Leukotriene B₄ (LTB₄) is a potent proinflammatory mediator that may be of particular relevance to the pathology of several respiratory diseases. We have previously reported that neutrophil chemotactic mediators in the lavage fluid of patients with diffuse panbronchiolitis (DPB) consist of many components. In this study, we evaluated the effect of erythromycin (EM) on the pathogenesis of DPB, by examining the level of LTB₄ in the bronchoalveolar lavage (BAL) fluid, and determining the relationship between the level and neutrophil accumulation into the respiratory tract. Pre-EM treatment neutrophil chemotactic activity (NCA) in the patients with DPB was significantly increased compared with that in five healthy nonsmoking volunteers (HVs) (p<0.001), and the level was markedly reduced after EM treatment (p<0.001). The amounts of LTB₄ detected in the BAL fluid from the patients, was also significantly higher than those in control subjects (3.5 ± 1.1 ng/mL vs 0.1 ± 0.0 ng/mL, p<0.001), and the level was significantly reduced after EM treatment (0.6 ± 0.3 ng/mL, p<0.01). In addition, the percent reduction of the level of LTB₄ was significantly correlated with NCA (r=0.832, p<0.01); the reduction was also significantly correlated with neutrophil percentage before and after EM treatment (r=0.778, p<0.05). These findings provide evidence for the potent role of LTB₄ in the respiratory tracts of patients with DPB and suggest that this lipooxygenase metabolite is involved in the recruitment of neutrophils into the airways of the patients. Our findings suggest that LTB₄ is one of the most important chemotactic mediators that has a pathogenic role in the airway damage of DPB. Erythromycin might inhibit the production of this mediator, restrict the neutrophil accumulation, modulate the excessive inflammation in the respiratory tract, and ultimately improve the pathogenesis of DPB.

Key words: bronchoalveolar lavage fluid; diffuse panbronchiolitis; erythromycin; leukotriene B₄

Diffuse panbronchiolitis (DPB), a clinicopathologic entity, is a disease of obscure etiology. The disorder is characterized by chronic inflammation predominantly localized in the respiratory bronchioles, with infiltration of inflammatory cells. Although DPB has been treated with steroid administration and oxygen inhalation in the early stage, and with antibiotics, expectorants, and bronchodilators in the advanced stage, most patients suffered from continuous infection with Pseudomonas aeruginosa and died of progressive lung disease in the past.

In 1987, Kudoh et al reported that the long-term low-dose oral administration of erythromycin (EM) was effective in chronic lower respiratory tract diseases, including DPB. Many investigators suggest that the beneficial effect of EM on the diseases is due to mechanisms other than antibacterial. However, the mechanism of the effect of EM in DPB is still not well characterized. Previously, we reported that neutrophil chemotactic activity (NCA) was markedly elevated in bronchoalveolar lavage (BAL) fluid obtained from the patients with DPB and was significantly reduced after EM treatment. This reduction correlated with the decreased percentages of neutrophils in the BAL fluid. Thus, EM might reduce the excessive inflammation in the respiratory tracts of patients with DPB by inhibiting the migration of neutrophils to the inflammatory sites. We also performed gel-filtration chromatography and revealed four NCA peaks in the elution profile, including a low molecular weight, in the BAL fluid before EM treatment.
tion-promoting factors for human neutrophils: this mediator is known to be released from human neutrophils and alveolar macrophages in response to various stimuli, including exposure to complement, immune complex, and endotoxin. There is evidence of a pathogenetic role for LTβ in several diseases, for example, rheumatoid arthritis and ulcerative colitis. This lipid mediator also increased in the sputum of patients with cystic fibrosis and in the BAL fluid of patients with bronchial asthma and idiopathic pulmonary fibrosis. However, this mediator has not been assayed in the BAL fluid of patients with DPB before and after EM treatment.

In this study, we assayed the LTβ in BAL fluids from patients with DPB to elucidate the relationship between LTβ and neutrophil accumulation and determine how EM takes effect in DPB.

MATERIALS AND METHODS

Patient Population

We studied 9 of 15 patients with DPB (6 men and 3 women; mean age, 37.6 ± 5.4 years; all nonsmokers, shown in Table 1) who were previously described. They could be evaluated for both the NCA and the level of LTβ in the lavage fluid and satisfied the clinical diagnostic criteria for DPB published by the Japanese Ministry of Health and Welfare. These criteria are as follows: (1) symptoms of chronic cough with sputum production and exertional dyspnea; (2) physical signs of coarse crackles and rhonchi; (3) typical radiologic features on chest radiograph of diffuse nodular shadows and hyperinflation; and (4) at least three conditions among the four abnormalities of FEV1 <70%, vital capacity <80% of the predicted value, residual volume >150% of the predicted value, or PaO2 <80 mm Hg. In eight of the nine patients, the disease was histologically confirmed in open lung biopsy specimens; in the remaining one patient (subject 6; shown in Table 1), it was diagnosed clinically.

All nine patients had persistent cough and sputum for more than 2 years (mean durations, 9.2 ± 2.3 years) with exertional dyspnea. All patients were suffering from chronic paranasal sinusitis, which was diagnosed by sinus radiograph, CT scan, or both, and showed diffuse fine nodular shadows in both lungs and overinflation on the chest radiograph. When patients had signs or radiographic findings suggesting pneumonia or acute exacerbation of the disease before enrollment in this study, adequate antibiotics were administered. Thus, none had a pulmonary infection in the 1 month before enrollment in the study.

All patients received 200 mg of EM stearate orally three times per day for more than 6 months until BAL was repeated (mean durations, 9.9 ± 1.0 months). The patients were instructed at each visit to the outpatient ward to take one tablet every 8 hours at home, and were given a 4-week supply. They were instructed to bring back any remaining tablets at each visit to check the numbers of remaining tablets.

None of the patients received corticosteroids or antibiotics other than EM for the duration of this study. No bacteria were cultured from BAL fluid in any of the patients. For comparison, five nonsmoking healthy subjects were also evaluated. In terms of chronic sinusitis, postnasal drip and the abnormal findings on sinus radiographs improved in two of nine patients (22.2%) and three of nine patients (33.3%), respectively.

Bronchoalveolar Lavage

The patients were premedicated intramuscularly with atropine (0.5 mg) and 2% lidocaine was used as a local anesthetic; the airway examination was then carried out with a flexible fiberoptic bronchoscope (Olympus BF-20 type, Olympus Corp, New Hyde Park, NY). The bronchoscope was securely wedged into the subsegmental bronchus of the right middle lobe and 150 mL of sterile 0.9% NaCl at 37°C was infused in three 50-mL aliquots and gently aspirated immediately after each infusion. The recovered lavage fluid was pooled, passed through a double layer of gauze to remove gross mucus, then centrifuged (Shandon Cytospin II) at 1,100 rpm for 2 min to obtain the cell preparation. The cell pellets were stained with May-Giemsa stain, and a differential cell count was performed on 200 cells. More than 90% of the cells were viable by the trypan blue exclusion test. The remaining fluid was centrifuged at 500×g for 5 min, and the supernatant was frozen at −80°C until studied.

Pulmonary Function Tests

Pulmonary function studies for each patient with DPB were performed before and after EM treatment and were expressed as a percent of predicted values (Autospirometer System 9, Minato Medical Science Corp, Osaka, Japan). Standardized methods for pulmonary function tests was described in detail. The resting PaO2 was measured using a gas analyzer (CCD288 Protagos system, Ciba Corning Diagnostics Corp, Medfield, Mass) and expressed in absolute values (mm Hg).

Assay of NCA

Neutrophil chemotactic activity was assessed using a 48-well microchemotaxis chamber (Neuroprobe Inc, Bethesda, Md) as described elsewhere. To each of the bottom wells was added a 25-μL aliquot of the sample. A polycarbonate filter sheet (3-μm pores), not containing polyvinylpyrrolidone (NFB3-PVPF, Costar Corp, Cambridge, Mass), was placed on top of the wells in the bottom plate. Neutrophils for the chemotactic assay were isolated from a healthy volunteer by a mono-poly resolving medium (M-PRM; Flow Laboratories, Irvine, Scotland) density gradient centrifugation. The neutrophils were suspended, at a density of 3X10⁶ cells per milliliter in Hanks’ balanced salt solution (HBSS, GibCO, Grand Island, NY), pH 7.2, containing 0.1% bovine serum albumin. More than 98% of the cells were neutrophils, as determined by Wright-Giemsa stain; cell viability was in excess of 95%, as determined by trypan blue exclusion test. The neutrophils (50-μL aliquots) were placed in each of the upper wells and incubated for 30 min at 37°C in a humidified atmosphere of 95% air:5% CO2. After incubation, the filters were removed, fixed in absolute methanol, and stained with a Giemsa-type stain Diff-

Table 1—Clinical Characteristics of Nine Patients With Diffuse Panbronchiolitis

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Duration of EM Treatment, m</th>
<th>Duration of the Disease, y</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>Chronic Sinusitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>+</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>1/62/F</td>
<td>+</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>2/21/M</td>
<td>+</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>3/49/F</td>
<td>+</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>4/52/M</td>
<td>+</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>5/34/M</td>
<td>+</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>6/19/F</td>
<td>+</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>7/41/M</td>
<td>+</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>8/60/M</td>
<td>+</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>9/25/M</td>
<td>+</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>37.6 ± 5.4</td>
<td>9.9 ± 1.0</td>
<td>9.2 ± 2.3</td>
</tr>
</tbody>
</table>

*OLB=open lung biopsy.
Quik, Harleco, Gibbstown, NJ). The cells that migrated through the filter to the other side were counted. The NCA was measured as the mean number of cells per 10 high-power fields (X1,000).

The results were expressed as the percentage of the chemotactic response to N-formyl-methionyl-leucyl-phenylalanine \(10^{-7}\) M. In each experiment, a negative control was assessed, using HBSS for the BAL fluid and the fraction fluid.

**Extraction Procedures**

The extraction was performed according to the method described previously. Briefly, the samples (each 2 mL) were added to three volumes of ethanol, and centrifuged at 2,000g for 15 min at 0°C. The supernatant was diluted with water to a 15% ethanol content and adjusted to a pH of 3.0 with 1N-HCl. The acidified supernatant was applied to a C18 reverse-phase extraction cartridge (Sep-Pak Cartridge, Waters Assoc, Milford, Mass), that had been preshaved with 15% aqueous ethanol (20 mL), water (20 mL), and the eluate of ethyl acetate/methanol (9:1, v/v) was evaporated under nitrogen. The residue was resuspended in 150 \(\mu\)L methanol/water (7:3) and analyzed by reverse-phase-high-performance liquid chromatography (RP-HPLC). The RP-HPLC was performed using a multisolvent delivery system (Waters model 600, Waters Assoc, Milford, Mass). Separations were achieved (LiChroCART Superspher RP-18, E. Merck, Darmstadt FR, Germany; particle size, 4 \(\mu\)m) at 30°C. The mobile phase consisted of methanol/water/acetic acid (60:40:0.08), and the flow rate was 1.0 mL/min. The residue was dissolved in 0.5 mL of radioimmunoassay (RIA) buffer. In this procedure, the recovery of radioactive standards using \(^3\)H-LTB4 from lavage fluids was 84.0 ± 4.3%.

**RIA LTB4**

Leukotriene B4 RIA was performed according to the manufacturer’s instructions (Amersham Int, Buckinghamshire, UK). Anti-LTB4 serum was diluted in RIA buffer (50 mM TRIS, pH 8.6, containing 0.1% gelatin) and aliquots (0.1 mL) were mixed with standard or samples (0.1 mL) in disposable polypropylene tubes. \(^3\)H-LTB4 in RIA buffer was added to give a total incubation volume of 0.4 mL, and the mixture was incubated at 4°C for 18 h. Free LTB4 was absorbed onto dextran-coated charcoal and, after centrifugation at 2,000g for 10 min at 4°C, the supernatant containing the antibody-bound LTB4 was decanted into scintillation vials. A scintillant cocktail (Clearsol I, Nakalai Tesque, Kyoto, Japan) was added, and the radioactivity was determined in a scintillation counter (Aloka, LSC-900 Liquid Scintillation System, Tokyo, Japan). Cross-reactivities at 50% B/B0 (as supplied by the manufacturer) were as follows: LTB4 (100%), 20-OH-LTB4 (3.9%), 12(R)-HETE (0.4%), 12(S)-HETE (<0.07%). Cross-reactivities to all other compounds tested (ie, LTC4, LTD4, LTE4, prostaglandin F2a, 5-, 12-, and 15-HETE, etc) were <0.05%. The detection limit was 12.5 pg.

**Statistical Analysis**

All results were expressed as mean values ± the standard error of the mean (SEM). For experiments with two groups, paired and unpaired Student’s t test was used for analysis. The regression line for the relationship between the NCA in the lavage fluids and the LTB4 concentration was computed by the least-squares method and the correlation coefficient was also obtained. Probability values of 0.05 or less were considered significant. The percent reduction of NCA was calculated according to the following formula:

\[
\text{Percent reduction of NCA} = \frac{\text{NCA in pre-EM treatment BAL fluid} - \text{NCA in post-EM BAL fluid}}{\text{NCA in pre-EM treatment BAL fluid}} \times 100.
\]

**Table 2—Bronchoalveolar Lavage Fluid Obtained From Nine Patients With Diffuse Panbronchiolitis Before and After Erythromycin Treatment**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Paired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, (\times 10^9)/mL</td>
<td>8.10 ± 2.10</td>
<td>3.50 ± 0.94</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>AM, (\times 10^9)/mL</td>
<td>2.00 ± 0.53</td>
<td>1.70 ± 0.52</td>
<td>NS*</td>
</tr>
<tr>
<td>Lym, (\times 10^9)/mL</td>
<td>1.20 ± 0.34</td>
<td>0.41 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Neu, (\times 10^9)/mL</td>
<td>6.26 ± 1.92</td>
<td>1.40 ± 0.75</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Es, (\times 10^9)/mL</td>
<td>0.07 ± 0.04</td>
<td>0.01 ± 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS=not significant; AM=alveolar macrophages; Lym=lymphocytes; Neu=neutrophils; Es= eosinophils.

The percent reduction of neutrophil percentage was calculated as:

\[
\% = \frac{\text{Neutrophil percentage in pre-EM treatment BAL fluid} - \text{Neutrophil percentage in post-EM treatment BAL fluid}}{\text{Neutrophil percentage in pre-EM treatment BAL fluid}} \times 100.
\]

The percent reduction of LTB4 was calculated as:

\[
\% = \frac{\text{LTB4 in pre-EM treatment BAL fluid} - \text{LTB4 in post-EM treatment BAL fluid}}{\text{LTB4 in pre-EM treatment BAL fluid}} \times 100.
\]

**RESULTS**

**Lavage Findings Before and After EM Treatment**

Table 2 shows the mean values for BAL parameters before and after EM treatment. The mean values for total cell and neutrophil number before EM treatment were significantly higher than those in healthy volunteers (total cell number, 8.10 ± 2.10 \(\times 10^5\)/mL vs 2.10 ± 0.75 \(\times 10^5\)/mL, p<0.05; neutrophil number, 6.26 ± 1.92 \(\times 10^5\)/mL vs 0.03 ± 0.01 \(\times 10^5\)/mL, p<0.05); the values were significantly reduced after EM treatment (Table 2), corresponding with an improvement in clinical symptoms and findings. The number of alveolar macrophages, lymphocytes, and eosinophils remained unchanged. The recovery rate before and after EM treatment was similar (47.3 ± 6.0% vs 43.5 ± 6.5%; n=9).

**Changes in Respiratory Function Test**

Table 3 shows the mean values for respiratory function tests after EM treatment. The mean values for FVC, FEV1, RV/TLC, and PaO2 were significantly reduced after EM treatment (Table 3), corresponding with an improvement in clinical symptoms and findings.

**Table 3—Respiratory Function Test and Blood Gas Analysis in Nine Patients With Diffuse Panbronchiolitis**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Paired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC, %</td>
<td>76.7 ± 5.7</td>
<td>94.8 ± 5.0</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>FEV1, %</td>
<td>66.1 ± 5.6</td>
<td>72.9 ± 5.1</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>RV/TLC, %</td>
<td>38.9 ± 3.7</td>
<td>54.1 ± 2.3</td>
<td>NS*</td>
</tr>
<tr>
<td>%Dco, %</td>
<td>59.8 ± 4.5</td>
<td>71.8 ± 4.0</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Dco/Va, mL/min/mm Hg</td>
<td>2.9 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>V25/HT, L/s/m</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>PaO2, mm Hg</td>
<td>75.1 ± 4.9</td>
<td>83.2 ± 3.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS=not significant.
function parameters and arterial blood gas analysis before and after EM treatment. After EM treatment, the mean values for the four parameters of respiratory function (\%VC, FEV\textsubscript{1}0\%, \%Dco and Dco/VA) significantly improved. There were no significant changes in the other parameters after EM treatment.

Changes in NCA in BAL Fluid

The mean NCA value was markedly reduced after EM treatment (from 53.0 ± 4.0\% to 26.4 ± 1.7\%; \textit{p}<0.001). The albumin concentration in the BAL fluid sample was not significantly changed after EM treatment (41.7 ± 3.9 mg/dL vs 37.5 ± 7.7 mg/dL). In addition, there were no significant correlations between the recovery rate of volume infused and NCA either in BAL fluid before EM treatment or after the treatment. The albumin concentration and NCA also did not correlate in BAL fluid. Thus, we regarded NCA as being unaffected by recovery rate or by albumin concentration; therefore, we expressed the value without correction. The BAL fluid samples from patients with DPB contain more bacterial endotoxin than samples from healthy subjects (before treatment, 1,500 ± 140 pg/mL; after treatment, 700 ± 48 pg/mL; healthy subjects, 130 ± 54 pg/mL). However, the endotoxin might have not affected the chemotaxis findings, because Issekutz and Bhimji\textsuperscript{21} reported that endotoxin did not induce neutrophil chemotaxis in the \textit{in vitro} assay and because of our finding that neither of the two endotoxin preparations \textit{(Pseudomonas aeruginosa} serotype 10 and \textit{Salmonella typhosa}; Sigma) tested induced neutrophil chemotaxis in the \textit{in vitro} assay at concentrations 10 to 100 times higher than those inducing leukocyte infiltration \textit{in vivo}.\textsuperscript{3}

The Level of LTB\textsubscript{4} in Lung Lavage

Figure 1 shows the level of LTB\textsubscript{4} in the lavage fluids obtained from patients with DPB before and after EM treatment and from healthy volunteers. Before EM treatment, there was a significant increase in the level of LTB\textsubscript{4} in the lavage fluid of patients with DPB, compared with that in healthy volunteers (3.5 ± 1.1 ng/mL to 0.1 ± 0.0 ng/mL; \textit{p}<0.001). By contrast, the level of LTB\textsubscript{4} in the lavage fluid of patients with DPB after EM treatment was markedly reduced (0.6 ± 0.3 ng/mL; \textit{p}<0.01). In addition, the level of LTB\textsubscript{4} in the BAL fluid of patients with DPB after EM treatment was still significantly elevated compared with that in healthy volunteers (\textit{p}<0.05).

Correlation Between Reduction in Neutrophil Percentage and the Level of LTB\textsubscript{4} Before and After EM Treatment

Figure 2 shows that the neutrophil percentage and the level of LTB\textsubscript{4} in the BAL fluid of the nine patients with DPB were compared before and after EM treatment. There was a significant correlation between the reduction in neutrophil percentage and the level of LTB\textsubscript{4} (\textit{r}=0.778, \textit{p}<0.05).
Correlation Between Reduction in NCA and the Level of LT\textsubscript{B4} Before and After EM Treatment

Neutrophil chemotactic activity and the level of LT\textsubscript{B4} in the BAL fluid of the nine patients with DPB were compared before and after EM treatment. There was a significant correlation between the reduction in NCA and the level of LT\textsubscript{B4} (r=0.832, p<0.01; Fig 3).

DISCUSSION

The sustained neutrophil accumulation in the airway is a characteristic clinical feature in DPB.\textsuperscript{3,4} We reported that the accumulation of neutrophils into the respiratory tract of this disease is induced by a variety of chemotactic mediators.\textsuperscript{4} Thus, we have hypothesized that these mediators, including LT\textsubscript{B4}, in the lavage fluid induce neutrophil accumulation into the airspace, cause injury to the host by generating the oxidative and proteolytic products of neutrophils, and then be associated with the severity of inflammation in the lower respiratory tracts of patients with DPB.

Leukotriene B\textsubscript{4} is the major product of arachidonic acid metabolism produced by the 5-lipoxygenase pathway in human neutrophils and alveolar macrophages in response to various stimuli.\textsuperscript{8-10} This lipid mediator is considered to be proinflammatory in vitro and in vivo since it promotes the chemotaxis of neutrophils, monocytes, and fibroblasts, causes neutrophil degranulation, and acts as a calcium ionophore.\textsuperscript{5,9,22-24} LT\textsubscript{B4} injection results in neutrophil accumulation within the human skin.\textsuperscript{25} Leukotriene B\textsubscript{4} inhalation was reported to induce neutrophil accumulation in the BAL fluid from dogs.\textsuperscript{26} Martin et al\textsuperscript{27} instilled LT\textsubscript{B4} into a subsegment of the right middle lobe using a fiberoptic bronchoscope in healthy human volunteers. They first showed that in normal healthy human lungs, this chemotactic mediator could recruit active neutrophils into the airspaces. Recently, LT\textsubscript{B4} has been reported to be responsible for chemotactic activity for neutrophils in various diseases, including rheumatoid arthritis and inflammatory bowel disease.\textsuperscript{11,12} Leukotriene B\textsubscript{4} has also been identified in sputum extracts from patients with chronic bronchitis, bronchiectasis, chronic obstructive pulmonary disease, and cystic fibrosis\textsuperscript{13-15} and in BAL fluids from patients with bronchial asthma and idiopathic pulmonary fibrosis.\textsuperscript{16} Thus, this oxygenated product of arachidonic acid metabolism has a wide range of pharmacologic actions that may be relevant to the pathologic processes associated with these chronic respiratory diseases, including DPB.

In this study, we showed that the level of LT\textsubscript{B4} in the BAL fluid from patients with DPB before EM treatment was significantly increased compared with that in healthy volunteers. These findings suggest that LT\textsubscript{B4} could cause cellular recruitment from the systemic circulation into the respiratory tract of patients with DPB and initiate an inflammatory reaction in vivo. Recently, the low-dose long-term oral administration of EM has been a preferred therapy for DPB since this antibiotic was reported to be effective in the improvement of clinicopathologic features of the chronic lower respiratory tract disorders.\textsuperscript{2} Erythromycin is a broad-spectrum macrolide antibiotic commonly used on patients with lower respiratory tract diseases. This antibiotic is most effective against Gram-positive cocci and bacilli, but is not active against most aerobic Gram-negative bacilli, e.g., P. aeruginosa. In addition, the antibacterial activity of EM depends on the concentration of the drug. However, the dosage used on the patients with DPB, 600 mg/d, is very low compared with the usual oral dose of EM for adults, ranging from 1 to 2 g/d.

Nagai et al\textsuperscript{28} reported that the maximal serum and sputum levels of EM in both responders and nonresponders to the low-dose long-term treatment were below the minimal inhibitory concentration (MIC) of several clinically pathogenic bacteria often isolated from the sputum of the patients, e.g., P. aeruginosa and Hemophilus influenzae. Various studies also have reported the influence of EM on the host defense mechanisms of patients with DPB, in terms of the changes in lymphocyte subsets,\textsuperscript{29} increased natural killer cell activity,\textsuperscript{30} and suppression of elastase activity.\textsuperscript{31} From these findings, the current concept...
is that EM might act on the pathogenesis of DPB as an anti-inflammatory agent rather than as a bactericidal. However, the mechanism of the effect of EM in DPB remains obscure.

We previously reported that in pre-EM treatment BAL fluid of patients with DPB, the percentages of neutrophils and NCA were significantly elevated, compared with those in healthy nonsmoking volunteers; these enhancing effects and neutrophilia in the BAL fluid of the patients were reduced to near normal after EM treatment; and the reduction of NCA correlated with improvements in neutrophil percentages.\(^3\)\(^4\) Herein we showed that the level of LTB\(_4\) in the BAL fluid of patients with DPB after EM treatment was significantly decreased in accordance with the reduction of neutrophil accumulation. In addition, we found that there was a significant correlation not only between the reduction of NCA and that of the level of LTB\(_4\) but also between the improvement of neutrophil percentages and LTB\(_4\) reduction before and after EM treatment. These findings suggest an association between this lipoxygenase metabolite and the severity of the bronchoalveolitis of patients with DPB. Taken together, EM reduces the accumulation of neutrophils in the respiratory tract of patients with DPB by suppressing the production of the chemoattractant, LTB\(_4\), and ultimately might inhibit inappropriate and excessive inflammation, which contributes to tissue damage by generating oxygen radicals and triggering the action of elastase.

In conclusion, in our study, the production of the 5-lipoxygenase product LTB\(_4\) was significantly increased in the BAL fluid of patients with DPB. The increased concentrations of this product in the patients could be associated with the severity of the inflammatory reactions. The synthesis and release of this arachidonate cascade in response to a number of naturally occurring stimuli was reduced after EM treatment, in accord with the reduction of neutrophil accumulation into the inflammatory sites in this disease. These findings suggest that the macrolide antibiotic agent, erythromycin, effectively modulates various immune and inflammatory processes via an activation of the lipoxygenase pathway of arachidonic acid in several kinds of inflammatory cells, including neutrophils and alveolar macrophages, reduces the excessive inflammation in the lower respiratory tract, and ultimately improves the pathogenesis of this disease, DPB.

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REFERENCES

20. Powell WS. Rapid extraction of arachidonic acid metabolites from biological samples using octadecylsilyl silica. Methods Enzymol 1982; 86:467-77
23 Rollins TE, Zanolari B, Springer MS, et al. Synthetic leukotriene B₄ is a potent chemotaxin but a weak secretagogue for human PMN. Prostaglandins 1983; 25:281-89

American Board of Internal Medicine 1995 Certification and Qualifying Examinations in Cardiovascular Disease

Registration Period: January 1 - April 1, 1995
Certification Examination Dates: November 9 - 10, 1995
Qualifying Examination Dates: November 9, 1995

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