Clinical and immunoregulatory effects of roxithromycin therapy for chronic respiratory tract infection

H. Nakamura, S. Fujishima, T. Inoue, Y. Ohkubo, K. Soejima, Y. Waki, M. Mori, T. Urano, F. Sakamaki, S. Tasaka, A. Ishizaka, M. Kanazawa, K. Yamaguchi

Clinical and immunoregulatory effects of roxithromycin therapy for chronic respiratory tract infection. H. Nakamura, S. Fujishima, T. Inoue, Y. Ohkubo, K. Soejima, Y. Waki, M. Mori, T. Urano, F. Sakamaki, S. Tasaka, A. Ishizaka, M. Kanazawa, K. Yamaguchi. ©ERS Journals Ltd 1999.

ABSTRACT: The clinical and immunoregulatory effects of long-term macrolide antibiotic therapy for chronic lower respiratory tract infections (CLRTI) were investigated.

Clinical parameters and neutrophil chemotactic mediators in the epithelial lining fluid (ELF) of CLRTI patients (n=10) were examined before and after 3 months oral administration of roxithromycin (RXM). The in vitro effects of RXM were also examined on the release of these mediators from alveolar macrophages (AM) and neutrophils.

Arterial oxygen tension (p<0.05), vital capacity (VC) (p<0.001), %VC (p<0.05) and forced expiratory volume in one second (p<0.01) were improved after RXM treatment, but airway bacteria were not eradicated. Among the mediators, the levels of interleukin (IL)-8, neutrophil elastase (NE) and leukotriene B4 (LTB4) were higher in ELF than in plasma of CLRTI patients and they decreased after RXM treatment (n=7, p<0.05 for each). RXM concentrations were significantly increased in the bronchoalveolar lavage cells of the treated patients. In in vitro experiments, RXM showed inhibitory effects on IL-8 release from AM and neutrophils.

In conclusion, interleukin-8, neutrophil elastase and leukotriene B4 contribute to the neutrophilic inflammation in the airways of chronic lower respiratory tract infection patients and the clinical effects of roxithromycin may, in part, be attributable to the suppression of excess release of the chemotactic mediators from inflammatory cells.

Eur Respir J 1999; 13: 1371–1379.

The clinical effectiveness of long-term macrolide antibiotic therapy has recently been reported in patients with diffuse panbronchiolitis (DPB) [1–3]. Before the introduction of macrolide therapy, DPB was a chronically progressive disease which resulted in chronic respiratory failure. DPB is characterized by chronic inflammation of the respiratory bronchioles and parabronchial and luminal infiltration of inflammatory cells, and is associated with continuous airway infection especially Pseudomonas aeruginosa [1, 2]. This disease is prevalent mainly in Japan, but was recently reported in the USA [4]. Similar airway inflammation, namely chronic lower respiratory tract infections (CLRTI), is also observed in patients with bronchiectasis (BE). Previous studies have suggested that this effect cannot be ascribed to its antibacterial action [3, 5] but rather, its immunoregulatory function, such as inhibition of neutrophil chemotaxis and superoxide production in vitro [5, 6]. An in vivo study suggested that macrolide treatment induced a decrease in the neutrophil chemotactic activities of bronchoalveolar lavage (BAL) fluid from patients with DPB [2]. However, the precise mechanisms responsible for this clinical efficacy remain unclear.

Neutrophil accumulation is a defining feature of airway inflammation in patients with CLRTI including DPB and BE [1, 2]. Although neutrophils are important effector cells against bacterial infections, neutrophil-derived oxygen metabolites and proteolytic enzymes could have harmful effects on lung tissues and may exacerbate clinical symptoms [1, 7]. Various mediators including interleukin (IL)-8, IL-1β, leukotriene (LT)B4 and neutrophil elastase (NE) may be involved in the recruitment and activation of neutrophils in patients with airway infections including CLRTI and cystic fibrosis [7–10]. Previous reports have indicated complex interactions between these mediators both in vivo and in vitro. For example, IL-8 is induced by IL-1β in various cells [11–13]. LTB4 upregulates neutrophil-derived IL-8 [14]. IL-8 correlates with NE in the airways of CLRTI patients [7], and NE from patients with cystic fibrosis induces IL-8 gene expression in epithelial cells [15]. Thus, it is reasonable to speculate that the combination of these mediators synergistically induces airway inflammation. However, precise roles of these mediators in the pathogenesis of CLRTI have not been fully elucidated. Therefore, the neutrophil chemotactic mediators IL-8, IL-1β, tumour necrosis.
Subjects

Investigated were veolar macrophages (AM) and neutrophils in culture on the release of these chemotactic mediators from al-

supernatant were then measured, and the effects of RXM in BAL cells, plasma and BAL supernatant were then measured, and the effects of RXM on the release of these chemotactic mediators from alveolar macrophages (AM) and neutrophils in culture investigated.

Patients and methods

Subjects

Ten patients with CLRTI, who had complained of chronic purulent sputum for at least 6 months, and whose chest radiographs suggested bronchiolitis and/or BE were studied. Seven patients were diagnosed as DPB and the age of the patients (mean±SEM) was 61.8±5.5 yrs, and none of the patients treated with RXM 150 mg b.i.d. for 3 months. No other antibiotics or glucocorticoids were administered during the period. Clinical symptoms were recorded by a pulmonologist in the outpatient clinic every 2 weeks. Clinical improvement after the 3-month treatment period was evaluated by three other pulmonologists who were not involved in this study. Based on physical examination and subjective findings, clinical symptoms were classified according to the criteria described in table 1.

Bronchoalveolar lavage

BAL was performed using a previously described standard protocol [17, 18]. In brief, a bronchoscope was wedged into a segment of the right middle lobe or lingula in which inflammatory findings were observed on a chest radiograph or CT scan. Sterile normal saline at room temperature was instilled through the bronchoscope in 50-mL volumes to a total of 150 mL. The recovered BAL fluid was filtered through sterile gauze, and the cells were counted. Cytospin preparations were made for cell differential counts. The fluid was centrifuged at 200 x g for 5 min, and the supernatant was collected. No significant adverse effects were observed after the procedure.

Quantification of neutrophil chemotactic mediators

Concentrations of neutrophil chemotactic mediators in BAL fluid and plasma of CLRTI patients and control subjects were determined as follows. IL-8 (Genentech Inc., San Francisco, CA, USA), TNF-α (Otsuka, Tokushima, Japan) and NE (E. Merck, Darmstadt, Germany) levels were measured with a sandwich enzyme-linked immunosorbent assay (ELISA). LTB4 (Amersham, Buckinghamshire, UK), IL-1β (Amersham) and C5a (Medgenix Diagnostics S.A., Brussels, Belgium) levels were measured.

Table 1. – Assessment of clinical symptoms and chest radiographic findings

| Score | Cough | Sputum | Wheeze | Dyspnoea | Sinusitis | Diffuse granular shadow | Peribronchial infiltrates |
|-------|-------|--------|--------|----------|-----------|------------------------|-------------------------|
| 3     | Persistent | Purulent | Diffuse severe |At rest |Severe purulent discharge | Severe |Severe |
| 2     | Frequent | Purulent | Diffuse mild |On mild exertion |Mild purulent discharge | Moderate |Moderate |
| 1     | Occasional | Serous | Localized mild |On moderate exertion |Serous discharge | Mild |Mild |
| 0     | None | None | None |None |None |None |None |
using a radioimmunoassay (RIA). Epithelial lining fluid (ELF) concentrations of these mediators were calculated using a BAL fluid to plasma ratio of urea nitrogen [19].

Quantification of roxithromycin concentrations

RXM concentrations in plasma, BAL supernatant and BAL cells were determined by disc method using *Micrococcus luteus* American Type Culture Collection (ATCC; Rockville, MD, USA) 9341 [20]. BAL was performed 16 h after the final ingestion of RXM. Blood was drawn 12 h after the intake. BAL cells were frozen at -70°C for 1 h and thawed at 37°C for 1 h. These procedures were repeated three times, and intracellular RXM was extracted [21]. Intracellular RXM concentrations of BAL cells were calculated using 2,491 mg/mL as the volume of a macrophage [22]. Neutrophil volume was not considered for this calculation because the total volume in BAL cells is much smaller and its life span is much shorter than a macrophage.

In vitro cytokine production and release from alveolar macrophages and neutrophils

BAL cells containing >90% AM from four control subjects were used to examine the potential of AM to produce and release cytokines. The cells were divided into four groups and each group was preincubated with RPMI-1640 containing either 0, 0.5, 5 or 50 μg/mL of RXM for 30 min. Then, 1 μg/mL of lipopolysaccharide (LPS; *Escherichia coli* 055: B5; Difco, Detroit, MI, USA) was added and the cells were cultured for 4 h. To quantify cell-associated IL-8, the cells were lysed, and cell-associated IL-8, and extracellular IL-8 and TNF-α were measured by ELISA, and extracellular IL-1β was measured by RIA, as described above. Peripheral blood neutrophils were separated by a density gradient centrifugation from seven healthy volunteers. Neutrophil purity was >98%. The preincubation and culture procedures were the same as those for AM except that extracellular TNF-α and IL-1β were not measured.

Statistical analysis

All data are presented as mean±SEM. Unpaired Student’s t-test and analysis of variance (ANOVA) were used to compare mean values. To analyse the relationship between paired values, paired Student’s t-test or Wilcoxon test was used based on the distribution of the data. Simple regression analysis was performed to evaluate correlation among neutrophil counts and mediator concentrations in ELF. Significance was accepted at p<0.05.

### Results

#### Clinical results

Table 2 represents clinical symptoms during treatment with RXM in CLRTI patients. CLRTI patients showed improvement of clinical symptoms based on the total scores (p<0.0001), but the percentage of improvement varied in each patient. Most of the clinical symptoms were improved four weeks after initiating treatment with RXM. Total symptom scores were higher in DPB (n=7) than in BE (n=3) before treatment (11.6±0.6 versus 7.7±1.2; p<0.01). The total scores decreased with 12 weeks of treatment in DPB (p<0.001). There was no difference in the percentage of improvement between DPB and BE (60.1±7.2 versus 59.0±5.9%).

Table 3 shows chest radiographic findings and sputum culture before and after treatment with RXM. Improvement of the total scores on chest radiographs was observed in all CLRTI patients (p<0.001), but the degree of the improvement differed in each patient. Total chest radiographic scores were higher in DPB (n=7) than in BE (n=3) before treatment (4.6±0.2 versus 2.0±0.6; p<0.001). The total scores decreased with 12 weeks of treatment in DPB (p<0.01). There was no difference in the percentage of improvement between DPB and BE (61.4±9.7 versus 72.3±14.7%). Sputum bacteria were cultured in blood agar, chocolate agar, Conradi-Dragilski agar, and Sabouraud agar media for 48 h and the numbers of bacteria were classified as: 3+, >500 colonies; 2+, 200–500 colonies; 1+, 1–199 colonies; or ND (not detected). Purulent sputum was obtained from all 10 patients, although no bacteria were detected in two patients (cases 7 and 8). Although the number of bacteria decreased in three patients including two with alteration of bacteria (cases 6 and 9), bacterial eradication was not observed in any of the patients.
patients. Treatment with RXM due to the inconvenience for the function tests were not performed in two patients, and (8,122±1,016 cells mm⁻³, white blood cell counts did not change significantly.

C-reactive protein (2.38±0.85 mg dL⁻¹ versus 0.56±0.25 mg dL⁻¹, p<0.05, n=9) and erythrocyte sedimentation rate (54.2±11.5 mm h⁻¹ versus 33.7±13.3 mm h⁻¹, p<0.001, n=9) were decreased after treatment with RXM. Peripheral white blood cell counts did not change significantly (8,122±1,016 cells mm⁻³ versus 6,244±566 cells mm⁻³, p=0.08, n=9). Arterial blood gas analysis and pulmonary function tests were not performed in two patients, and blood testing was not carried out in one patient after treatment with RXM due to the inconvenience for the patients.

The results of arterial blood gas analysis and pulmonary function tests are shown in table 4. Arterial oxygen tension (PₐO₂), vital capacity (VC), %VC and forced expiratory volume in one second (FEV₁) were significantly improved in patients with CLRTI after treatment with RXM. PₐCO₂: arterial carbon dioxide tension; VC: vital capacity; FEV₁: forced expiratory volume in one second. *: p<0.05; **: p<0.01; ***: p<0.001, compared with the value before treatment with RXM. (1 Torr=0.133 kPa.)

Table 3. – Changes in chest radiograph and bacteriological findings before and after treatment with roxithromycin in chronic lower respiratory tract infections

| Case | Diag. | Chest radiograph | Improvement | Sputum culture |
|------|-------|------------------|-------------|----------------|
|      |       | Diffuse granular shadow | | |
|      |       | Before | After | % | Before | After | |
|      |       | Peribronchial infiltrates | | |
|      |       | Before | After | | |
|      |       | Total | | | |
| 1    | BE    | 0      | 0     | 100 | Pneumococcus | H. influenzae |
| 2    | DPB   | 1      | 0     | 60  | P. aeruginosa | H. influenzae |
| 3    | DPB   | 2      | 1     | 50  | P. aeruginosa | H. influenzae |
| 4    | DPB   | 3      | 0     | 100 | Pneumococcus  | H. influenzae |
| 5    | DPB   | 3      | 2     | 40  | P. aeruginosa | H. influenzae |
| 6    | BE    | 1      | 0     | 67  | H. influenzae | H. parahaemolyticus |
| 7    | BE    | 0      | 0     | 50  | ND | ND |
| 8    | DPB   | 2      | 2     | 25  | ND | ND |
| 9    | DPB   | 3      | 1     | 80  | H. influenzae | Pneumococcus |
| 10   | DPB   | 3      | 1     | 75  | Pneumococcus | Pneumococcus |

Mean 2.0 0.8 1.8 0.6 3.8 1.4 65

Diag.: diagnosis; BE: bronchectasis; DPB: diffuse panbronchiolitis. H. influenzae: Haemophilus influenzae; P. aeruginosa: Pseudomonas aeruginosa; H. parahaemolyticus: Haemophilus parahaemolyticus. Sputum culture, 3+: >500 colonies; 2+: 200–500 colonies; 1+: 1–199 colonies; ND: not detected.

Bronchoalveolar lavage cells and chemotactic mediators in plasma and bronchoalveolar lavage fluid of untreated patients

BAL cell counts and differentials before and after RXM treatment are shown in table 5. BAL was performed in all 10 CLRTI patients before treatment and in seven patients after RXM treatment. Three patients did not agree to the second bronchoscopy. The percentage of neutrophils was increased in CLRTI patients compared with that in the control subjects before treatment (p<0.05). After treatment with RXM, total cell, lymphocyte, and neutrophil counts were significantly decreased (p<0.05, for each by Wilcoxon test). The decrease in total cell and neutrophil counts was also significant in DPB (n=5) (p<0.05 for both). The percentage of macrophages increased and that of neutrophils decreased after treatment in DPB (p<0.05 for both) as well as in all CLRTI patients. The percentage of neutrophils was higher in untreated DPB (n=5) than in control subjects (p<0.0001). Total cell counts, neutrophil counts and percentage before treatment in BE (n=3) were 97.3±57.9 × 10⁶ cells mL⁻¹ ELF⁻¹, 69.3±60.2 × 10⁶ cells mL⁻¹ ELF⁻¹, and 46.4±22.3%, respectively.

Pretreatment levels of neutrophil chemotactic mediators in ELF and plasma are shown in table 6. IL-8 concentrations in the ELF of CLRTI patients were higher than those of control subjects (p<0.05). IL-8, IL-1β, NE and LTB₄ concentrations in the ELF from CLRTI patients were increased compared with plasma concentrations (n=8, p<0.01, p<0.05, p<0.05 and p<0.05, respectively). The mediator concentrations in ELF were compared between DPB (n=7) and BE (n=3). IL-8 levels were lower in DPB than in BE (34.1±8.5 versus 88.2±19.4 ng mL⁻¹, p<0.05) and were higher in both DPB and BE than in...
control subjects (p<0.05 for both). There were no significant differences in ELF concentrations of TNF-α, IL-1β, NE, LTB4, and C5a between DBP and BE (0.16±0.07 versus 0±0 ng·mL⁻¹, 4.95±1.50 versus 9.01±0.17 ng·mL⁻¹, 75.1±39.2 versus 171.3±59.7 μg·mL⁻¹, 4.31±1.64 versus 3.14±1.66 ng·mL⁻¹, 360±188 versus 267±267 ng·mL⁻¹, respectively).

Roxithromycin concentrations in plasma, epithelial lining fluid and bronchoalveolar lavage cells

After three months of administration, RXM concentrations in plasma, BAL supernatants and BAL cells were measured in CLRTI patients. As shown in table 7, intracellular RXM concentrations in AM were markedly increased compared with those in plasma and ELF (p<0.01), but ELF concentrations were similar to those in plasma.

Comparison of epithelial lining fluid mediators before and after the treatment with roxithromycin

As shown in figure 1, the ELF levels of IL-8 (60.9±13.8 versus 17.3±40 ng·mL⁻¹, p<0.05), NE, LTB4 and C5a between DBP and BE (0.16±0.07 versus 0±0 ng·mL⁻¹, 4.95±1.50 versus 9.01±0.17 ng·mL⁻¹, 75.1±39.2 versus 171.3±59.7 μg·mL⁻¹, 4.31±1.64 versus 3.14±1.66 ng·mL⁻¹, 360±188 versus 267±267 ng·mL⁻¹, respectively).

Table 5. – Bronchoalveolar lavage cell counts and differentials before and after treatment with roxithromycin

|                     | Patients with CLRTI (n=7) | Control subjects (n=3) |
|---------------------|---------------------------|------------------------|
|                     | Before treatment          | After treatment         |
| Total cell count    | 81.7±32.6                 | 13.8±5.3               |
| ×10⁶·mL ELF⁻¹       | (45.8)                    | (11.2)*                |
| Macrophage count    | 10.2±4.1                  | 4.0±1.2                |
| ×10⁵·mL ELF⁻¹       | (5.9)                     | (4.0)                  |
| % Macrophage        | 19.2±8.5***               | 48.0±13.9*             |
| Lymphocyte count    | 3.1±1.0                   | 0.6±0.2                |
| ×10⁵·mL ELF⁻¹       | (2.0)                     | (0.9)*                 |
| % Lymphocyte        | 6.9±3.0                   | 7.1±2.6                |
| Neutrophil count    | 68.5±31.8                 | 9.2±5.8                |
| ×10⁵·mL ELF⁻¹       | (34.0)                    | (2.6)*                 |
| % Neutrophil        | 73.9±11.0**               | 44.8±14.8*             |

Values are presented as mean±SEM with median values in parentheses. CLRTI: chronic lower respiratory tract infections; ELF: epithelial lining fluid. **: p<0.01; ***: p<0.001, compared with control subjects; *: p<0.05, compared with the value before treatment.

Effects of roxithromycin on interleukin-8, tumour necrosis factor-α and interleukin-1β release from alveolar macrophages

The ELF concentrations of IL-8, NE and C5a correlated with neutrophil counts before treatment with RXM in 10 CLRTI patients (r=0.642, p<0.05; r=0.845, p<0.01; r=0.845, p<0.01, respectively) but this was only due to two outliers. TNF-α, IL-1β, and LTB4 concentrations did not correlate with the neutrophil counts. There was a significant correlation between IL-8 and either NE (r=0.837, p<0.01) or IL-1β (r=0.670, p<0.05). However, C5a, LTB4, nor TNF-α correlated with IL-8. In addition, NE correlated with C5a or IL-1β, (r=0.770, p<0.01; r=0.650, p<0.05, respectively). There were no significant correlations between any other combinations of the mediators before treatment with RXM in CLRTI patients.

Table 6. – Neutrophil chemotactic mediator concentrations in epithelial lining fluid (ELF) and plasma from patients with chronic lower respiratory tract infections (CLRTI) and control subjects

|                     | CLRTI                     | Control                    |
|---------------------|---------------------------|----------------------------|
|                     | ELF (n=10)                | Plasma (n=8)               |
| IL-8 ng·mL⁻¹        | 50.3±11.3***              | <0.05                      |
| TNF-α ng·mL⁻¹       | 0.11±0.06                 | 0.018±0.012                |
| IL-1β ng·mL⁻¹       | 6.17±1.23***              | <0.01                      |
| NE μg·mL⁻¹          | 103.9±34.2*               | 0.34±0.07                  |
| LTB4 ng·mL⁻¹        | 3.95±1.21*                | 0.053±0.008                |
| C5a ng·mL⁻¹         | 332±146                   | 18.0±4.7                   |

|                     | ELF (n=3)                 | Plasma (n=3)               |
|                     | <0.05                     | <0.005                     |
| IL-8 ng·mL⁻¹        | 1.66±0.29                 | 0.016±0.002                |
| TNF-α ng·mL⁻¹       | <0.03                     | 0.15±0.02                  |
| IL-1β ng·mL⁻¹       | 6.1±0.08                  | 0.053±0.008                |
| NE μg·mL⁻¹          | 210±43                    | 22.0±0.6                   |

Values are presented as mean±SEM. IL: interleukin; TNF-α: tumour necrosis factor-α; NE: neutrophil elastase; LTB4: leukotriene B4; **: p<0.01, compared with ELF concentrations in control subjects; *: p<0.05; **: p<0.01, compared with plasma concentrations in CLRTI.
Table 7. – Roxithromycin concentrations in plasma, epithelial lining fluid (ELF), and bronchoalveolar lavage (BAL) cells from patients with chronic lower respiratory tract infections after three months of treatment

| Case | Plasma µg·mL⁻¹ | BAL fluid µg·mL⁻¹ | Calculated ELF µg·mL⁻¹ | BAL cell lysate µg·mL⁻¹ | AM count in BAL cell lysate ×10⁶·mL⁻¹ | Calculated BAL cell µg·mL⁻¹ |
|------|----------------|------------------|------------------------|-------------------------|--------------------------------------|-----------------------------|
| 1    | 4.29           | <0.05            | <0.14                  | ND                      | ND                                   | ND                          |
| 2    | 2.55           | 0.10             | 1.56                   | ND                      | ND                                   | ND                          |
| 3    | 4.27           | <0.05            | <2.67                  | <0.05                   | 2.05                                 | <97.8                       |
| 4    | 8.78           | 0.11             | 4.77                   | 0.64                    | 3.06                                 | 840                         |
| 7    | 1.94           | <0.05            | <1.13                  | 0.56                    | 2.63                                 | 855                         |
| 9    | ND             | <0.05            | <1.41                  | 2.63                    | 11.04                                | 956                         |
| 10   | 0.59           | <0.05            | <2.07                  | 4.72                    | 9.49                                 | 1967                        |
| Mean±SEM | 3.74±1.16 | 0.03±0.02    | 0.90±0.68              | 1.71±0.88              | 5.65±1.91                           | 924±313³, ***               |

AM: alveolar macrophage; ND: not done. ³: calculated including the data below detection limit as 0; ***: p<0.001, compared with plasma and ELF concentrations.

Discussion

RXM significantly improved the clinical symptoms of CLRTI patients despite its failure to eliminate airway bacteria. It has been demonstrated in this study that various chemotactic mediators including IL-8, NE, and C5a contribute to the airway inflammation of CLRTI patients. The clinical improvement after the RXM treatment was associated with a decrease in IL-8, NE, and LTB4 concentrations in ELF and the resulting attenuation of neutrophilic inflammation. A marked RXM accumulation in BAL cells was demonstrated in vivo and the inhibitory effects of RXM on the release of pro-inflammatory cytokines were studied based on the author’s scoring criteria, but the percentage of improvement varied in each patient. There was no significant difference in the improvement of these scores between DBP and BE. Further investigations will be required to elucidate the factors determining the response of CLRTI patients to macrolide antibiotic therapy, including chest radiographic findings, infected bacteria, pulmonary function, etc. However, these results suggested that improvement in clinical and chest radiographic findings paralleled the decreased neutrophil accumulation in the airspaces, but was not always accompanied by a decrease in the number of airway bacteria. Although clinical findings paralleled decreases in the amount of bacteria in 30% of patients, they were improved despite an increased or unchanged number of bacteria in 50% of patients. The uncontrolled production of neutrophil chemotactic mediators, including IL-8, by inflammatory cells may play major roles in recruiting neutrophils to the airways and may exacerbate respiratory symptoms, especially in DBP patients. Decreased ELF levels of IL-8, NE, and LTB4 after the RXM treatment suggest that RXM could inhibit the excess release of these mediators from inflammatory cells in vivo, which was supported by the inhibitory effects of RXM on the release of IL-8 from AM and neutrophils in vitro. Although the in vitro RXM effects were significant only at a high concentration, RXM was markedly concentrated in AM after 3 months of administration. In addition, the in vivo results demonstrated that a decrease in IL-8 levels can lead to a decrease in other chemotactic mediators including NE and IL-1ß, which may efficiently attenuate neutrophilic inflammation in the airways. Therefore, RXM may actually inhibit the release of IL-8 and improve neutrophilic inflammation in vivo. Previous reports also suggest that macrolide antibiotics can

NE levels in ELF, but the ELF C5a levels in CLRTI patients were similar to those in control subjects. Furthermore, they did not decrease after RXM treatment, and did not correlate with IL-8. Therefore, RXM effects may not be mediated by a decrease in C5a levels. It is unlikely that TNF-α plays important roles in the pathogenesis of CLRTI as its ELF levels were much lower than other mediators and did not change after treatment with RXM.

The effects of RXM on clinical symptoms, pulmonary functions and chest radiographic findings in CLRTI patients were remarkable. Clinical symptoms and chest radiographic findings were improved in all CLRTI patients studied based on the author’s scoring criteria, but the percentage of improvement varied in each patient. There was no significant difference in the improvement of these scores between DBP and BE. Further investigations will be required to elucidate the factors determining the response of CLRTI patients to macrolide antibiotic therapy, including chest radiographic findings, infected bacteria, pulmonary function, etc. However, these results suggested that improvement in clinical and chest radiographic findings paralleled the decreased neutrophil accumulation in the airspaces, but was not always accompanied by a decrease in the number of airway bacteria. Although clinical findings paralleled decreases in the amount of bacteria in 30% of patients, they were improved despite an increased or unchanged number of bacteria in 50% of patients. The uncontrolled production of neutrophil chemotactic mediators, including IL-8, by inflammatory cells may play major roles in recruiting neutrophils to the airways and may exacerbate respiratory symptoms, especially in DBP patients. Decreased ELF levels of IL-8, NE, and LTB4 after the RXM treatment suggest that RXM could inhibit the excess release of these mediators from inflammatory cells in vivo, which was supported by the inhibitory effects of RXM on the release of IL-8 from AM and neutrophils in vitro. Although the in vitro RXM effects were significant only at a high concentration, RXM was markedly concentrated in AM after 3 months of administration. In addition, the in vivo results demonstrated that a decrease in IL-8 levels can lead to a decrease in other chemotactic mediators including NE and IL-1ß, which may efficiently attenuate neutrophilic inflammation in the airways. Therefore, RXM may actually inhibit the release of IL-8 and improve neutrophilic inflammation in vivo. Previous reports also suggest that macrolide antibiotics can
accumulate within phagocytes to higher levels than the other antibiotics, and that this process is more pronounced for RXM than for erythromycin (EM) [26–29]. These results may partly explain the unique effects of macrolide antibiotics on CLRTI patients, and suggest a potential superior action of RXM over EM.

In addition to the inhibitory effects on IL-8 release, various immunoregulatory effects have been reported for macrolide antibiotics. These effects include inhibition of neutrophil chemotaxis [5] and superoxide production [6], resolution of biofilms surrounding bacteria [30], activation of cilial movement [31] and a decrease in mucus secretion [32]. The clinical efficacy of macrolides may be a consequence of all these actions. Since the attenuation of chemotaxis and activation of activated neutrophils by macrolides is distinct among these mechanisms, macrolides may be applicable to other neutrophil-mediated pulmonary disorders, such as cystic fibrosis, adult respiratory distress syndrome and subacutely exacerbating idiopathic pulmonary fibrosis [4, 5, 18, 33].

RXM has a longer plasma half-life than EM [16]. Sufficient plasma concentrations can be obtained when a 150 mg tablet is administered twice a day, which is equivalent
tion could recur after the discontinuation of RXM. Longer observation is necessary to establish a standard protocol for the treatment of CLRTI patients with macrolide antibiotics.

In summary, high levels of the chemotactic mediators interleukin-8, neutrophil elastase and leukotriene B4 exist in the airway of chronic lower respiratory tract infection patients. Treatment with roxithromycin resulted in a remarkable clinical improvement, which is associated with decreased levels of these mediators. In combination with the results of in vitro experiments, it is speculated that the clinical efficacy may be mediated by the inhibitory effects of intracellularly accumulated roxithromycin on the excess release of chemotactic mediators, especially interleukin-8, from inflammatory cells.

Acknowledgements. The authors thank T. Kawa-shiro (National Higashi-Saitama Hospital) and M. Sato (Eisai Co., Ltd.) for their invaluable assistance.

References

1. Ichikawa Y, Nimomiya H, Koga H, et al. Erythromycin reduces neutrophils and neutrophil derived elastolytic-like activity in the lower respiratory tract of bronchiolitis patients. *Am Rev Respir Dis* 1992; 146: 196–203.

2. Kadota J, Sakito O, Kohno S, et al. A mechanism of erythromycin treatment in patients with diffuse panbronchiolitis. *Am Rev Respir Dis* 1993; 147: 153–159.

3. Nagai H, Shishido H, Yoneda R, Yamaguchi E, Tamura A, Kurashima A. Long-term low dose administration of erythromycin to patients with diffuse panbronchiolitis. *Respiration* 1991; 58: 145–149.

4. Fitzgerald JE, King TE Jr, Lynch DA, Tuder RM, Schwarz MI. Diffuse panbronchiolitis in the United States. *Am J Respir Crit Care Med* 1996; 154: 497–503.

5. Oda H, Kadota J, Kohno S, Hara K. Erythromycin inhibits neutrophil chemotaxis in bronchoalveoli of diffuse panbronchiolitis. *Chest* 1994; 106: 1116–1123.

6. Umeki S. Anti-inflammatory action of erythromycin: its inhibitory effect on neutrophil NADPH oxidase activity. *Chest* 1993; 104: 1191–1193.

7. Oishi K, Sonoda F, Kobayashi S, et al. Role of interleukin-8 (IL-8) and an inhibitory effect of erythromycin on IL-8 release in the airways of patients with chronic airway diseases. *Infect Immun* 1994; 62: 4145–4152.

8. O’Connor CM, Gaffney K, Keane J, et al. α₂-protease inhibitor, elastase activity, and lung disease severity in cystic fibrosis. *Am Rev Respir Dis* 1993; 148: 1665–1670.

9. Konstan MW, Walenga RW, Hilliard KA, Hilliard JB. Leukotriene B4 markedly elevated in the epithelial lining fluid of patients with cystic fibrosis. *Am Rev Respir Dis* 1993; 148: 896–901.

10. Sakito O, Kadota J, Kohno S, Abe K, Shirai R, Hara K. Interleukin 1β, tumor necrosis factor alpha, and interleukin 8 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis: a potential mechanism of macrolide therapy. *Respiration* 1996; 63: 42–48.

11. Matsushima K, Morishita K, Yoshimura T, et al. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MD-NCF mRNA by interleukin 1 and tumor necrosis factor. *Am J Respir Crit Care Med* 1996; 154: 497–503.

12. Elner VM, Strieer RM, Elner SG, Baggiolini M, Lindley I, Kunkel SL. Neutrophil chemotactic factor (IL-8) gene
expression by cytokine-treated retinal pigment epithelial cells. Am J Pathol 1990; 136: 745–750.

13. Fujishima S, Hoffman AR, Vu T, et al. Regulation of neutrophil interleukin-8 gene expression and protein secretion by LPS, TNFα, and IL-1β. J Cell Physiol 1993; 154: 478–485.

14. McCain RW, Holden EP, Blackwell TR, Christman JW. Leukotriene B4 stimulates human polymorphonuclear leukocytes to synthesize and release interleukin-8 in vitro. Am J Respir Cell Mol Biol 1994; 10: 651–657.

15. Nakamura H, Yoshimura K, McElvaney NG, Crystal RG. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. J Clin Invest 1992; 89: 1478–1484.

16. Konno S, Adachi M, Asano K, Kawazoe T, Okamoto K, Takahashi T. Influences of roxithromycin on cell-mediated immune responses. Life Sci 1992; 51: 107–112.

17. The BAL Cooperative Group Steering Committee. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. Am Rev Respir Dis 1990; 141: 169–202.

18. Nakamura H, Fujishima S, Waki Y, et al. Priming of alveolar macrophages for interleukin-8 production in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1995; 152: 1579–1586.

19. Remillard SI, Basset G, Lecossier D, et al. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. J Appl Physiol 1986; 60: 532–538.

20. Poupad JA, Dewees LB, Morton HE. Antibiotic susceptibility of Klebsiella enterobacter as determined by a single high-concentration disc method. Antimicrob Agents Chemother 1969; 9: 489–494.

21. Munoz C, Misset B, Fitting C, Blériot JP, Carlet J, Cavallion JM. Dissociation between plasma and monocyte-associated cytokines during sepsis. Eur J Immunol 1991; 21: 2177–2184.

22. Crapo JD, Barry BE, Gehr P, Bachofen M, Weibel ER. Cell number and cell characteristics of the normal human lung. Am Rev Respir Dis 1982; 125: 332–337.

23. Nakamura H, Yoshimura K, Jaffe HA, Crystal RG. Interleukin-8 gene expression in human bronchial epithelial cells. J Biol Chem 1991; 266: 19611–19617.

24. Thelen M, Peveri P, Kemen P, Von Tscarner V, Waltz A, Baggioni M. Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. FASEB J 1988; 2: 2702–2706.

25. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJH. Leukotriene B4, potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 1980; 286: 264–265.

26. Hand WL, King-Thompson NL. Contrasts between phagocyte antibiotic uptake and subsequent intracellular bacterial activity. Antimicrob Agents Chemother 1986; 29: 135–140.

27. Stamlar DA, Edelstein MAC, Edelstein PH. Azithromycin pharmacokinetics and intracellular concentrations in Legionella pneumophila-infected and uninfected guinea pigs and their alveolar macrophages. Antimicrob Agents Chemother 1994; 38: 217–222.

28. Hand WL, King-Thompson N, Holman JW. Entry of roxithromycin (RU 965), imipenem, cefotaxime, trimethoprim, and metronidazole into human polymorphonuclear leukocytes. Antimicrob Agents Chemother 1987; 31: 1553–1557.

29. Carlier M, Zenebergh A, Tulkens PM. Cellular uptake and subcellular distribution of roxithromycin and erythromycin in phagocytic cells. J Antimicrobial Chemotherapy 1987; 20: 47–56.

30. Yasuda H, Ajiki Y, Koga T, Kawada H, Yokota T. Interaction between biofilms formed by Pseudomonas aeruginosa and clarithromycin. Antimicrob Agents Chemother 1993; 37: 1749–1755.

31. Takeyama K, Tamaoki J, Chiyotani A, Tagaya E, Konno K. Effect of macrolide antibiotics on ciliary motility in rabbit airway epithelium in vitro. J Pharm Pharmacol 1993; 45: 756–758.

32. Tamaoki J, Isoko K, Sakai N, Kamemura T, Konno K. Erythromycin inhibits Cl secretion across canine tracheal epithelial cells. Eur Respir J 1992; 5: 234–238.

33. Fujishima S, Sasaki J, Shinozawa Y, Takuma K, Hori S, Aikawa N. Interleukin 8 in ARDS. Lancet 1993; 342: 237–238.

34. Konno S, Asano K, Kurokawa M, Ikeda K, Okamoto K, Adachi M. Antiasthmatic activity of a macrolide antibiotic, roxithromycin: analysis of possible mechanisms in vitro and in vivo. Int Arch Allergy Immunol 1994; 105: 308–316.