Pulmonary arterial hypertension (PAH) is characterized by progressive arteriopathy leading to elevation in pulmonary arterial pressure and, if untreated, right ventricular failure and death. Pathogenetically, several abnormalities in endothelial function have been noted, including prostacyclin (1) and nitric oxide (2) deficiency and endothelin excess (3), abnormalities which contribute to the development and progression of disease.

In recent years, highly effective therapies targeting these specific endothelial abnormalities have emerged. Treatments, including the prostacyclin analogues epoprostenol, treprostinil, and iloprost; endothelin receptor antagonists (ERAs) such as bosentan; and the phosphodiesterase 5 inhibitor sildenafil, have been demonstrated to have both short- and long-term efficacy (4–12). Because of the availability of several agents, working via different mechanisms, interest has developed in combination therapy.

One possible approach is combining an ERA with a prostacyclin derivative. The dual ERA, bosentan, the only approved drug in this class, is widely utilized as first-line oral therapy for PAH, and has been shown to improve short- and long-term exercise capacity, clinical status, and survival (9–11). However, even on bosentan therapy, many patients remain symptomatic with significant residual pulmonary hypertension, representing a group for which additional therapy might be beneficial.

Treprostinil, a relatively long-acting prostacyclin derivative, also has proven efficacy in PAH, when given as a continuous subcutaneous infusion (7). The possibility of delivering treprostinil via intermittent inhalation led us to perform this pilot study. In this 12-week open-label safety and efficacy trial, inhaled treprostinil was added to PAH patients already on oral bosentan.

METHODS

Patient population. Twelve patients with a diagnosis of PAH, either idiopathic or associated with connective tissue disease, were enrolled in the trial. Patients had to be symptomatic with a New York Heart Association functional class of III or IV despite oral bosentan for at least 12 weeks.

RESULTS

One patient was excluded from analysis due to the subsequent finding of pulmonary capillary hemangiomatosis. In the remaining 11 patients, inhaled treprostinil was safe and well tolerated. Inhaled treprostinil was associated with an increase in 6MWD at 12 weeks (baseline 339 ± 86, 12 week, 1 h post-inhalation 406 ± 121 m, 67-m change, p = 0.01). An improvement in 6MWD of 49 m from baseline was noted during the trough period, just before inhalation of treprostinil (p = 0.009). The 6MWD improvement of at least 10% was noted in 1 of 6 patients receiving 30 μg versus 5 of 6 receiving 45 μg. Over 12 weeks, significant decreases were noted in mean pulmonary arterial pressure (−10%) and in pulmonary vascular resistance (−26%). Functional class improved from III to II in 9 of 11 patients.

CONCLUSIONS

This trial suggests that inhaled treprostinil is safe, well tolerated, and associated with significant improvements in exercise capacity, functional class, and pulmonary hemodynamics in symptomatic patients with PAH on bosentan. (J Am Coll Cardiol 2006;48:1433–7) © 2006 by the American College of Cardiology Foundation
before enrollment in the study. The institutional review board approved the consent and protocol, and all patients were informed of alternative treatment options.

**Study assessments.** Baseline assessments included 6-min walk distance (6MWD), functional class determination, and hemodynamics by right heart catheterization. Inhaled nitric oxide was given, and repeat hemodynamic assessment was performed after 5 min. Inhaled treprostinil was then delivered, and hemodynamic assessment was repeated every 15 min for 1 h, then every 30 min until 3 h after inhalation. Patients were then sent home and instructed to inhale treprostinil 4 times daily.

Repeat functional assessment and 6-min walk testing was performed 1 h after treprostinil inhalation at 6 and 12 weeks, with an additional 6-min walk done at trough (just before inhalation of treprostinil) at week 12. At week 12, right heart catheterization was repeated within 1 h of the last dosing of inhaled treprostinil.

Adverse effects, compliance, and tolerability were assessed at each study visit. After week 12, patients were offered continued therapy.

**Drug delivery.** Treprostinil, 5 μg per inhalation, was delivered via a hand-held ultrasonic, single-breath nebulizer (Opti-Neb, Metropolitan Medical, Inc., Winchester, Virginia). Two dosing regimens were evaluated: 30 μg (6 inhalations) 4 times daily for the first 6 subjects and 45 μg (9 inhalations) for the second 6 subjects. The treprostinil sodium solution used in this study was produced specifically for inhalation with a 0.6-mg/ml concentration of the active ingredient treprostinil. The inhalation solution does not contain the preservative, metacresol, used in the commercially available treprostinil sodium product remodulin. The commercially available product is designed for subcutaneous and intravenous delivery only. The inhalation solution is preservative free.

**Statistical analysis.** Because this was a pilot trial, there were no specific hypotheses, and the trial was not powered to detect a specific effect. Summary statistics were prepared for most results. All values are given as mean ± SD. A Student paired t test (2-sided) was used to compare key variables at baseline and after 3 months of treatment; p < 0.05 was considered statistically significant.

**RESULTS**

Of the 12 patients enrolled, 11 patients were analyzed. One patient, who was suspected of having pulmonary capillary hemangiomatosis (PCH), underwent lung transplantation and was excluded from the data analysis. The diagnosis of PCH was confirmed by pathologic examination of the explanted lung tissue. This patient had no subjective improvement and a worsening 6MWD distance through the trial.

Baseline characteristics for the 11 reported subjects are shown in Table 1. Six patients had idiopathic pulmonary arterial hypertension (IPAH), 3 had PAH associated with systemic sclerosis, and 2 had PAH associated with prior drug use (1 methamphetamine, 1 fenfluramine). Mean duration of bosentan use was 72 ± 60 weeks (range 12 to 225 weeks).

**Hemodynamics.** The acute effects of inhaled nitric oxide and inhaled treprostinil on mean pulmonary arterial pressure (PAPmean) are shown in Figure 1. Both inhaled nitric oxide and inhaled treprostinil (at doses of 30 μg and 45 μg) acutely decreased PAPmean. The pulmonary hemodynamic effects of inhaled treprostinil peaked at 45 min; pulmonary arterial pressure returned to baseline values by 180 min.

Hemodynamic results at 12 weeks for the group are shown in Table 2. Significant decreases were noted in

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**Table 1.** Baseline Characteristics of Study Patients (n = 11)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>NYHA Class</th>
<th>6MWD (m)</th>
<th>PAPmean (mm Hg)</th>
<th>PCW (mm Hg)</th>
<th>C.I. (l/min/m²)</th>
<th>PVR (mm Hg/l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>M</td>
<td>APAH (drugs)</td>
<td>III</td>
<td>415</td>
<td>68</td>
<td>9</td>
<td>1.4</td>
<td>21</td>
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<tr>
<td>2</td>
<td>55</td>
<td>F</td>
<td>APAH (scleroderma)</td>
<td>III</td>
<td>296</td>
<td>39</td>
<td>14</td>
<td>4.0</td>
<td>2.6</td>
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<tr>
<td>3</td>
<td>43</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>371</td>
<td>63</td>
<td>14</td>
<td>2.0</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>59</td>
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<td>5</td>
<td>38</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>444</td>
<td>39</td>
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<td>1.3</td>
<td>7.2</td>
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<td>6</td>
<td>36</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>390</td>
<td>44</td>
<td>5</td>
<td>2.2</td>
<td>9.8</td>
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<td>7</td>
<td>58</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>360</td>
<td>44</td>
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<td>2.1</td>
<td>8.5</td>
</tr>
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<td>8</td>
<td>46</td>
<td>F</td>
<td>APAH (HHT)</td>
<td>III</td>
<td>218</td>
<td>56</td>
<td>14</td>
<td>3.0</td>
<td>6.5</td>
</tr>
<tr>
<td>9</td>
<td>64</td>
<td>F</td>
<td>APAH (scleroderma)</td>
<td>III</td>
<td>326</td>
<td>41</td>
<td>11</td>
<td>3.6</td>
<td>4.9</td>
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<tr>
<td>10</td>
<td>73</td>
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<td>III</td>
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<td>50</td>
<td>8</td>
<td>4.4</td>
<td>5.4</td>
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<tr>
<td>11</td>
<td>50</td>
<td>F</td>
<td>APAH (scleroderma)</td>
<td>III</td>
<td>215</td>
<td>47</td>
<td>10</td>
<td>3.0</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Mean 51 ± 12

339 ± 86 49 ± 9.6 10.3 ± 3.4 2.6 ± 1.1 9.2 ± 4.6

APAH = associated pulmonary arterial hypertension; C.I. = cardiac index; HHT = hereditary hemorrhagic telangiectasia; IPAH = idiopathic pulmonary arterial hypertension; NYHA = New York Heart Association; PAPmean = mean pulmonary arterial pressure; PCW = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; 6MWD = 6-min walk distance.
PAPmean and pulmonary vascular resistance (PVR) (10% decrease) measured at peak post-inhalation (approximately 1 h) with a trend toward improvement in cardiac index (15% increase) and PVR (26% decrease). No significant changes in systemic blood pressure occurred.

6MWD. Inhaled treprostinil was associated with a trend toward improvement 12 weeks in peak 6MWD (baseline 339 ± 86, 12 week, peak post-inhalation 406 ± 121 m, 67-m change; p = 0.01) (Table 3). A significant improvement in 6MWD was noted at the trough period, just before a treatment (49-m improvement, p = 0.009). Individual 6-min walk data are shown in Figure 2 and demonstrate improvement in walk distance of at least 10% in 6 of 11 subjects. Of note, a 10% or greater improvement in 6MWD was noted in 5 or 6 patients in the 45-µg dosing group versus only 1 of 6 in the 30-µg dosing group.

Functional class. Functional class significantly improved at week 12, with 9 of 11 patients improving from class III to II. No patient deteriorated to class IV during the study period.

Safety and tolerability. All subjects tolerated the therapy. The delivery device functioned well with each treatment completed in less than 1 min. No serious adverse events occurred in the trial. Transient cough (5 patients), headache (4 patients), and sore throat (2 patients) insufficient to limit compliance were reported. No obvious “rebound” effects between treatments were noted; specifically, no syncope or clinical deteriorations were reported.

Pharmacokinetics. Maximum plasma concentration and half-life of inhaled treprostinil demonstrated that, when inhaled, treprostinil reached the systemic circulation in a dose-dependent fashion. The maximum concentration of the 30-µg and 45-µg groups was 0.33 ng/ml and 0.96 ng/ml, respectively. Similarly, the time until maximum concentration for the 30-µg and 45-µg groups was 15 min and 45 min, respectively, with a half-life of 44 to 52.

**DISCUSSION**

In this pilot study, the addition of inhaled treprostinil in symptomatic PAH patients despite bosentan therapy led to significant improvements in clinically important end points and was well tolerated. No safety concerns or delivery device problems emerged during the 12-week trial.

Previously published studies support the concept of combination therapy with bosentan and prostanoids for PAH. Hoeper et al. (13) found that, in 20 patients treated with prostanoids (11 beraprost, 9 iloprost), the addition of bosentan led to improvements in maximal oxygen consumption and 6MWD. In a multicenter trial (BREATHE-2 [Combination of Bosentan With Epoprostenol in Pulmonary Arterial

<table>
<thead>
<tr>
<th>Table 3. Change in 6-Min Walk Distance at 12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meters Walked</strong></td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Week 12 (m)</td>
</tr>
<tr>
<td>Pre-inhalation (trough)</td>
</tr>
<tr>
<td>Post-inhalation (peak)</td>
</tr>
</tbody>
</table>

**Table 2.** Hemodynamic Effects of Inhaled Treprostinil at 12 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Baseline</th>
<th>12 Weeks</th>
<th>Change</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPAP (mm Hg)</td>
<td>49 ± 10</td>
<td>44 ± 12</td>
<td>−10%</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>PCW (mm Hg)</td>
<td>10.3 ± 3.4</td>
<td>9.5 ± 2.5</td>
<td>−8%</td>
<td>0.381</td>
<td></td>
</tr>
<tr>
<td>CI (/min/m²)</td>
<td>2.6 ± 1</td>
<td>3.0 ± 0.9</td>
<td>+15%</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>PVR (Wood U)</td>
<td>9.3 ± 4.9</td>
<td>6.9 ± 3.5</td>
<td>−26%</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>mSAP (mm Hg)</td>
<td>85 ± 15</td>
<td>86 ± 14</td>
<td>+1%</td>
<td>0.83</td>
<td></td>
</tr>
</tbody>
</table>

mSAP = mean systemic arterial pressure; other abbreviations as in Table 1.
of 33 patients starting intravenous epoprostenol, the simultaneous addition of bosentan resulted in a trend toward greater improvement in total pulmonary resistance index (14).

Preliminary data from a recent randomized, placebo-controlled trial (STEP [Randomized, Double-Blind, Placebo-Controlled Study of Iloprost Inhalation as Add-On Therapy to Bosentan in Pulmonary Arterial Hypertension]) (15) in PAH patients already on bosentan compared another inhaled prostacyclin derivative, iloprost, with placebo. Compared with placebo, patients treated with inhaled iloprost achieved a significant improvement in pulmonary hemodynamics, 6MWD, functional class, and delay in clinical worsening. Although iloprost (Ventavis, CoTherix, Inc., South San Francisco, California) is now approved for use as both monotherapy and in combination with other treatments, potential limitations include the frequency of treatments (6 to 9 treatments per day) and duration of each treatment (7 to 10 min).

In our trial, the improvements seen in 6MWD and functional class were encouraging, because both of these parameters have been shown to correlate with survival in PAH (16,17) and are easily measurable. The mean improvement in walk distance of 67 m was, in fact, greater than reported in the pivotal monotherapy trials for epoprostenol (31 m), subcutaneous treprostinil (16 m), iloprost (36 m), bosentan (35 m), and sildenafil (45 m). In addition, there appeared to be a sustained effect of inhaled treprostinil, with trough 6MWD improving by 49 m.

There appears to be a trend to improved exercise capacity with the higher (45-μg) dose. However, given the small number of subjects and the lack of a control group, firm conclusions regarding “ideal” dose of inhaled treprostinil cannot yet be made.

Significant pulmonary hemodynamic improvements were observed in this study. Acutely, small effects on pulmonary arterial pressure, cardiac output, and PVR were noted. These hemodynamic effects were even greater at week 12, consistent with the long-term effects of prostanoid therapy delivered by the intravenous route.

Inhaled treprostinil was generally well tolerated in this 12-week trial. Although transient sore throat, headache, and cough were noted by some patients, compliance with the therapy was excellent. The small size of the nebulizer, 4 times daily dosing, and short (less than 1 min) treatment times are all attractive aspects to inhaled treprostinil therapy.

Pharmacokinetic measurements confirmed a dose-dependent plasma concentration of treprostinil. Despite inhaled treprostinil reaching the circulation, there were no systemic hemodynamic effects, suggesting localized pulmonary vascular activity. In addition, the half-life of inhaled treprostinil was less than 1 h, although the pulmonary hemodynamic effects lasted as long as 3 h, again supporting a local, sustained effect on the pulmonary vessels. In addition, the finding of improvements in trough 6MWD (when there would be no measurable plasma treprostinil) further supports a sustained, chronic effect on the pulmonary vasculature.

There are several limitations to this study. The trial was open label, and therefore a placebo effect on functional class and walk distance cannot be excluded. However, the significant improvements in pulmonary hemodynamics, in some cases to near-normal or normal values, are strong evidence for a potent drug effect. Another limitation of this small trial is that optimal or lowest effective dose could not be determined. Finally, no conclusions regarding long-term safety or efficacy can be reached. A large, phase III, multinational, randomized, placebo-controlled trial examining the efficacy and safety of inhaled treprostinil as add-on therapy to bosentan (Treprostinil Inha-
lation Used for the Management of Pulmonary Hypertension (TRIUMPH I) is currently ongoing.

In conclusion, the addition of the prostanoid, treprostinil, as an inhaled therapy, to bosentan in patients with symptomatic PAH resulted in improvements in subjective and objective measures and appeared safe and well tolerated. These results lend preliminary support to the approach of add-on combination therapy for this serious disease.

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REFERENCES