High Dose of Inhaled Fluticasone Reduces High Levels of Urinary Leukotriene E₄ in the Early Morning in Mild and Moderate Nocturnal Asthma*

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Background: The circadian variation in urinary leukotriene E₄ (LTE₄) excretion with a morning acrophase has recently been reported in nocturnal asthma (NA); however, the effects of inhaled corticosteroids (ICS) on this circadian rhythmicity of leukotriene (LT) in patients with NA are controversial.

Methods: We first measured peak expiratory flow (PEF), urinary LTE₄, 11-dehydro-thromboxane B₂ (TXB₂), and creatinine levels six times every 4 h for 24 h in two groups: patients with mild-to-moderate, steroid-naive NA (n = 10, group A), and patients with severe NA treated with high-dose ICS (n = 10, group B). Next, group A patients received 2 weeks of treatment with 800 µg/d of inhaled fluticasone propionate (FP), and we compared the measured parameters before and after treatment.

Results: In group A, a circadian rhythm in urinary LTE₄ with peak levels at approximately 4 AM associated with reduced PEF was observed. Group B had suppression of urinary LTE₄ excretion and had no circadian rhythmicity, as seen in group A, despite a dip in PEF at 4 AM. A high dose of FP in group A significantly (p < 0.05) reduced LTE₄ levels and abolished the circadian rhythm, as well as improving PEF. We found no significant difference in the circadian rhythm of urinary 11-dehydro-TXB₂ between groups A and B, and high-dose FP partially decreased urinary 11-dehydro-TXB₂ levels but not significantly.

Conclusions: A high-dose of ICS reduced urinary LTE₄ levels and abolished their circadian variation in patients with asthma, suggesting that LT might contribute to the mechanism of NA.

(CHEST 2003; 124:1768–1773)

Key words: circadian rhythm; inhaled corticosteroid; leukotriene; nocturnal asthma

Abbreviations: BDP = beclomethasone dipropionate; FP = fluticasone propionate; ICS = inhaled corticosteroids; LT = leukotriene; LTE₄ = leukotriene E₄; LTE₄ = leukotriene E₂; NA = nocturnal asthma; NFkB = nuclear factor-kB; PEF = peak expiratory flow; TX = thromboxane; TXB₂ = thromboxane B₂
morning acrophase in patients with NA, but not in nonnocturnal asthmatic subjects.\textsuperscript{11} NA is associated with a significant increase of eosinophils in BAL fluid, and of eosinophils and macrophages in the alveolar tissue sampled at 4 AM.\textsuperscript{12,13} These inflammatory cells are known to produce LTs and thromboxane (TX). Inhaled corticosteroids (ICS) are the first line of anti-inflammatory treatment for asthma, but whether they reduce LT synthesis \textit{in vivo} remains controversial. Several studies\textsuperscript{14–16} suggest that inhaled or oral steroids do not change urinary LTE\textsubscript{4} levels either in healthy people or in patients with mild asthma; however, IV administration of corticosteroids inhibited leukotriene production in acute asthma, while inhaled fluticasone propionate (FP) [500 µg/d for 4 weeks] significantly decreased sputum leukotriene B\textsubscript{4} (LTB\textsubscript{4}) levels in severe bronchectasis.\textsuperscript{17,18} Our aims were to determine whether high-dose therapy with ICS alters the circadian rhythm of LT and TX in patients with NA, and to examine the contribution of arachidonic acid metabolites to the pathogenesis of NA.

\section*{Materials and Methods}

\subsection*{Study Population}

All subjects were recruited from Sapporo Medical University Hospital. Twenty patients with NA (8 men and 12 women) were chosen at random from a larger group of non-aspirin–sensitive asthmatics (Table 1). We defined asthma using the American Thoracic Society criteria with a documented daytime improvement in percentage of predicted FEV\textsubscript{1} of \textgtrless 15% after \beta\textsubscript{2}-agonist inhalation.\textsuperscript{19} NA was defined as a documented fall in the overnight PEF of \textgtrless 15% and asthma symptoms in the early morning on at least 5 nights in the 2-week period preceding the study.\textsuperscript{20} Patients were excluded if they had had other acute respiratory illnesses or had had a moderate-to-severe asthma exacerbation within 1 month before the study. All had normal renal function, and had not had symptoms of gastroesophageal reflux and excess obesity, which cause sleep disturbance. Only people with a \textgtrless 2 + reaction to antigen-specific serum IgE of 23 common antigens as tested by a commercial kit (CAP RAST system; Pharmacia Diagnostics; Uppsala, Sweden) were considered for atopy. There were 10 asthmatic patients not receiving ICS therapy (group A) and 10 patients receiving ICS (group B). Asthma severity was classified according to published guidelines.\textsuperscript{21} \textit{Severe asthma} is defined as having continuous symptoms with FEV\textsubscript{1} < 60% predicted before asthma treatment, and high-dose inhaled steroid therapy (beclomethasone dipropionate [BDP], \textgtrless 800µg/d) needs for best possible results. \textit{Moderate asthma} is defined as daily symptoms with FEV\textsubscript{1} > 60% and \textless 80% predicted before treatment. \textit{Mild asthma} is defined as FEV\textsubscript{1} > 80% predicted before treatment. In group A, five patients had mild asthma and another five patients had moderate asthma, while all patients in group B had severe asthma. Patient characteristics are summarized in Table 1. All subjects gave written informed consent to participate in this study.

\subsection*{Study Design}

All subjects were admitted to Sapporo Medical University Hospital during this study. Theophylline and inhaled \beta\textsubscript{2}-agonists were discontinued 24 h before the study began, and patients refrained from exercise and rested during the 24 h of the study. Urine collection for determination of creatinine, LTE\textsubscript{4}, and 11-dehydro-TXB\textsubscript{2} began at noon on the first study day, and was performed every 4 h (4 PM, 8 PM, midnight, 4 AM, and noon) along with PEF measurements. Each patient received instruction on PEF measurements using a Mini-Wright Peak Flowmeter (Clement Clarke International; Harlow, Essex, UK). To confirm the effects of short-term ICS, we approached all group A patients about an additional 2-week treatment with FP and re-examination by 24-h monitoring of PEF, urinary LTE\textsubscript{4}, and 11-dehydro-TXB\textsubscript{2} excretion. Seven of 10 patients in group A agreed to the second study and received inhaled FP dry powder (800 µg/d for 2 weeks). We compared measurements before and after this treatment.

\subsection*{Measurement of Urinary LTE\textsubscript{4}}

Each patient provided approximately 30 mL of urine in a polypropylene container. Immediately after collection, 4 mL of

\begin{table}[h]
\centering
\caption{Subject Characteristics*}
\begin{tabular}{|l|c|c|c|}
\hline
Characteristics & Mild and Moderate NA Without ICS (Group A) & Severe NA With ICS (Group B) & p Value \\
\hline
Subjects, No. & 10 & 10 & \\
Female/male gender, No. & 4/6 & 8/2 & NS \\
Age, yr & 59 ± 6 & 40 ± 7 & NS \\
Atropy/nonatopy, No. & 5/5 & 4/6 & NS \\
Peripheral blood eosinophil,/mL & 766.9 ± 183.6 & 266.9 ± 41.6 & <0.001 \\
FEV\textsubscript{1}, mL & 1965 ± 288 & 2318 ± 251 & NS \\
FEV\textsubscript{1}, % predicted & 74.0 ± 5.9 & 83.3 ± 6.5 & NS \\
Reversibility of FEV\textsubscript{1} after \beta\textsubscript{2}-agonist, % & 24.0 ± 3.2 & 23.5 ± 1.1 & NS \\
Duration of asthma treatment, yr & 3.0 ± 1.6 & 8.8 ± 2.0 & 0.027 \\
Dose of BDP, µg/d & 0 & 1544.4 ± 247.8 & \\
Oral \beta\textsubscript{2}-agonist, No. & 1 & 2 & NS \\
Theophylline, No. & 5 & 7 & NS \\
Cysteinyl leukotriene receptor 1 antagonist, No. & 0 & 0 & NS \\
\hline
\end{tabular}
\footnote{Data are presented mean ± SE unless otherwise indicated; NS = not significant.}
\end{table}
solution (ethyl acetate: methanol, 2:1) was added to each 1 mL of urine specimen to eliminate proteins; the specimens were then frozen at −70°C until assayed for urinary creatinine and LTE₄. We have reported previously our assay method.²² Using a reverse-phase, high-pressure liquid chromatography gradient system equipped with a Nova-Pak C18 column (Waters Associates; Milford, MA), LTE₄ was measured with a Leukotriene [³H] Radioimmunoassay Kit (DuPont New England Nuclear Research; Boston, MA). Measurements were corrected according to the creatinine content of urine, and levels were expressed as picograms per milligram of creatinine. The recovery rate of tritiated LTE₄ was from 50 to 60%. The intra-assay and interassay coefficients of variation were 11% and 10%, respectively.

Measurement of Urinary 11-dehydro-TXB₂

Indomethacin was added to the urine samples, which were stored at −70°C. We have previously reported our method of measuring of urinary 11-dehydro-TXB₂.²² After deproteinization and defatting, the samples were applied to a Si minicolumn BOND ELUT SI (Varian; Harbor City, CA) and subsequently fractionated by eluent 1 (chloroform:acetic acid, 100:0.5), eluent 2 (acetonitrile:chloroform: acetic acid, 10:90:0.5), and eluent 3 (acetonitrile:chloroform:acetic acid, 20:80:0.5). The fraction obtained by eluent 3 was evaporated using N₂ gas and reconstituted with the buffer of the 11-dehydro-thromboxane B₂ [¹²⁵I] Radioimmunoassay Kit (DuPont New England Nuclear Research). 11-dehydro-TXB₂ was measured using this kit, and the recovery rate of tritiated 11-dehydro-TXB₂ was 61.5%.

Statistical Analysis

Results were expressed as mean ± SE. χ² test and Mann-Whitney U test were used for analysis of patients’ backgrounds, and the Mann-Whitney U test was used to compare mean values of PEF, and levels of urinary LTE₄ and 11-dehydro-TXB₂ between groups A and B. In the second study, for comparisons of PEF, LTE₄, and 11-dehydro-TXB₂ levels before and after ICS therapy and between two different clock times, we employed the Wilcoxon signed-rank test; p < 0.05 was considered statistically significant.

RESULTS

Using values from the patients’ asthma diaries, PEF before and during the study was not different. No patients needed rescue medications during the study. Subjects’ characteristics and pulmonary function test results are shown in Table 1. Group A patients had significantly higher (p < 0.01) peripheral blood eosinophils than those in group B. There were no significant differences in pulmonary function. Eleven patients were nonatopic and 9 patients were atopic in groups A and B. The mean value of PEF in atopic asthmatics did not differ from that in nonatopic asthmatics. The mean levels of urinary LTE₄ and 11-dehydro-TXB₂ levels of the six measurements were not significantly different between atopic and nonatopic patients.

In the first study, the mean value and circadian rhythmicity of PEF was not different between groups A and B (Fig 1, top; Table 2). Mean urinary LTE₄ levels in group A were significantly (p < 0.05) higher in the midnight to 4 AM and 4 AM to 8 AM time periods as compared with group B (Fig 1, middle), and circadian rhythmicity with a morning acrophase was detected in group A but not in group B (Table 2). Urinary 11-dehydro-TXB₂ levels in each time period were not different between groups A and B (Fig 1, bottom). Group A exhibited circadian

![Figure 1. Comparisons of PEF (top), urinary LTE₄ (middle), and 11-dehydro-TXB₂ (bottom) between patients with steroid-naive, mild-to-moderate NA (group A) and patients with severe NA receiving high-dose ICS (group B). Although the morning dip in PEF is similar between groups A and B, urinary LTE₄ levels are elevated only in group A. There is no difference in urinary 11-dehydro-TXB₂ between the groups.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22000/ on 04/19/2017)
variation with a morning acrophase, but this was not seen in group B (Table 2).

In the second study, asthma symptoms in the early morning disappeared and PEF levels at 8 PM, midnight, 4 AM, and 8 AM were significantly (p < 0.05) improved after high-dose FP therapy (Fig 2, top). The mean PEF value in group A at 4 AM after ICS therapy was significantly (p < 0.05) higher than before FP therapy (Table 3), and the morning dip was abolished (Fig 2, top). The mean levels of LTE4 in the 4 to 8 PM, 8 PM to midnight, midnight to 4 AM, 4 to 8 AM time periods were significantly (p < 0.05) decreased by 2 weeks of FP therapy in steroid-naive asthmatic patients (Fig 2, middle). Furthermore, the circadian rhythmicity was lost after this therapy (Table 3). There were no significant differences between before and after FP therapy in the levels of urinary 11-dehydro-TXB2 (Fig 2, bottom; Table 3).

### Table 2—Comparisons of PEF, Urinary LTE4, and 11-dehydro-TXB2 Between NA Without ICS (Group A) and NA With ICS (Group B)*

<table>
<thead>
<tr>
<th>Time Periods</th>
<th>Group A</th>
<th>Group B</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEF, L/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 PM</td>
<td>376.7 ± 39.2</td>
<td>362.2 ± 23.7</td>
<td>NS</td>
</tr>
<tr>
<td>4 AM</td>
<td>264.4 ± 30.4</td>
<td>295.6 ± 32.1</td>
<td>NS</td>
</tr>
<tr>
<td>p value†</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>LTE4, pg/mg creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 to 8 PM</td>
<td>270.1 ± 66.3</td>
<td>186.1 ± 34.9</td>
<td>NS</td>
</tr>
<tr>
<td>4 to 8 AM</td>
<td>509.7 ± 185.6</td>
<td>159.7 ± 39.0</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>p value‡</td>
<td>&lt; 0.03</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>11-dehydro-TXB2, pg/mg creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 to 8 PM</td>
<td>751.0 ± 108.0</td>
<td>769.2 ± 149.0</td>
<td>NS</td>
</tr>
<tr>
<td>4 to 8 AM</td>
<td>904.3 ± 121.8</td>
<td>1029.3 ± 236.0</td>
<td>NS</td>
</tr>
<tr>
<td>p value§</td>
<td>&lt; 0.01</td>
<td>NS</td>
<td></td>
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</tbody>
</table>

*Data are presented as mean ± SE; see Table 1 for expansion of abbreviation.
†Group A vs group B.
‡4 PM vs 4 AM.
§4 to 8 PM vs 4 to 8 AM.

**Table 3—Effect of 2 Weeks of Inhaled FP in Patients With Mild and Moderate NA**

<table>
<thead>
<tr>
<th>Time Periods</th>
<th>Before Therapy</th>
<th>After Therapy</th>
<th>p Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEF, L/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 PM</td>
<td>352.0 ± 59.0</td>
<td>382.0 ± 57.9</td>
<td>NS</td>
</tr>
<tr>
<td>4 AM</td>
<td>208.0 ± 49.6</td>
<td>370.0 ± 58.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>p value†</td>
<td>&lt; 0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LTE4, pg/mg creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 to 8 PM</td>
<td>369.2 ± 98.4</td>
<td>196.4 ± 25.7</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>4 to 8 AM</td>
<td>776.0 ± 225.4</td>
<td>285.2 ± 73.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>p value‡</td>
<td>&lt; 0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>11-dehydro-TXB2, pg/mg creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 to 8 PM</td>
<td>808.4 ± 198.4</td>
<td>629.6 ± 110.8</td>
<td>NS</td>
</tr>
<tr>
<td>4 to 8 AM</td>
<td>984.2 ± 212.7</td>
<td>724.2 ± 230.0</td>
<td>NS</td>
</tr>
<tr>
<td>p value§</td>
<td>&lt; 0.05</td>
<td>NS</td>
<td></td>
</tr>
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</table>

*Data are presented as mean ± SEM; see Table 1 for expansion of abbreviation.
†Before vs after.
‡4 PM vs 4 AM.
§4 to 8 PM vs 4 to 8 AM.

**Figure 2. Comparisons of PEF (top), urinary LTE4 (middle), and urinary 11-dehydro-TXB2 (bottom) before and after 2 weeks of treatment with inhaled FP (800 μg/d) in patients with steroid-naive mild-to-moderate NA (group A). The morning dip in PEF and morning elevation in urinary LTE4 are abolished by therapy, but urinary 11-dehydro-TXB2 is unchanged.**

4 to 8 AM time periods were significantly (p < 0.05) decreased by 2 weeks of FP therapy in steroid-naive asthmatic patients (Fig 2, middle). Furthermore, the circadian rhythmicity was lost after this therapy (Table 3). There were no significant differences between before and after FP therapy in the levels of urinary 11-dehydro-TXB2 (Fig 2, bottom; Table 3).

**DISCUSSION**

We recently observed the existence of circadian rhythm of urinary LTE4 with a morning peak in
steroid-naive NA but not in steroid-naive non-NA. Although we changed sampling method from every 3 h to every 4 h in the present study, the same circadian rhythm was found in steroid-naive NA. LTE4 appeared in urine within the first 1 to 4 h after IV infusion of cysteinyl-LTs; therefore, a 4-h urine sample would reflect the detail changes of LTs that occurred over the 4 h prior to sampling, suggesting that morning dip of PEF at approximately 4 AM reflects on urinary LTE4 levels during 4 to 8 AM. In this study, the high dose of FP simultaneously reduced the mean levels of LTE4, abolished the circadian rhythm in urinary LTE4 and PEF, and disappeared asthma symptoms in the early morning in steroid-naive NA. On the contrary, in patients with severe NA who had been treated with high-dose BDP, the urinary LTE4 excretion was lowered and the circadian rhythm abolished but nocturnal symptoms and a dip of PEF at 4 AM remained. It was reported that 14 of 17 patients with severe asthma who received >20 mg of prednisolone for ≥1 year had reduced LTE4 levels in BAL fluid to the levels of normal control subjects. Jatakamon suggested a potential role of neutrophils rather than eosinophils in more severe asthma because the number of neutrophils, interleukin-8, and myeloperoxidase levels in sputum were high. LTC4 is mainly produced in neutrophils, interleukin-8, and myeloperoxidase levels in sputum were high. LTC4 is mainly produced in epithelial cells, eosinophils, and mast cells. Moreover, cysteinyl LT-1 receptor antagonists are more effective in mild asthma as compared to severe asthma. We suggested that LTs might make a greater contribution to nocturnal exacerbations in patients with mild-to-moderate, steroid-naive asthma than in those treated with high-dose ICS. It was speculated that the morning dip in patients with severe NA with high-dose ICS might occur due to LTs and other bronchoconstrictive factors.

This is the first study to investigate the effect of short-term, high-dose ICS on the circadian rhythmicity of LT production in NA. Previous studies of the effects of ICS on LT production were inconclusive. Powell et al. suggested that glucocorticoids reduced the biliary levels of cysteinyl-LTs in allergen-induced airway responses in rats, and Tsang et al. reported that FP therapy (500 μg/d for 4 weeks) significantly reduced serum LTB4 in severe bronchiectasis. However, Manso et al. found that 7 days of therapy with 1,600 μg/d of budesonide did not decrease urinary LTE4, but this study was probably invalid because the values in the study subjects (healthy subjects) before treatment were already as low as 20 pg/mg creatinine, making any effect of ICS difficult to detect. O’Shaughnessy et al. reported no effects of FP (1,000 μg/d for 14 days) on urinary LTE4 excretion 4 h after allergen challenge in mild atopic asthma, while Dworski et al. also failed to find any inhibition of LTE4 in BAL fluid by glucocorticoids in atopic asthma after allergen instillation. In both studies, the effects of steroid on LTE4 levels in urine or BAL were evaluated by the change after allergen challenge. The differences in pretreatment lower LTE4 levels (probably due to nonasthmatic healthy subjects) and in method of evaluation (the change a short time after allergen challenge) may explain why previous studies failed to find an ICS inhibitory effect on LT production.

In the present study, a high-dose of ICS had a more suppressive effect on LT production as compared to TX synthesis. These results agree with those of previous studies. Wenzel et al. reported that a single dose of prednisolone decreased LTB4 production, but not 11-dehydro-TXB2 generation, from pulmonary alveolar macrophages obtained by BAL at 4 AM in patients with NA. Nakamura et al. reported that the effects of steroid on the production of eicosanoids differed in TX and LTs: steroid therapy slightly lowered urinary 11-dehydro-TXB2, but significantly decreased urinary LTE4, in patients with rheumatoid arthritis. We also observed that 7 days of methylprednisolone treatment significantly reduced urinary LTE4, but only had a trend to decrease urinary 11-dehydro-TXB2 in asthmatic patients with acute exacerbations (H. Tanaka, MD; unpublished data; May 1998).

We could not determine the mechanism by which ICS suppressed LT production. Hancox et al. reported that 6 weeks of treatment with inhaled budesonide (400 μg/d) reduced the binding activity of the proinflammatory transcription factor nuclear factor-κB (NFκB) in bronchial mucosa in asthmatic subjects, which supports the finding that corticosteroids reduce the level of NFκB binding DNA in human lung tissue and peripheral blood mononuclear cells. Wilson et al. also demonstrated that the anti-inflammatory effects of ICS are mediated through inhibition of NFκB-regulated gene expression. Possibly, inhibition by ICS may be both of the following: (1) direct suppression of the activity of LT producing inflammatory cells, and (2) indirect by reducing eosinophils, lymphocytes, and other inflammatory cells from the airway, and down-regulating cytokine and chemokine, which leads to decreased LTs. We conclude that high-dose ICS therapy can reduce urinary LTE4 levels and abolish urinary LTE4 circadian rhythmicity in patients with asthma, and that LTs may play an important role in the mechanism of nocturnal exacerbations.

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