Amoxicillin Modulates Leukosequestration and Proinflammatory Cytokine Release in Airway of Patients with Bronchiectasis

Fu-Tsai Chung, Horng-Chyuan Lin

Background: Bronchiectasis is a chronic airway disease of diverse etiology, characterised by persistent bacterial colonization, bronchial inflammation, and progressive tissue damage. Neutrophil influx with oxidants and pro-inflammatory cytokines production not only provides phagocytic protection from microbes, but is also implicated in further airway inflammation. This study was designed to investigate whether amoxicillin affects neutrophil-mediated airway inflammation in bronchiectasis.

Methods: A 2-week course of therapy with amoxicillin (250 mg, 4 times per day) or duracef (250 mg, twice per day) was administered for bronchiectasis patients. Twenty-one bronchiectasis patients in stable condition after adequate chest care and hydration were enrolled in a randomized fashion. The neutrophil cellularity in 3 ml induced sputum was counted before and after treatment. The sputum IL-8 and TNF-α levels were measured using the ELISA method. Leukocyte adhesion molecules CD11b/CD18 and DCFH in induced sputum were determined by flow cytometric assay.

Results: The total cell count of neutrophils in 3 ml induced sputum was significantly reduced in patients receiving amoxicillin from 14.4 ± 5.1 to 9.3 ± 5.2 ($x10^6$ cells) ($p < 0.05$). There was no change in total cell counts in the duracef group ($p = 0.13$). Amoxicillin significantly decreased the TNF-α and IL-8 levels in a supernatant of sputum, from 168.7 ± 65.6 pg/ml to 50.3 ± 26.8 pg/ml ($p < 0.01$), and from 9538.4 ± 1650.1 pg/ml to 5664.4 ± 1384.4 pg/ml ($p < 0.01$), respectively, whereas the TNF-α and IL-8 levels in the duracef group did not significantly change after treatment.

In the amoxicillin group, the change in the sputum IL-8 level was significantly related to the change in the total cell count of leukocytes ($r = 0.67$, $n = 11$, $p < 0.05$). There was also a significant correlation between the percentage of change in the sputum IL-8 level and total cell counts of leukocytes after antibiotic therapy in the amoxicillin group ($r = 0.76$, $n = 11$, $p < 0.01$). The expression of CD11b, CD18 and DCFH did not significantly change after treatment in both groups.

Conclusion: Different antibiotics have different effects on patients with bronchiectasis. Amoxicillin downregulates the TNF-α and IL-8 levels in sputum, thus leading to a decrease of airway neutrophil sequestration and preventing further airway damage. ***(Thorac Med 2006; 21: 392-405)***

Key words: bronchiectasis, amoxicillin, neutrophil, tumor necrosis factor-α, interleukin-8

Department of Thoracic Medicine, Chang Gung Memorial Hospital, Chang Gung University, College of Medicine, Taipei, Taiwan

Address reprint requests to: Dr. Horng-Chyuan Lin, Department of Thoracic Medicine, Chang Gung Memorial Hospital, Chang Gung University 199 Tun-Hwa N. Rd., Taipei, Taiwan

Introduction

Bronchiectasis is a disease involving recurrent pulmonary infections and chronic airway inflammation that causes destruction of the ciliated epithelium and submucosa with elastic and muscular tissue degeneration [1]. Dense polymorphonuclear neutrophil (PMN) infiltrates in the airways is a characteristic of bronchiectasis, and significantly increases during airway infection, thus exaggerating the inflammatory burden [2]. Recruitment or sequestration of neutrophils appears to be a crucial response of the host against bacterial and fungal invasion [3-4]. When the mucociliary clearance and alveolar macrophages are overwhelmed, the rapid influx of neutrophils from the lung vasculature, releasing substantial amounts of reactive oxygen metabolites, together with hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and hydroxyl radical (OH·), accounts for a large part of PMN microbicidal activity [5]. However, activated PMNs in the bronchial lumen are also capable of releasing pro-inflammatory mediators, including IL-8 and TNF-α [6], which when combined with toxic oxygen radical species, including H2O2 and $O2^-$ [7], may also subsequently lead to epithelial injury and impairment of mucociliary clearance [8], and increase mucus secretion. In particular, the chemotaxis, transendothelial and transepithelial migration, as well as phagocytosis and degranulation of PMNs, are regulated by the expression of adhesion molecules on PMNs, especially β-2 integrins (CD11/CD18) [9]. Thus, a fine-tuned regulation of neutrophil activation may occur after the adherence of neutrophils to epithelial cells. Moreover, the adherence between leukocytes and epithelial cells may perpetuate the activation of leukocytes by the production of pro-inflammatory cytokines from the epithelial cells, leading to mucus hypersecretion. Therefore, the initiation of PMN adherence and oxidant production, and then the decrease in pro-inflammatory cytokine production in patients with bronchiectasis may be of therapeutic significance.

Antibiotic therapy is the mainstay treatment for infective exacerbations in bronchiectasis [10]. Broncicctasis runs a chronic course and patients are given short-term antibiotics intermittently for clinical exacerbations. Besides the respective interactions between antibiotics and between the immune system and bacteria, antibiotics also directly interact with the immune system [11]. Agents which potentiate neutrophil function and interfere with pro-inflammatory cytokine release may have a beneficial effect on the development and progression of such disease. Amoxicillin has also been widely used in the treatment of excess sputum production in chronic bronchitis. Recent reports have revealed that amoxicillin could be a immunodepressing antibiotic through its neutralizing effect on cytokine production [11]. Therefore, amoxicillin may have a potential role in the neutrophil-regulated host defences and neutrophil-derived airway inflammatory responses in patients with bronchiectasis.

Neutrophils are recruited in abundance in the airways in bronchiectasis, as shown by sputum cytology [12]. Previous studies [13] have reported that cellular and biochemical analysis of induced sputum is feasible in healthy and asthmatic subjects. Therefore, the changes in neutrophil activity and cytokine level in induced sputum in the airways of bronchiectasis patients provide the responses to antibiotic therapy.

The present study was designed to investigate the immunomodulatory effects of amoxicillin on airway neutrophil sequestration and the associated cytokine production in patients with bronchiectasis.

Methods

Patients

Twenty-one patients (11 men and 10 women) with bronchiectasis were enrolled in this study. Bronchiectasis was diagnosed by history and clinical symptoms, as well as chest X-ray and/or computerized tomography scan of the thorax. Bronchiectasis was a result of prior pneumonia in 16 patients and prior tuberculosis (TB) in 5 patients. None of them had immunoglobulin deficiency or immotile cilia syndrome. All patients had suffered from a production of mucopurulent sputum of more than 30 ml/day at home, for at least 3 weeks before the study, with clinically significant symptoms of cough. Their ages ranged from 52 to 68 (a mean of 59.8 ± 5.1) years. All subjects received regular postural drainage and chest care before the study. They were conscious, co-operative, and able to produce an effective cough. Patients with other active pulmonary or systemic disorders were excluded. No oral or inhaled corticosteroids, non-steroid anti-inflammatory agents or maintenance antibiotics were used at least 4 weeks before the study. Theophylline was used throughout the study without changing the dosage, and inhaled β2-agonists were permitted on an "as required" basis. Moreover, the patients had not had a pulmonary (respiratory rate > 30/min, severe hypoxemia with SaO2 < 90% in room air or hypercapnia with pH < 7.35) or systemic (high fever, body temperature > 38.5°C, chills or bacteremia) exacerbation in the past 4 weeks. Female patients were not pregnant or breast feeding. Patients with a known hypersensitivity to any of the constituents of the test drugs were excluded.

Study design and antibiotic treatment

The subjects were randomly divided into 2 groups: 11 patients used amoxicillin (250 mg orally 4 times per day) and 10 patients served as a control group, continuing to take duracef (250 mg orally 2 times per day). The treatment period consisted of 14 consecutive days for each group.

At the baseline of the study period, all patients underwent a pulmonary function test. The best of at least 3 attempts at reproducible forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) (with a difference within 200 ml or less than 5%) were measured by a Spiroanalyzer ST-350R (Fukuda Sangyo. Co Ltd. Chiba, Japan). Before the start and end of treatment, sputum was collected for analysis. Maintenance treatment, such as postural drainage and chest care, continued at home without a change throughout the study period.

Twenty-three patients were initially recruited for this study. One male (aged 56) in the amoxicillin group, and 1 female (aged 57 years) in the duracef group dropped out due to incomplete sputum study (the male patient) and massive hemoptysis (the female patient) during the study period. Totally, 11 patients (7 men and 4 women) in the amoxicillin group and 10 patients (4 men and 6 women) in the duracef group completed the entire course of treatment. Their characteristics, initial clinical assessments and sputum cellularity are presented in Table 1. The protocol was approved by the Medical Ethics Committee of Chang Gung Memorial Hospital, and informed consent was obtained from all patients.

Sputum collection

On the morning of day 1 (the start) and the morning of day 15 (the end) of the study, the patients' mouth and tongue were swabbed dry with a gauze, and the outlets of the salivary glands were occluded by cotton pads. After receiving postural drainage and chest percussion, the

Table 1. Characteristics of patients and their sputum cellurity and cytokines level before and after therapy

	Amoxicillin Group (n = 11)		Duracef Group (n = 10)	
Age (yrs)	60.3 ± 5.4		59.3 ± 4.4	
Sex (M/F)	7 / 4		4 / 6	
Total Numbers of Involved Lobes on CxR	2.8 ± 0.4		2.5 ± 0.3	
Cause of bronchiectasis (n)				
prior pneumonia	9		7	
prior tuberculosis	2		3	
FEV1, L (% pred)	1.37 ± 0.20 (57.0 ± 7.3)		1.42 ± 0.22 (54.6 ± 8.4)	
FVC, L (% pred)	1.94 ± 0.21 (70.6 ± 7.3)		2.09 ± 0.18 (71.9 ± 7.5)	
	Pre-Treatment	Post-Treatment	Pre-Treatment	Post-Treatment
Cellurity in sputum				
Total cell count (x 10^6 PMNs/3 ml)	14.4 ± 5.1	9.3 ± 5.2**	18.8 ± 7.1	23.7 ± 13.0
Viability (%)	94.6 ± 0.8	94.8 ± 1.2	97.0 ± 1.0	97.3 ± 1.0

Definitions of abbreviation: PMNs = polymorphic neutrophils; TNF-α = tumor necrosis factor-α; IL-8 = interleukine-8.

* Values are mean ± SEM;

** $p < 0.05$, compared with pretreatment level.

patients inhaled nebulized sterile 3% saline solution for 20 minutes from an ultrasonic neubulizer (Ultra-NEB'99, DEVILBISS Co. Pascay-Meslay, France), the reservoir of which was filled with 3% saline solution (100 ml). The patients were asked to cough and the sputum was collected and sent for analysis. The sputum production was collected under the supervision of our rehabilitative therapeutist in a hospital set-up.

Sputum Processing

Preparation of the supernatant Three milliliters of sputum were diluted 1:3 with Hanks' balanced salt solution (HBSS), vortexed briefly, and centrifuged for 5 minutes at 2000 rpm. The supernatants were collected and stored at –80°C for cytokine analysis.

Preparation of the cellular component (neutrophils) The pellet containing the cells was washed twice by suspension in Ca- and Mg-free HBSS containing 10% fetal calf serum (FCS), mixing gently by vortex mixer, and then centrifuging for 5 minutes at 2000 rpm. The pellet was suspended again in 10 ml HBSS with 10% FCS, and the suspension was filtrated through a nylon mesh (with a 60 μm hole size) to yield the neutrophils. This suspension of neutrophils was at least 95% pure, using Liu's stain. The cell counts were measured by a hemocytocounter. The viability of neutrophils was determined by trypan blue dye exclusion.

Cytokine assay in the supernatant of induced sputum

interleukin-8 (IL-8) The immunoreactivity of IL-8 in supernatants was measured by a sandwich-ELISA method, using a commercial-kit (Bender MedSystems Vienna, Austria, Europe). The IL-8/NAP-1 ELISA was an assay for the quantitative detection of IL-8/NAP-1 levels in cell culture supernatants, human serum,

plasma, and amniotic or other body fluids. An anti-IL-8/NAP-1 monoclonal coating antibody was adsorbed into microwells. IL-8/NAP-1 present in the sample or standard bound to antibodies adsorbed to the microwells. A HRP-conjugated polyclonal anti-IL-8/NAP-1 antibody was added and bound to IL-8/NAP-1 captured by the first antibody. Following incubation, unbound enzyme conjugated anti-IL-8/NAP-1 was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of IL-8/NAP-1 present in the sample. The reaction was terminated by the addition of acid, and absorbance was measured at 450 nm. A standard curve was prepared.

Tumor necrosis factor-α (TNF-α) The TNF-α level in supernatants was also measured by an ELISA method, using a commercial-kit (Bender MedSystems Vienna, Austria, Europe). An anti-TNF-α monoclonal coating antibody was adsorbed into microwells. TNF-α present in the sample or standard bound to antibodies adsorbed to the microwells. A HRP-conjugated polyclonal anti-TNF-α antibody was added and bound to TNF-α captured by the first antibody. Following incubation, unbound enzyme conjugated anti-TNF-α was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of TNF-α present in the sample. The reaction was terminated by the addition of acid, and absorbance was measured at 450 nm. A standard curve was prepared.

Analysis of adhesion molecule expression on neutrophils

For the analysis of the increased plasma membrane expression of CD11b, CD18 and ICAM-1 on neutrophils (1 ml, 10^6 cells/ml), immunofluorescence flow cytometry was performed by staining cells with 5 μL monoclonal mouse antibody to human and 5 μL FITC-conjugated F (ab)'2 rabbit anti-mouse IgG (DAKOPATTS a/s, Denmark). Analysis was accomplished with a FACScan (Becton Dickinson, Mountain View, CA) by gating 10,000 counts in the neutrophil region of the forward and right angular scattergrams and using the nonbinding IgG1 as a control. Results were expressed as the mean of fluorescence intensities (M.F.I) of patient samples, taking the mean channel number of the positive cells in arbitrary units of log amplified green fluorescent signals.

Analysis of oxidative metabolism capacity in neutrophil

intracellular hydrogen peroxide production The individual leukocyte respiratory burst response was assessed using 2'.7' dichlorofluorescin diacetate (DCFH-DA) and flow cytometry [14]. In brief, after incubation in the presence or absence of stimulation, neutrophils were pelleted and resuspended in PBS containing 5 mM glucose and 0.1% gelatin, but lacking $Ca^{2+}$and Mg^{2+} (PBSg) at 10^5 cells/ml, and then loaded with DCFH-DA (1 μM) for 15 min at 37°C with shaking. Cells were washed twice with PBSg and transferred to an ice bath, then were further incubated with PE-conjugated anti-CD11b/CD18 (Beton Dickinson, Mountain View, CA, USA) for 20 min at 4°C, and subsequently analyzed by flow cytometry after 2 extensive washings with cold PBSg containing 10% FCS.

Flow cytometric analysis

Analysis was performed with a FAScan flow cytometer (Becton Dickson, Mountain View, CA) equipped with a 5 W argon ion laser at 300 MW with a 75 high voltage setting and an excitation

wavelength of 488 nm. The emitted light was collected through 488 nm and 550 nm dichroic filters. A 525-nm band-pass filter was used for gating fluorescence emission, and a 570-nm long-pass filter for scattering emission. All fluorescence was measured using logarithmic amplification. Granulocytes were identified based on forward angle light scatter and log 90° scatter parameters. Ten μl of propidium iodide were added to each sample to distinguish leukocytes from nonviable cells. After stimulation, the cells might have had a different forward angle and side (90°) light scatter, which required a change in the gates. In cases of dual-color analysis, overlap of the green fluorescence and PE emission spectra was eliminated by electronic substration. For the histogram, 5000 leukocytes were analyzed. The FACScan cytometer was always operated at the same settings.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). One-way analysis of variance for mixed design was used to compare the values of more than 2 different experimental groups. If variance among groups was noted, a Bonferroni test wa used to determine significant differences between specific points within groups. Comparisons of only 2 different time points were made with a paired *t* test. For data with uneven variation, a Mann- Whitney *U* test (2-tailed) or Wilcoxon signed ranks test was used for unpaired or paired experimental/baseline values, respectively. The relationships between IL-8 and TNF-α, as well as changes in IL-8 and total cell counts in the amoxicillin group, CD11b M.F.I. and CD18 M.F.I., and changes in CD11b M.F.I. and CD18 M.F.I. after treatment with amoxicillin, were sought by Spearman's rank correlation test. Significance was set at $p < 0.05$.

Results

Baseline data of the study subjects

There were no significant differences in baseline data between the 2 groups in terms of age, sex, lobar involvement of bronchiectasis on chest X-ray [15], pulmonary functions (FEV_1 and FVC), and sputum cellularities (total cell count and viability) (Table 1). All bacterial culture of sputa before and after treatment showed no particular pattern for specific pathogens, such as pseudomonas. All bacterial cultures from our patients grew mixed flora, which, in our laboratory, meant that the colony counts for specific pathogens were too low to reach clinical significance.

Effect on cellularity in induced sputum

The total cell count of neutrophils in 3 ml induced sputum was significantly reduced in patients receiving amoxicillin, from 14.4 ± 5.1 to 9.3 ± 5.2 (x 10^6 cells) ($p < 0.05$). The total cell counts in the duracef group did not change ($p = 0.13$) (Table 1). The viability did not significantly change in either group. The viabilities of neutrophils before and after treatment in both groups were all above 94%.

Effect on cytokine levels in induced sputum

Amoxicillin significantly decreased the TNF-α level in the supernatants of sputum (from 168.7 ± 65.6 pg/ml to 50.3 ± 26.8 pg/ml; $p < 0.01$), whereas the TNF-α level in the duracef group did not significantly change after treatment (from 173.2 ± 47.6 pg/ml to 117.3 ± 30.9 pg/ml; $p > 0.05$) (Figure 1).

Similiary, amoxicillin also significantly decreased the IL-8 level in supernatants of sputum (from 9538.4 ± 1650.1 pg/ml to 5664.4 ± 1384.4 pg/ml; $p < 0.01$), whereas the IL-8 level

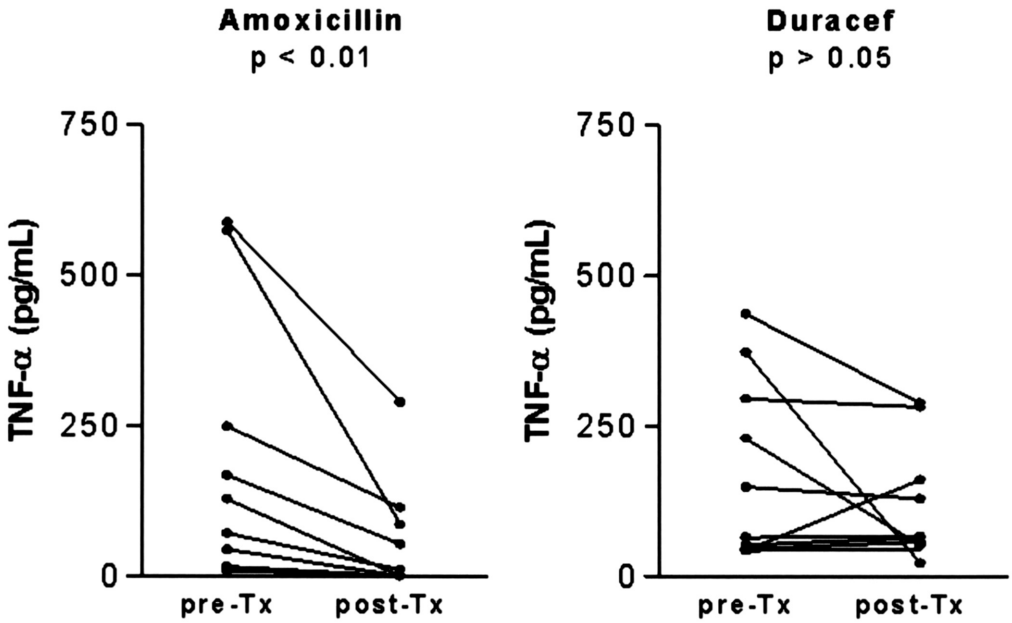

Fig. 1. The effect of amoxicillin (n=11) or duracef (n=10) on the expression of TNF-α before and after treatment in patients with bronchiectasis. The significance is indicated.

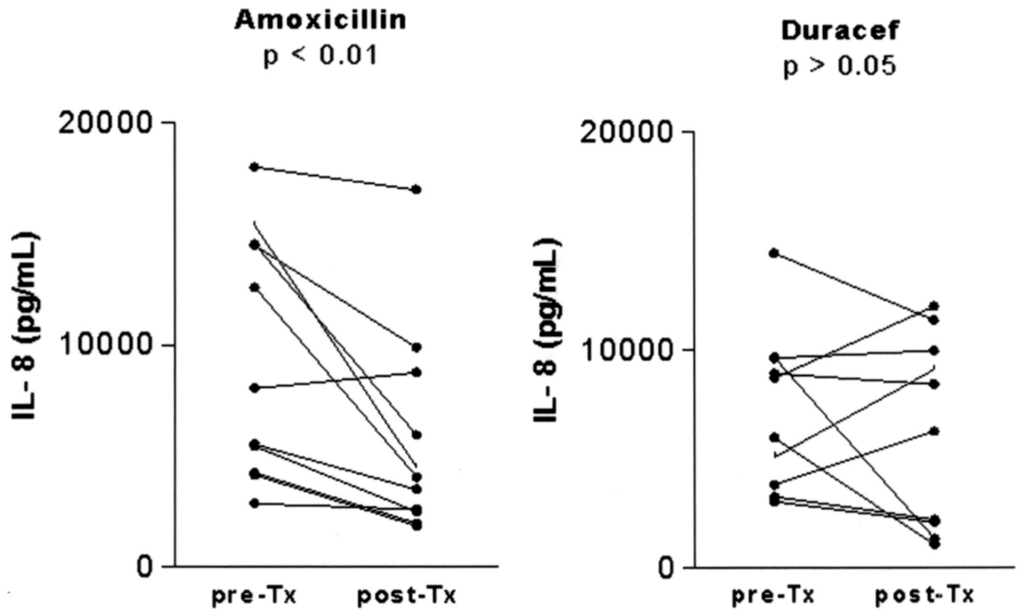

Fig. 2. The effect of amoxicillin (n=11) or duracef (n=10) on the expression of IL-8 before and after treatment in patients with bronchiectasis. The significance is indicated.

in the duracef group did not significantly change after treatment (from 7233.2 ± 1150.64 pg/ml to 6364.1 ± 1372.3 pg/ml; $p > 0.05$) (Figure 2).

In the amoxicillin group, the change in the sputum IL-8 level was significantly related to the change in the total cell count of leukocytes (r =

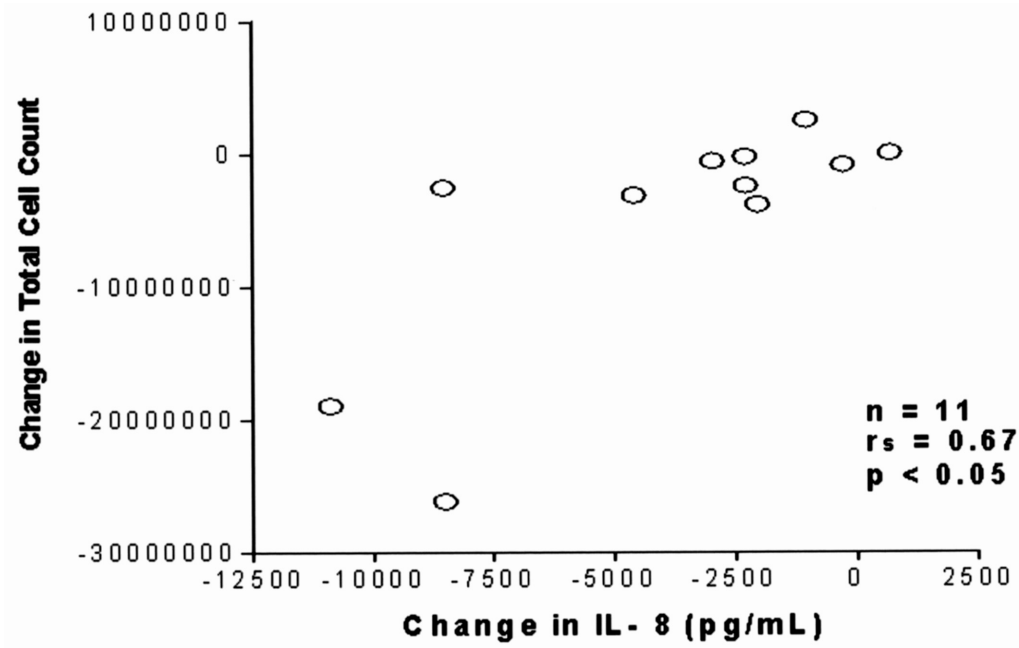

Fig. 3. The relationship between change in total cell counts of leukocytes and interleukin-8 (IL-8) level in induced sputum before and after therapy in patients receiving amoxicillin. The significance is indicated.

0.67, n = 11, $p < 0.05$) (Figure 3). There was also a significant correlation between the percentage of change in the sputum IL-8 level and total cell counts of leukocytes after antibiotic therapy in the amoxicillin group (r = 0.76, n = 11, $p < 0.01$) (Figure 4).

Effect on adhesion molecular (β2-integrin) expression of airway neutrophils

There was a trend toward a decrease in CD11b, CD18 in neutrophils in the amoxicillin groups (from 309.9 ± 43.6 to 270.6 ± 32.6 and 264.3 ± 37.2 to 215.9 ± 33.7) [expressed as mean fluorescence intensity (M.F.I.) of 10000 cells], although it was not signficantly different. The expression of leukocyte adhesion molecules CD11b and CD18 in induced sputum in the duracef group showed no significance (308.2 ± 19.8 to 325.9 ± 19.8 and 280.2 ± 19.3 to 277.6 ± 14.9) (Figure 5).

Effect on intracellular oxidative metabolism of airway neutrophils

The mean DCF fluorescence intensity of neutrophils in induced sputum, representing the airway oxidative metabolism of patients receiving amoxicillin, also showed a decreasing trend (673.7 ± 183.3 to 567.1 ± 158.0) after treatment, although it did not reach statistical significance; there was no significant change in the duracef group (495.6 ± 141.0 to 531.2 ± 129.9; $p > 0.05$) (Figure 5).

Patients tolerated antibiotics well, without any complaints. No acute infective exacerbation was found in either group during the 2-week therapy. No study subjects developed infection in the week following therapy.

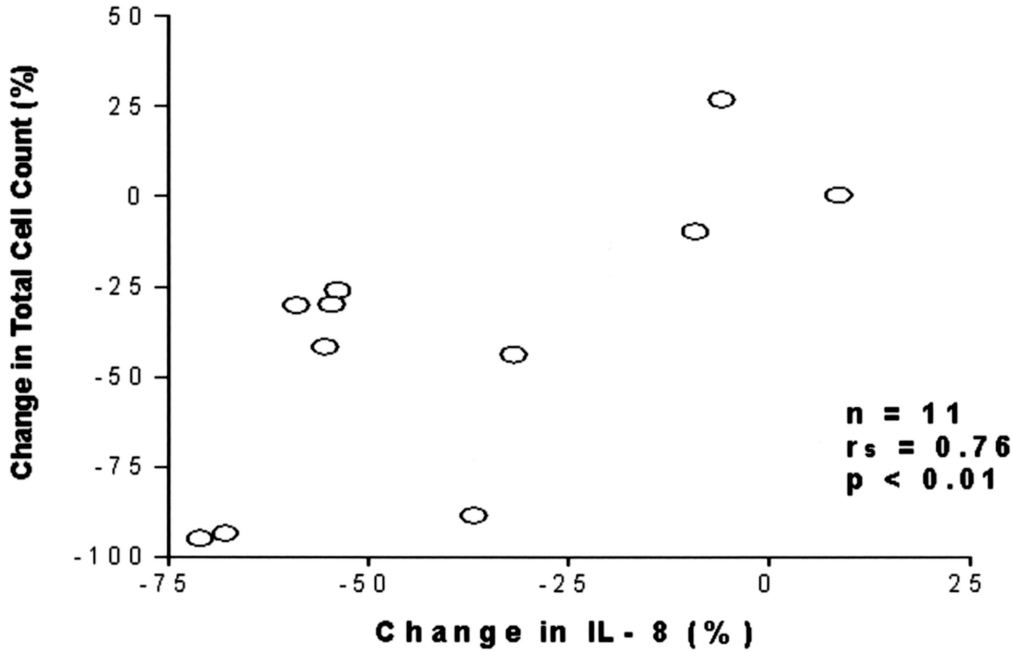

Fig. 4. The relationship between percentage change in total cell counts of leukocytes and interleukin-8 (IL-8) level in induced sputum before and after therapy in patients receiving amoxicillin. The significance is indicated.

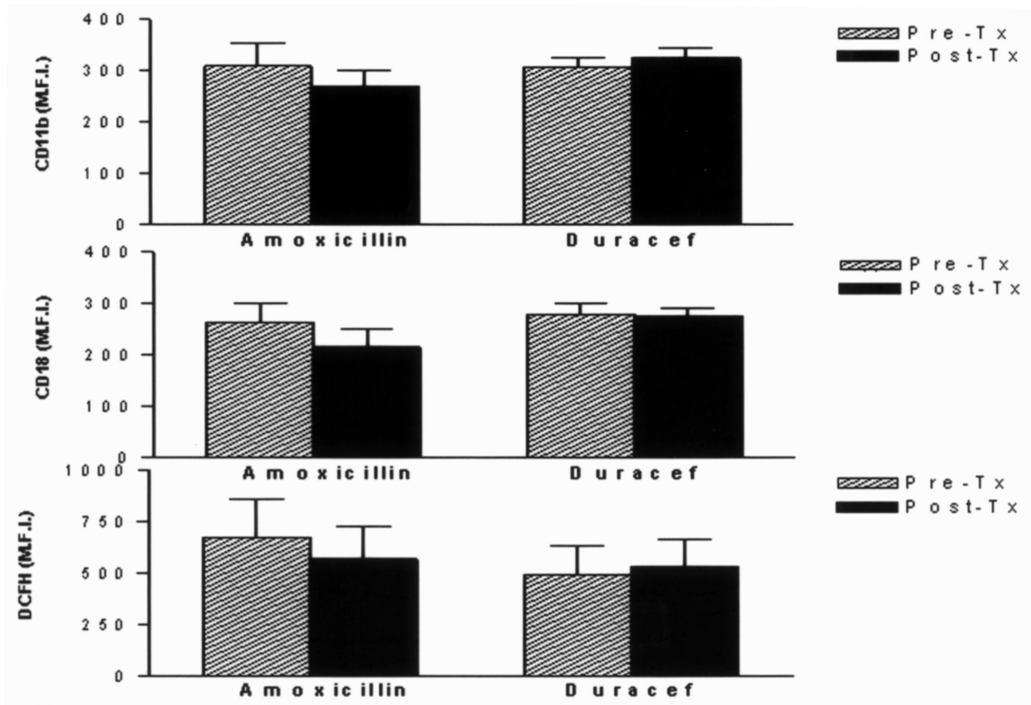

Fig. 5. The changes in leukocyte CD11b, CD18 and intracellular hydrogen peroxide [expressed by mean DCF fluorescence intensity (M.F.I.)] in patients after receiving (post-Tx) amoxicillen (n=11) or duracef (n=10), compared with the pre-treatment values (pre-Tx). The mean ± SEM values and the stastistical significance are indicated.

Discussion

Bronchiectasis is a chronic airway disease of diverse etiology, which is characterised by persistent bacterial colonization, bronchial inflammation, and progressive tissue damage [1]. The load of colonized bacteria is often associated with the production of large volumes of purulent sputum containing neutrophils [10, 16]. Neutrophil influx with reactive oxygen species and pro-inflammatory cytokines production not only provides opsonophagocytic protection from microbes [3], but is also implicated in further airway inflammation [17]. Aside from regular chest physiotherapy, current therapy with an emphasis on frequent courses of antibiotics [18] is still not able to gain a satisfactory understanding of their pharmacologic mechanisms. The present study demonstrated that amoxicillin was clinically useful in patients with bronchiectasis through its reduction of the level of pro-inflammatory cytokines, including TNF-α and IL-8, and associated total neutrophil counts in induced sputum, thus leading to a decrease in further airway injury in bronchiectasis. In contrast, duracef did not affect neutrophil activity and cytokine production in bronchiectasis.

Neutrophils mediate host defenses and tissue damage through the production of ROI and performed proteins (proteases and antibiotic proteins), which synergize for a fully destructive response [5]. Neutrophil-derived protease has been shown to stimulate interleukin-8 (IL-8) released by epithelial cells, thereby enhancing recruitment, and to inactivate the neutrophil C3Bi receptor, thus reducing the opsonophagocytic function of the cells. Janice *et al*. [19] reported that IL-8 is an important chemoattractant and presents a high concentration in the sputum of patients with chronic inflammatory airway diseases, such as bronchiectasis and cystic fibrosis. TNF-α has been implicated in the pathogenesis of many inflammatory airway diseases, such as asthma. TNF-α is known to upregulate the expression of cell adhesion molecules on endothelial cells in vitro [20] and also enhance extracellular proteolysis by neutrophils, thus leading to disease progression in bronchiectasis. Therefore, therapeutic antibiotics which reduce the IL-8 and TNF-α in the lung may have a potentially beneficial effect on disease progression in patients with bronchiectasis. Amoxicillin is used worldwide in the treatment of chronic inflammatory airway diseases. The present study demonstrated that amoxicillin, given via the oral route for 2 weeks, reduced the level of IL-8 and TNF-α in induced sputum from patients with bronchiectasis, suggesting that this antibiotic may profoundly suppress inflammatory reactions and resultant tissue injury.

The source of the IL-8 and TNF-α found in the sputum of our patients is unknown. Vera *et al*. [6] concluded that there were increased concentrations of IL-8 and TNF-α in induced sputum from patients with chronic obstructive pulmonary disease, thus leading to airway inflammation. These concentrations might be produced by the many inflammatory cells that are present in the airways, such as neutrophils and monocytes. The cellular source of IL-8 may be macrophages, neutrophils, or epithelial cells, and the TNF-α may be derived from macrophages, mast cells, or other inflammatory cells. [6, 20]. TNF-α was reported to increase IL-8 production from epithelial cells and neitrophils. The existence of a baseline high concentration of IL-8 and TNF-α in sputum suggested that epithelial cells may be another major source of IL-8 and TNF-α. A study specifically designed to address the effect of an antibiotic (amoxicillin) on the cytokine expression of differ-

ent airway cells is needed.

Neutrophil recruitment in the airway is the hallmark of bronchiectasis. IL-8 is a chemotactic cytokine with the main actions of neutrophil recruitment and activation. Pin *et al*. [21] concluded that cell counts in induced sputum could be used to investigate airway inflammation. There is increasing evidence that amoxicillin could be an immunodepressing antibiotic through a neutral effect on cytokine production [11]. Therefore, the action of amoxicillin in decreasing the neutrophil count in the sputum from patients with bronchiectasis may be through its direct effect on the inhibition of IL-8 release from airway epithelial cells.

Currie *et al*. [22] reported in their study that amoxicillin reduced the prurulent sputum volume in patients with bronchiectasis, and may improve morbidity. This double-blind randomized study, investigating the clinical improvement with amoxicillin treatment, found that there was no bactericidal effect, because the sputum culture showed no important changes in the bacterial flora and concentration of *Haemophilus spp*. after amoxicillin treatment. They suggested that amoxicillin may significantly reduce the host inflammatory response in patients with bronchiectasis. Data in the present study showed that amoxicillin modulates TNF-α and IL-8, and reduces the neutrophil count in the airway of patients with bronchiectasis.

PMNs are paramount in host response mechanisms against bacteria and fungi [23]. The ability of PMNs to combat infectious agents in the lung is due to a number of specific activities, including chemostaxis, adherence to vessel endothelium and airway epithelium, and transmigration to tissues, as well as phagocytosis and microbial killing [3]. Genetic deficiencies in cellular CD11b/CD18 are associated with the decreased mobilization of neutrophils to sites of infection and increased susceptibility to infection, reflecting the importance of CD11b/CD18 in several adherence-dependent neutrophil inflammatory and host defense functions [24]. Neutrophils sequestrated at the airways with infection and inflammation will exert their microbicidal action via ingestion and the subsequent destruction of invading micro-organisms via the change in reactive oxygen intermediates (ROI) [23]. Shappell *et al*. [25-26] also suggested that H2O2 production is adherence-dependent and mediated by the expression of Mac-1 (CD11b/CD18). In the present study, amoxicillin in the treatment of patients with bronchiectasis did not statistically decrease the expression of leukocyte β2 integrins (CD11b/CD18) on neutrophils, and the production of ROI (H2O2) by neutrophils in induced sputum; however, amoxicillin showed a greater trend toward lowering the β2 integrins (CD11b/CD18) on neutrophils, and the production of ROI (H2O2) by neutrophils, than duracef, suggesting that amoxicillin exerts a potential role in bronchiectasis by blocking the neutrophil adherence and migration to airways to produce opsonophagocytic protection. Perhaps a large scale study is needed to confirm this point.

Sampling airway secretions by means of bronchoscopy has a number of disadvantages. Previous reports [13], with an emphasis on induced sputum, had concluded that the analysis of induced sputum was a useful noninvasive method to reveal changes in inflammatory cells and markers which were similar to those reported in bronchoalveolar lavage fluid. Many studies have demonstrated that determining the IL-8 level in sputum was a good means of evaluating chronic inflammatory airway disease [19]. That is why we selected to measure the changes in neutrophil functions and cytokines in induced sputum as the responses of antibiotic therapy for airway infec-

tion and inflammation.

Taken together, the antibiotics had some effect on the patients with bronchiectasis. Amoxicillin downregulated the TNF-α and IL-8 levels in sputum. It may have decreased the total cell counts of leukocytes in sputum by means of decreasing the IL-8 expression. Through these processes, amoxicillin decreased airway neutrophil sequestration to prevent further airway damage. Our conclusion provides a new therapeutic direction in bronchiectasis treatment.

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Amoxicillin 調節支氣管擴張病人之呼吸道內白血球及前發炎細胞素之表現

鍾福財　林鴻銓

目的：探討 Amoxicillin 調節支氣管擴張病人之呼吸道內中性球及前發炎細胞素之表現。

方法：21 位穩定支氣管擴張病人隨機分為兩組，分別接受為期兩週之 Amoxicillin (250 毫克每天四次)或 duracef (250 毫克每天兩次)治療；比較治療前後，痰液內中性球數量，及 TNF-α 與 IL-8 濃度；並分析中性球附著分子表現及氧化代謝能力。

結果：接受 Amoxicillin 治療組，治療前後痰液內中性球數量(14.4 ± 5.1 降到 9.3 ± 5.2)(× 10^6 細胞) ($p < 0.05$)及 TNF-α (168.7 ± 65.6 pg/ml 降到 50.3 ± 26.8 pg/ml) ($p < 0.01$)與 IL-8 (9538.4 ± 1650.1 pg/ml 降到 5664.4 ± 1384.4 pg/ml) ($p < 0.01$)濃度皆呈現顯著減少。同時痰液內 IL-8 降低量與中性球減少數之絕對值($r = 0.67$, $n = 11$, $p < 0.05$)與相對值($r = 0.76$, $n = 11$, $p < 0.01$)皆呈現正相關。接受 duracef 治療組，治療前後則無顯著變化。而痰液內中性球附著分子表現及氧化代謝能力，兩組皆無顯著變化。

結論：Amoxicillin 可藉由調節支氣管擴張病人痰液內 TNF-α 與 IL-8 濃度，以減少呼吸道內中性球數量，及可能對呼吸道的損傷。*(胸腔醫學* ***2006; 21: 392-405****)*

關鍵詞：支氣管擴張， Amoxicillin ，中性球，腫瘤壞死因子-α ，細胞間素-8

長庚大學暨林口長庚紀念醫院　胸腔內科
索取抽印本請聯絡：林鴻銓醫師，林口長庚紀念醫院　胸腔內科，桃園縣龜山鄉復興街 5 號