

PHARMACOTHERAPY

Iloprost Inhalation in Mild Asthma

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Objective. To determine the feasibility of administering iloprost by inhalation in patients with mild atopic asthma. **Methods.** Volunteers underwent supervised inhalation of iloprost in the clinic with measurement of spirometry and blood pressure for 2 hours. The volunteers then inhaled iloprost four times daily at a dose of 2.5 or 5 µg for 14 days. Spirometry, asthma questionnaires, peak flow diaries, measurement of methacholine responsiveness, and exhaled nitric oxide concentrations were obtained prior to and after the treatment period. **Results.** Chronic inhalation of iloprost (2.5–5 µg) did not alter spirometry or methacholine responsiveness. **Conclusion.** Inhaled iloprost in carefully selected volunteers with mild asthma appears to be a suitable intervention to explore the effects of prostacyclin in human asthma.

Keywords human, methacholine, nitric oxide, prostacyclin, spirometry

INTRODUCTION

Asthma is a chronic inflammatory airway disease characterized by reversible airflow obstruction. In 2007, 34 million Americans had been diagnosed with asthma (1). Medications that treat the airway inflammation, such as inhaled corticosteroids or leukotriene receptor modulators, are the mainstay of therapy. However, there are a significant proportion of asthmatics who do not respond to these medications (2). Martin and colleagues (3) showed that 33 of 72 subjects with uncontrolled asthma did not respond to short-term use of inhaled corticosteroids. They concluded that different therapeutic strategies would need to be established for those who did not respond. Similarly, 55% of children with mild persistent asthma did not respond to inhaled corticosteroids or leukotriene receptor antagonists (4). Severe asthma by definition represents a condition where inhaled corticosteroids are not sufficient, and there is a molecular basis for this steroid resistance (5, 6). Thus, new controller medications for asthma are needed.

Prostaglandin I₂ (PGI₂ or prostacyclin) is a cyclooxygenase product of arachidonic acid that has the ability to relax vascular smooth muscle and inhibit platelet activation. However, there is increasing evidence that prostacyclin has effects on inflammatory processes ranging from allergic inflammation to acute lung injury.

Mice lacking the prostacyclin (IP) receptor have increased infiltration of eosinophils in the peribronchial and perivascular regions of the lungs when challenged with ovalbumin when compared to wild-type mice (7). These mice also have elevated levels of IgE and IgG when compared to control mice, suggesting an enhancement of

allergic inflammation in the IP receptor-deficient mice. Treatment ex vivo of OVA-specific CD4 T lymphocytes with prostacyclin abrogates the development of allergic inflammation (8). Lambrecht and colleagues (9) showed that administration of the prostacyclin analog iloprost to mice with established allergic inflammation causes a down-regulation of dendritic cell function and reduction in inflammation. Contemporaneous with this report, Peebles and colleagues (10) demonstrated that prostacyclin-mediated reduction of endotoxin induced cytokine release from dendritic cells. A prostacyclin agonist with thromboxane inhibitory properties has been shown to ameliorate experimental mouse asthma (11). These publications and others provide a strong rationale for testing the effect of prostacyclin agonists in human asthma (9, 12).

Prostacyclin analogs are used primarily for the treatment of pulmonary arterial hypertension and can be given intravenously, subcutaneously, orally, or by inhalation. The most convenient method of delivery to asthmatics appears to be by inhalation. There are two compounds, iloprost and treprostinil, that are approved by the FDA for inhalational use in the treatment of pulmonary arterial hypertension. Iloprost was used because it was the drug used by Lambrecht and colleagues. In their study, iloprost at a dose of 0.20 µg intranasally prevented inflammation caused by subsequent inhalation of allergen. The maximal dose of inhaled iloprost in humans is 5.0 µg, and it is inhaled six to nine times daily in the treatment of pulmonary arterial hypertension. We therefore sought to have volunteers inhale 5.0 µg four times daily for 2 weeks, a duration of treatment long enough to show effects of inhaled steroids. However, there are concerns about bronchoconstriction and cough in response to inhaled prostacyclin, especially in patients with pre-existing obstructive lung disease. To explore the feasibility of chronic administration of prostacyclin to the airways of asthmatic subjects, we conducted an unblinded pilot study of inhaled iloprost given four times daily for 14 days,

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measuring spirometry and methacholine responsiveness as objective measures of airway function.

METHODS

The study was designed and executed solely by the investigators who independently controlled all the data. The study was approved by the Vanderbilt University Institutional Review Board and volunteers provided written consent. Nonsmoking atopic volunteers between the ages of 18–60 with mild persistent asthma were recruited by advertisement and via an Institutional Review Board (IRB)-approved database. The study was registered with the NIH Clinical Trials Registration NCT01209533.

Volunteers were screened with a brief history and physical examination. If qualified, spirometry was performed, and for females with childbearing potential, a serum pregnancy test was performed. The Mini Asthma Quality of Life Questionnaire (MiniAQLQ) and the Asthma Control Questionnaire (ACQ) were administered (13, 14). Exhaled breath was collected for measurement of nitric oxide. A methacholine challenge test was performed. Volunteers were taught how to perform measurements of peak flow and recorded these daily in the morning before inhaling iloprost.

On a subsequent day, measurements of pulmonary function were made before and at 15-minute intervals for 2 hrs after inhalation of 2.5 μ g of iloprost via the I-neb[®] AAD[®] System (Phillips Respironics, Andover, MA). Blood pressure, pulse rate, and arterial oxygen saturation were measured before and at 15-minute intervals after inhalation. Symptoms such as cough, dyspnea, flushing, headache, and chest tightness were recorded. In volunteers willing to proceed to the higher dose of 5 μ g, a study was conducted on the subsequent day using a similar protocol with the dose of 5 μ g of inhaled iloprost.

The volunteers then started to inhale iloprost (2.5 or 5 mcg) four times daily separated by at least 2 hrs for 2 weeks. Daily diaries of symptoms (Juniper Asthma Control Diary) and recording of morning peak flow were done (15). The volunteers were contacted by telephone by the study nurse or investigators within the first week of beginning iloprost with enquiries specific to possible side effects of iloprost.

After 2 weeks of inhaled iloprost, volunteers returned to the clinical research center 8–20 hours after the last dose of iloprost and a final methacholine challenge and exhaled nitric oxide (ENO) measurement were performed. The ACQ and MiniAQLQ were administered. The daily peak flow data were collected.

The methacholine challenge was performed as previously described (16). Volunteers did not use albuterol within 8 hrs of the study. No volunteer had a viral upper respiratory tract infection in the 6 weeks preceding study. Methacholine dilutions were made fresh each day (Provacholine, Methapharm Inc., Coral Spring, FL). After confirmation of an FEV₁ > 79% of predicted, doubly increasing aerosolized concentrations were given by inhalation of 5 slow vital

capacity breaths every 5 minutes starting at a methacholine concentration of 0.075 mg/ml to a maximum of 20.0 mg/ml or until a drop of >20% in the baseline FEV₁ occurred. Methacholine solutions were aerosolized through a DeVilbiss 646 nebulizer (DeVilbiss Healthcare, Somerset, PA) connected via a dosimeter set to open for the first 0.6 seconds of inspiration to a wall air source at 20 psi. Measurements of FEV₁ were made using a computerized spirometer (Flow Screen VIASYS Healthcare GmbH, Hoechberg, Germany) using American Thoracic Society (ATS) standards.

ENO was measured using an on-line restricted breath technique that employs exhalation via a high resistance and positive mouth pressure to close the velum, thus excluding nasal NO. Subjects without a nose clamp inhaled to total lung capacity (TLC) via a NO filter, reducing the NO concentration to less than 5 ppb, and then they exhaled via the filter while targeting a pressure of 20 mm Hg. The NO and pressure profiles, as displayed on a computer monitor in real time, showed a washout phase and plateau (Sievers Nitric Oxide Analyzer NOA 280i, Boulder, CO). The plateau ENO level was recorded. Exhalations were repeated and suboptimal maneuvers were rejected until three ENO values that varied by less than 7% were obtained.

Administration of iloprost was accomplished using the I-neb[™] AAD[®] System. Volunteers were instructed in the use of the delivery system, its cleaning, and medication storage. Written instructions were also provided. For each inhalation session, the entire contents of one opened ampule of Ventavis were transferred into the I-neb[™] AAD[®] System immediately before use. The device provided cues for inhalation and recorded the dose administered, time, and date.

Statistics

To assess the effect of inhaled iloprost on nonspecific airway responsiveness to inhaled methacholine, the dose of methacholine causing a 20% fall from baseline FEV₁ (PD₂₀FEV₁) at screening and post-iloprost values were compared by paired *t* test. The effect of inhaled iloprost on FEV₁ was assessed by analysis of variance (ANOVA) with repeated measures. Other paired data (FEV₁, ENO, and questionnaire data) were tested using paired *t* test. Study data were collected and managed using REDCap electronic data capture tools hosted at Vanderbilt University (17). Analyses were performed using statistical software (GraphPad Software, La Jolla, CA).

RESULTS

Twenty-three volunteers with a physician diagnosis of asthma were screened. One patient was excluded because of a baseline FEV₁ < 79% and another was unable to perform spirometry. One subject dropped out of the study after screening because of scheduling problems. Four subjects were excluded because of nonresponsive methacholine challenges. One subject began treatment with iloprost

TABLE 1.—Characteristics and responses of 11 subjects.

Age (yrs)	Lowest FEV ₁ (%)	Symptoms with iloprost	FEV ₁ base (l)	FEV ₁ post (l)	PD ₂₀ base (mg/ml)	PD ₂₀ post (mg/ml)	ENO base (ppb)	ENO post (ppb)
40	7.4	None	3.36	3.09	2.93	0.47	58	76
23	3.2	Transient light-headedness	3.24	2.97	0.14	0.31	101	47
46	8.7	None	2.88	2.82	1.59	0.42	60	28
23	2.6	Transient light-headedness	2.42	2.79	1.12	1.54	62	58
26	3.5	None	2.79	2.71	9.19	25.00	9	13
26	18.1	Dry throat "wheeziness"	2.67	2.38	0.27	0.83	25	28
38	5.0	Transient headache	2.24	2.21	17.00	17.58	16	13
37	5.7	Transient light-headedness	2.83	2.7	3.25	12.07	34	27
34	3.2	Transient headache	2.58	2.59	4.64	3.29	13	9
32	6.0	Transient chest congestion	2.96	2.95	0.84	2.46	57	63
42	8.0	Transient headache	2.68	2.69	0.51	0.47	36	17

Notes: All were female, atopic, had a physician diagnosis of asthma and used albuterol prn. Lowest FEV₁ as % of baseline at any time point in the 2 hrs following inhalation of iloprost (5 µg). Symptoms during initial iloprost inhalation (5 µg). FEV₁, PD₂₀FEV₁ to methacholine, and ENO at baseline and after 2 weeks of four times daily iloprost inhalation.

but dropped out of the trial after 48 hours secondary to an unremitting headache. Fifteen subjects completed the study procedure. Two subjects were excluded because of low compliance (less than 75% inhalation of the expected 56 treatments). Two subjects were excluded because of inability to return on day 15 after completion of medication. The results of 11 subjects were analyzed (10 taking 5 mcg and one 2.5 mcg four times daily). Selected features and outcomes of the subjects are given in Table 1.

After inhalation of iloprost (2.5 mcg), there was no statistically significant change in FEV₁ over 120 minutes (data not shown). Within 30 minutes of nebulizer treatment, one patient experienced facial flushing, one patient experienced a dry throat, and one patient felt slightly light-headed. These symptoms resolved in all patients within 45 minutes. On a subsequent day, all 16 volunteers returned to inhale iloprost (5 mcg) with repeat spirometry every 15 minutes for 120 minutes. A statistically significant drop in mean FEV₁ was observed at 15 and 90 minutes after inhalation, which was not symptomatic (Figure 1).

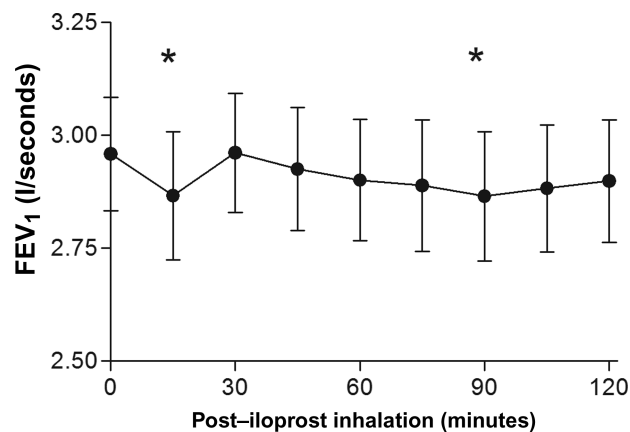


FIGURE 1.—Mean and SEM of FEV₁ ($n = 16$) measured at baseline and serially for 2 hrs after inhalation of 5 mcg of iloprost.

* indicates statistically significant change from baseline (ANOVA with repeated measures with post hoc Dunnett's multiple comparison test). $p < .05$ at 30 minutes (95% CI: 2.68–3.24) and at 90 minutes (95% CI: 2.56–3.17).

The maximal fall in FEV₁ from baseline at any time point was less than 5% in 11 and less than 10% in four, and one had maximal fall of 18%. This subject completed the study with no significant change in PD₂₀ for methacholine. Three volunteers experienced "light-headedness" within 15 minutes of treatment which resolved within 45 minutes of treatment. Four volunteers noted a headache which resolved within 60 minutes of treatment. One volunteer experienced a dry throat which resolved within 30 minutes of treatment. Two volunteers noted chest tightness that resolved and one noted transient nausea. Blood pressure and oxygen saturation remained normal for the 120 minutes after the nebulizer treatment. All but one subject were able to start four times daily treatment with iloprost at the 5 mcg dose. The one patient used 2.5 mcg of iloprost.

The baseline FEV₁ did not change significantly after the 14-day course of inhaled iloprost. (2.79 ± 0.10 vs. 2.72 ± 0.08 , $p > .25$, (95% confidence interval (CI) of difference: -0.056 to 0.19)). Similarly, the PD₂₀ did not change after 14 days of treatment with inhaled iloprost (Figure 2). The mean PD₂₀ prior to the inhalation of iloprost was 3.8 ± 1.5 mg/ml and after iloprost was

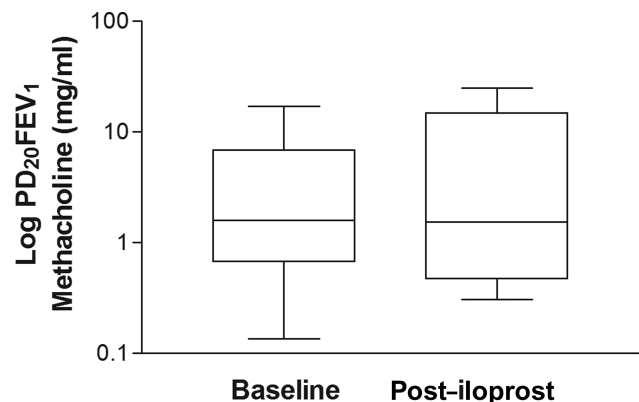


FIGURE 2.—Median, 25% and 75% quartiles, and maximum and minimum values of PD₂₀FEV₁ in 11 subjects before and after 14 days of inhaled iloprost. There was no statistically significant change in the response to inhaled methacholine ($p = .55$).

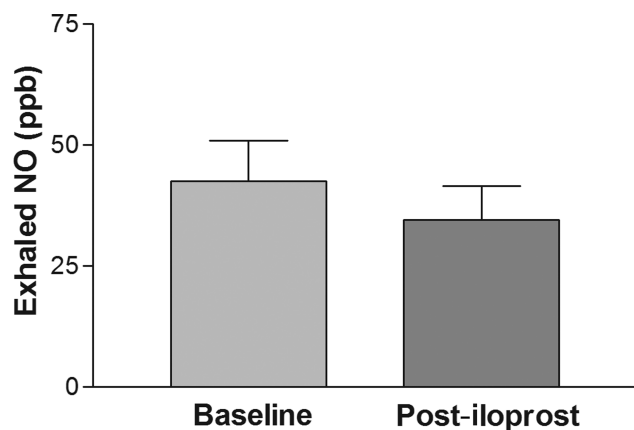


FIGURE 3.—ENO measured at baseline and after 14 days of inhaled iloprost. Height of bars represents mean + SEM ppb from 11 subjects. There was no significant change in exhaled NO (95% CI: -5.485 to 21.35).

5.9 ± 2.6 ($p > .5$). ENO level decreased from the pretreatment baseline, but the decrease was not statistically significant (Figure 3).

While on the treatment protocol, volunteers performed daily peak flow measurements and kept an asthma control diary. There was no significant change in morning peak and no worsening of asthma control based on asthma control diary. Volunteers completed the Juniper ACQ and AQLQ before and after treatment with iloprost. There was no statistically significant difference in scores after treatment with iloprost.

DISCUSSION

Ours is the first study to demonstrate that iloprost is tolerated and appears to be safe in patients with mild asthma when inhaled four times daily for 2 weeks. The lack of change in FEV₁ and PD₂₀FEV₁ after treatment with iloprost four times daily for 14 days implies that iloprost does not worsen asthma control. This is supported by the lack of change in ACQ or AQLQ scores. Although not statistically significant, the slight decrease in exhaled NO suggests improvement in airway inflammation in this group of mild asthmatics (18–20). The most common side effects experienced by volunteers were dry and/or sore throat immediately after nebulizer treatment which did not preclude volunteers from continuing the medication. The dose, duration, and frequency of iloprost inhalation in our study may not be the optimal parameters for use of iloprost in asthma.

CONCLUSION

Iloprost is approved by FDA for the treatment of pulmonary hypertension, but specific warnings note that iloprost inhalation can induce bronchospasm, which may be more severe or frequent in patients with a history of hyper-reactive airways. Iloprost has not been evaluated in patients with chronic obstructive pulmonary disease,

with severe asthma, or with acute pulmonary infections. Our results suggest that under carefully monitored conditions, selected patients with mild asthma do not experience deterioration in asthma control with four times daily inhalation of iloprost for 14 days. Our data should not be interpreted as indicating safety or efficacy of iloprost in obstructive airway disease. Further studies will be necessary to determine whether inhaled iloprost can favorably affect allergic asthma.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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