Decreed Exhaled Nitric Oxide in Mild Persistent Asthma Patients Treated with a Leukotriene Receptor Antagonist, Pranlukast

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Abstract: Exhaled nitric oxide (NO) level decreases after corticosteroid treatment in asthmatics, but the effect of a leukotriene receptor antagonist, pranlukast, on exhaled NO has not been elucidated. Pranlukast treatment in mild persistent asthmatics for 4 weeks decreased the exhaled NO level, which did not differ from the levels in healthy subjects. [Japanese Journal of Physiology, 49, 541–544, 1999]

Key words: nitric oxide, bronchial asthma, leukotrienes.

Nitric oxide (NO) is detected in exhaled air, and the exhaled NO level is considered to be determined by the balance between NO production in the airway (conducting airway and/or the transition zone) and NO removal into alveolar vessels [1]. The exhaled NO level is influenced by several factors, such as breath-holding time before the measurement [2], expired flow speed during the measurement [3], and cigarette smoking [4].

Another factor which influences the exhaled NO level is airway inflammation. Several studies have demonstrated significantly higher exhaled NO levels in asthma patients than in healthy subjects [5, 6]. Although it has been demonstrated that the exhaled NO level decreases after corticosteroid treatment of asthma patients [7, 8], and that glucocorticoids inhibit cytokine-induced iNOS formation in epithelial cells [9], the effect of other drugs which suppress airway inflammation and iNOS-derived NO production on exhaled NO level has not been elucidated.

Treatmet with leukotriene receptor antagonists may reduce airway inflammation [10, 11], and antileukotriene therapies are becoming a potential alternative to inhaled corticosteroids for the treatment of mild persistent asthmatics [12]. An antileukotriene, pranlukast hydrate, is reported to inhibit the elevation of exhaled NO levels during the reduction of high-dose inhaled corticosteroid in moderate to severe asthma patients [13]. However, whether antileukotriene therapy without corticosteroid therapies reduces exhaled NO levels still remain unclear [14].

In this study, we examined the exhaled NO level in mild persistent asthma patients after treatment with an oral competitive leukotriene receptor antagonist, pranlukast hydrate, in an attempt to investigate the pathophysiological significance of leukotrienes in exhaled NO levels in mild persistent asthma patients. This treatment, for 4 weeks in mild persistent asthmatics without corticosteroid therapies, decreased the exhaled NO level which did not differ from the levels in healthy subjects and stable asthma patients who were treated with beclomethasone dipropionate (BDP) inhalation 200 µg twice a day.

Methods

Subjects. A total of 57 healthy non-smoker volunteers with no history of pulmonary disease and normal spirometric values served as the healthy control in this study. Twenty-two mild persistent asthma patients that were treated only with a β2 stimulant as relief therapy were included. Each patient was carefully interviewed to determine their history, and was evalu-
ated to confirm that they were in the asthma category of “mild persistent asthma” and that their asthma symptoms had been stable for at least 2 months. Mild persistent asthma is defined by the United States National Asthma Education Program’s “Guidelines for the Diagnosis and Treatment of Asthma II” [15] as asthma that occurs on a less than daily basis, with brief attacks associated with nocturnal awakenings less than once a week but more than twice a month, and patients with normal lung function (FEV₁ or peak flow value to be more than 80% of the predicted value).

Eighteen stable asthma patients on BDP inhalation 200 μg twice a day were also included. Smokers were excluded from this study.

**Pranlukast study.** Twelve of the 22 mild persistent asthma patients were randomly chosen and treated with pranlukast hydrate (225 mg twice a day) for 4 weeks. As a control, the others (10 patients) were observed without pranlukast hydrate for 4 weeks.

This investigation was performed in accordance with the principles outlined in the Declaration of Helsinki, and all the subjects gave their informed consent.

**Measurement of exhaled NO.** After dilation of the airways by inhalation of a β₂ agonist (salbutamol sulfate 200 μg) to minimize the effect of heterogeneous ventilation on the exhaled NO level, standing subjects expired from the maximal inspiration level after two deep breaths into a Teflon tube via a mouthpiece, and subjects were instructed to exhale against the resistance of the flow regulator to eliminate contamination by air from the nose and sinuses by closing the velum palatinum [3]. Expiration flow rate was adjusted at a constant rate of 2 l/min using a flow regulator. A tube with a 1.5-mm inside diameter was connected to a side port of the Teflon tube and to a chemiluminescence NO/NOₓ analyzer (CLM-500, Shimadzu, Kyoto). Part of the expired air was introduced into the NO/NOₓ analyzer at a constant flow rate of 50 ml/min, while the main stream was exhausted through the constant flow regulator. The exhaled NO concentration was continuously displayed on a chart recorder, and the exhaled NO level was defined as the late plateau concentration to exclude contamination by nasal air. Two measured values were averaged. To validate our method, we checked the dependency of the exhaled NO level on the expiration flow speed in healthy subjects and it was identical to the report by Silkoff et al. [3].

**Statistical analysis.** All mean values are reported with the corresponding standard deviation unless otherwise stated. The mean exhaled NO levels in the various groups were compared by ANOVA with post hoc Scheffé’s test. The Friedman test was used to compare the exhaled NO levels before, 2 weeks after, and 4 weeks after the pranlukast-treatment, and the Mann-Whitney U-test was used to compare the exhaled NO level 4 weeks after the pranlukast-treatment and the level after 4 weeks observation of control asthma patients. p values less than 0.05 were considered significant.
Results

Subjects. Healthy subjects (n=57, 34 females, 23 males; age: 39.0±13.3 years; %VC, 113.3±15.7%; and %FEV1; 104.0±14.7%), stable asthma patients on BDP inhalation therapy (n=18, 5 females, 13 males; 50.7±14.5 years; %VC, 111.2±13.0%; and %FEV1, 96.9±14.1%), and 10 control patients (5 females, 5 males; age: 49.7±9.4 years; %VC, 99.4±20.9%; and %FEV1, 94.6±14.5%) were observed for 4 weeks without changing the asthma treatment (β2 stimulant inhalation as a reliever were investigated: 12 of the 22 mild persistent asthma patients were treated only with pranlukast hydrate (8 females, 4 males; age: 44.5±10.4 years; %VC, 98.1±6.1%; and %FEV1, 87.3±9.0%), and 10 control patients (5 females, 5 males; age: 49.7±9.4 years; %VC, 99.4±20.9%; and %FEV1, 94.6±14.5%) were observed for 4 weeks without changing the asthma treatment (β2 stimulant inhalation as a reliever).

Exhaled NO levels. The exhaled NO levels of mild persistent asthma patients without the administration of pranlukast hydrate were significantly higher than the levels in healthy subjects (37.9±32.5 ppb, Fig. 1): control asthma patients (132.8±73.1 ppb, p=0.0009), after 4 weeks observation in control patients (132.6±73.1 ppb, p=0.0009), and before administration of pranlukast hydrate in pranlukast-treated patients (150.4±110.8 ppb, p<0.0001). There were no significant differences in exhaled NO values among the three groups not treated with pranlukast hydrate. After administration of pranlukast hydrate for 2 weeks, the exhaled NO level was 119.5±84.1 ppb, which was significantly higher than the level in healthy subjects (p=0.0046). After the administration of pranlukast hydrate for 4 weeks, the exhaled NO level further decreased to 72.9±57.3 ppb, and it did not differ from the level in healthy subjects and stable asthma patients with BDP inhalation (58.2±17.7 ppb).

In the group of asthma patients treated only with pranlukast hydrate, the exhaled NO level significantly increased dependent on the duration of the treatment (p=0.0051, Table 1), and the exhaled NO level after 4 weeks administration of pranlukast hydrate was significantly lower than the level of the control asthma patients after 4 weeks observation without pranlukast hydrate (p=0.0376).

Discussion

Similar to the non-specific anti-inflammatory drug glucocorticoid, a 4-week administration of an oral leukotriene receptor antagonist, pranlukast hydrate, to mild persistent asthma patients also decreased exhaled NO levels to the levels of healthy subjects.

Since we did not stop BDP inhalation in the stable asthma patients who inhaled BDP, the baseline asthma status without BDP treatment in those patients could not be determined. They might be stable due to the BDP treatment, or they might be stable even without BDP treatment. However, since the exhaled NO level in the stable asthma group with BDP inhalation in this study was similar to the healthy level, BDP inhalation in mild persistent asthma patients is expected to reduce the exhaled NO level to a level equal to or slightly more than the level in healthy subjects and pranlukast-treated patients.

The 5-lipoxygenase products of arachidonic acid metabolism have been shown to be important mediators of airway inflammation and obstruction in asthma [16]. Pranlukast hydrate, a competitive leukotriene receptor antagonist, attenuates allergen-induced early and late airway responses and airway hyper-respon-

### Table 1. Exhaled NO level in patients with mild persistent asthma with and without pranlukast.

<table>
<thead>
<tr>
<th>No.</th>
<th>Baseline (ppb)</th>
<th>2 weeks (ppb)</th>
<th>4 weeks (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (with pranlukast)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>285</td>
<td>140</td>
<td>n.a.</td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>90</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>275</td>
<td>200</td>
<td>n.a.</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
<td>n.a.</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>115</td>
<td>100</td>
<td>79</td>
</tr>
<tr>
<td>7</td>
<td>150</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>n.a.</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>115</td>
<td>90</td>
<td>65</td>
</tr>
<tr>
<td>11</td>
<td>95</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>12</td>
<td>395</td>
<td>320</td>
<td>225</td>
</tr>
<tr>
<td>Control (without pranlukast)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>110</td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>115</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>40</td>
<td></td>
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<tr>
<td>4</td>
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<td>55</td>
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<tr>
<td>5</td>
<td>140</td>
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<tr>
<td>6</td>
<td>160</td>
<td>115</td>
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<tr>
<td>7</td>
<td>165</td>
<td>155</td>
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<tr>
<td>8</td>
<td>112</td>
<td>147</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>314</td>
<td>245</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>132±73</td>
<td>133±60</td>
<td></td>
</tr>
</tbody>
</table>

n.a., not available, since these patients did not appear on the day to examine their exhaled NO level. The Friedman test revealed that the exhaled NO level significantly decreased dependent on the duration of the treatment (p=0.0051), and the exhaled NO level after 4 weeks administration of pranlukast hydrate was significantly lower than the level of the control asthma patients after 4 weeks observation without pranlukast hydrate (p=0.0376).
siveness [17], and allergen-induced late asthmatic re-
actions have been found to be associated with the ele-
vation of exhaled NO levels [18]. Since cysteinyl 
leukotrienes are produced almost exclusively by in-
flammatory leukocytes in humans, a blockade of the 
receptors may blunt TNF-α release [11], thereby re-
ducing iNOS induction in leukocytes and bronchial 
epithelial cells. Furthermore, the inhibition of neu-
rophil recruitment will decrease the number of iNOS-
positive cells. Therefore, iNOS-derived NO produc-
tion would be suppressed by the blockade of 
leukotriene receptors.

In conclusion, a specific treatment targeted against 
leukotriene activity decreased the exhaled NO level in 
mild persistent asthma patients without glucocorticoid 
treatment. NO production in the airway is related to 
the activity of leukotrienes in mild persistent asthma.

The authors thank Ms. Masumi Tanaka for her excellent 
technical assistance. This study was supported by the Re-
search Grant for Cardiovascular Disease (8C-5) from the 
Ministry of Health and Welfare of Japan.

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