostenol may trigger flash pulmonary edema^{3,4}.

Drug hypersensitivity reactions to epoprostenol are extremely rare and unpredictable but, as with other instances of drug hypersensitivity, they can have serious consequences⁵. Here, we present the second case of epoprostenol-induced severe interstitial pneumonitis in a woman with IPAH. Our case highlights the novelty and utility of the drug T cell proliferation assay to identify patients at risk of developing this life-threatening complication.

Case Report

A 44-year-old female presented with a three-year history of progressive shortness of breath and recurrent episodes of lower extremity edema treated with intermittent diuretics. Six weeks prior to admission, her dyspnea became more pronounced. Her past medical history was significant for stage I systemic hypertension, asthma, hypothyroidism and no known drug allergies. She smoked 1/3 pack of cigarettes daily for 25 years and admitted to five episodes of methamphetamine use twenty years prior to presentation.

The patient was admitted to an outside hospital and underwent a transthoracic echocardiogram (TTE) which revealed severe pulmonary hypertension with an estimated right ventricular systolic pressure (RVSP) of 92 mmHg. At this time, the patient was given a diagnosis of right ventricular failure secondary to IPAH and was initiated on furosemide and oral sildenafil. In addition, the patient was started on 200 mg of amiodarone daily following three episodes of non-sustained ventricular tachycardia. Eventually, the patient was transferred to our center for further care.

On examination, the patient was hemodynamically stable but in mild respiratory distress with a systemic oxygen saturation of 100% on 15L oxygen via facial mask. Cardiac exam revealed a normal S1/S2, an III/VI holosystolic murmur at the left lower sternal border and a jugular venous pressure of 8 cmH₂O. Her lungs were clear to auscultation bilaterally and her extremities showed evidence of 2+ pitting edema to the knees bilaterally.

Her imaging studies and laboratory tests were unremarkable except for an NT-pro BNP of 896 pg/ml (normal <100 pg/ml). A right heart catheterization confirmed the diagnosis of pulmonary arterial hypertension, demonstrating a mean pulmonary artery pressure of 43 mm Hg, a pulmonary vascular resistance of 16.05 Wood units and an estimated cardiac output (CO) of 2.35 L/min. The patient was transferred to the cardiac intensive care unit (CICU) and started on intravenous (IV) epoprostenol within 24 hours. On hospital day 12, she was sent home on epoprostenol 10 ng/kg/min and her previous medications.

Two weeks later, she returned to our hospital with persistent malaise, fevers and productive cough. Upon readmission, she was toxic-appearing and febrile to 102.5 °F. Lung exam was significant for new diffuse bilateral wheezes with occasional crackles upon inspiration while her admission CXR revealed no new infiltrates. Her labs were remarkable for a WBC of 27.2 with 94.6% neutrophils and 20% bands post-antibiotics. Serial cultures were negative. In the setting of persistent high fevers, an escalating WBC and new hypoxia and hypotension, she was transferred to the CICU on hospital day 3. Her antibiotic coverage was broadened. By hospital day 5, her respiratory status deteriorated in conjunction with the development of diffuse bilateral fluffy infiltrates on CXR. A non-contrast chest CT demonstrated the presence of patchy, diffuse, bilateral ground-glass opacities with areas of consolidation most severely affecting the right upper lobe (Figure 1a).

On hospital day 6, she was intubated secondary to respiratory fatigue. A bedside bronchoscopy with right upper lobe bronchoalveolar lavage (BAL) was performed which revealed the following: 53% neutrophils, 35% lymphocytes, 6% mono/macrophages (no foamy macrophages), 6% atypical lymphocytes. Amiodarone was discontinued out of concern for potential amiodarone lung toxicity. After BAL smears and gram stain returned negative, a total of 1 g IV methylprednisolone was administered over 12 hours. This was followed by 250 mg bid IV methylprednisolone over three days. Subsequently, the patient's FiO₂ requirement fell to 70%, a follow-up CXR showed rapid clearance of her infiltrates and her WBC dropped to 25.5. The patient continued to improve and on hospital day 12, she was extubated to 6L NC and NO at 15 PPM. By hospital day 19, the patient was transitioned to oral prednisone at 75 mg daily and oxygen by nasal cannula alone.

While amiodarone-induced lung toxicity was initially thought to be the most likely diagnosis, the lack of foamy macrophages in the BAL along with the short exposure time and low maintenance dose used by the patient prompted us to review her medication list for other agents linked to drug-induced pneumonitis. Our review of the literature led us to a report which linked epoprostenol therapy to interstitial pneumonitis (IP) in patients with IPAH⁶. Given that initiation of epoprostenol in our patient correlated temporally with the development of pneumonitis, we sought to explore this possibility further. Due to the risk of acute right ventricular failure with epoprostenol cessation, we decided to solidify our diagnosis prior to stopping the medication by performing a drug T cell proliferation assay as described below.

Drug T cell proliferation assay

The patient's blood was collected while still on IV epoprostenol at a steady dose of 10 ng/kg/min (1 μ g assay dose) as well as prednisone 75 mg qd and proliferation assays were performed in the presence of IV epoprostenol, the epoprostenol diluent alone, three other prostacyclin therapies and normal saline. The three other prostacyclin therapies included: the longer-acting prostacyclin, IV treprostinil, at a comparable dose of 30 ng/kg/min (100 μ g assay dose), inhaled iloprost (1 μ g assay dose) and IV alprostadil (50 μ g assay dose), or prostaglandin E₂, which is currently approved for use only in the pediatric population in the setting of patent ductus arteriosus. Proliferation assays were carried out in both a healthy control (HC) and our patient (Pt). The HC had no history of asthma, allergy, or autoimmune disease and was on no medications. The assays were performed in round-bottom 96-well microtiter plates. 3.75×10^3 CD4⁺CD25^{neg} responder T cells, chemical compound as indicated (at 0.5 ng/ml final concentration), anti CD3 and anti CD28 (BD Biosciences) were added to test stimulation as a positive control⁷ to control wells and 3.75×10^4 allogeneic irradiated CD3-depleted peripheral blood mononuclear cells were added to all wells as antigen presenting cells. All wells were supplemented with anti-CD3 (clone HIT3a at 5.0 μ g/ml). T cells were cultured for 7 days at 37°C in RPMI 1640 medium (Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum. Sixteen hours before the end of the incubation, 1.0 μ Ci of ³H-thymidine was added to each well. Plates were harvested using a Tomtec (Hamden, CT, USA) cell harvester and ³H-thymidine incorporation determined using a PerkinElmer (Waltham, MA, USA) scintillation counter. Antigen-presenting cells consisted of peripheral blood mononuclear cells depleted of T cells using StemSep human CD3+ T cell depletion (STEMCELL Technologies, Vancouver, BC, Canada) followed by 40 Gy of irradiation. Statistical analysis was performed using GraphPad Prism (LaJolla, CA, USA) software. Comparisons between two groups were made by using the two-tailed, unpaired Student's t-test. P values less than 0.05 were considered significant (two-tailed).

A significant T-cell proliferation was observed in response to epoprostenol (proliferation measured as ^3H -thymidine incorporation was 9.5 counts per minute (cpm) $\times 10^3$ for epoprostenol versus 3.5 in a healthy control, $P < 0.001$). There was a trend towards an increased T cell response to treprostinil but this was statistically insignificant ($P = \text{NS}$). There was no significant T cell response either to the diluent alone, normal saline, iloprost or alprostadil (Figure 2).

As a result, we decided to avoid further use of prostanoids and to switch the patient to oral therapy with ambrisentan and sildenafil. The patient was weaned off epoprostenol and discharged on oral therapy with plans for a slow steroid taper over the next few months. After two months, the patient was off all prednisone and no recurrence of pneumonitis was observed. Her oxygenation was 97% on room air and she ambulated 1730 feet on a six-minute walk test. A chest CT at five weeks post-discharge revealed marked improvement in the previously noted parenchymal opacities (Figure 1b).

Discussion

Drug-induced lung disease encompasses a wide range of pulmonary manifestations and is a challenging diagnosis of exclusion. Epoprostenol was the only new drug added to our patient's regimen prior to the onset of her symptoms. She developed symptoms within a month of starting therapy and therefore would have had time to produce a memory T cell response. The BAL we performed demonstrated elements that have been associated with hypersensitivity reactions in the lung⁸⁻¹⁰ and lacked the foamy macrophages characteristic of amiodarone pulmonary toxicity (APT). Furthermore, APT typically develops in older patients ($>60\text{y.o.}$) on higher doses ($>400\text{mg/day}$ or cumulative dose 101 to 150g), at least 18 to 24 months into treatment¹³⁻¹⁵ or on a background of parenchymal lung disease. Finally, our patient responded rapidly to the cessation of epoprostenol thus supporting the tenet of remission upon drug removal.

In terms of allergen potential, the development of non-specific interstitial pneumonitis and severe erythroderma in response to epoprostenol therapy has been described^{11,12}. Another group presented a case which mimicked ours; their patient was safely transitioned on pulse steroids to prostaglandin E1 after a T cell proliferation assay for epoprostenol returned grossly positive (273% versus control)⁶.

We found the drug T cell proliferation assay to be vital to the diagnosis of epoprostenol-induced IP in our patient. Given our dependence on epoprostenol as first-line therapy for our most ill IPAH patients, we were hesitant to stop treatment in our patient who had experienced a clinically favorable response. The positive assay, however, lent significant objective weight to the link between epoprostenol, perhaps treprostinil, and hypersensitivity. Notably, the sensitivity and specificity of the assay is estimated at 62 to 78% and at least 85%, respectively^{16,17}, and the assay was conducted while our patient was on 1.5mg/kg prednisone daily. This is relevant as immunosuppressive drugs such as prednisone can confound results by suppressing in vitro T cell stimulation¹⁶. Thus, our patient's response may have been grossly underestimated.

We tested several similar drugs in both our patient and a healthy control to uncover or eliminate the possibility of a prostanoid class effect. A T cell response to treprostinil was close to significance in our patient alone and further response could have been suppressed by high-dose prednisone therapy. Thus, we decided to avoid experimenting with another prostacyclin agent in our patient. Finally, we tested vehicle alone to ensure the diluent was not the culprit in the hypersensitivity reaction.

In summary, we learned that epoprostenol can incite a profound inflammatory response in the pulmonary interstitium. We believe the positive drug T cell proliferation assay served as the definitive link between epoprostenol and hypersensitivity in our patient and further empowered us to withdraw a treatment known to be critical to patients with IPAH. We are convinced this assay should be considered in the appropriate clinical setting given its potential utility in the confirmation of hazardous drug reactions.

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Abbreviations

IPAH	idiopathic pulmonary arterial hypertension
TTE	transthoracic echocardiogram
RVSP	right ventricular systolic pressure
CO	cardiac output
iNO	inhaled nitric oxide
CICU	cardiac intensive care unit
IV	intravenous
BAL	bronchoalveolar lavage
IP	interstitial pneumonitis
APT	amiodarone pulmonary toxicity

FIGURE 1a

FIGURE 1b

Figure 1.

Figure 1a: Chest CT image at level of tracheal carina shows patchy, bilateral areas of parenchymal opacification consisting of admixed ground-glass and consolidation. A fine reticular network is seen superimposed over ground-glass opacities in a “crazy paving” pattern (arrows).

Figure 1b: Follow-up chest CT scan acquired during steroid taper shows marked improvement in parenchymal opacities.

Figure 2.

Proliferation assays were performed using age-matched healthy control (HC) vs. patient (Pt). ^3H thymidine counts per minute $\times 10^3$ (cpm) indicative of T cell proliferation is on the y-axis and conditions for HC vs. Pt proliferation are given on the x-axis. Vehicle indicates drug diluent alone. Controls were performed using antiCD3 and antiCD28 as positive controls to elicit proliferation (HC stim and Pt stim).