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Macrolide effects on the prevention of COPD exacerbations

by

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ABSTRACT

The number of senile patients with chronic obstructive pulmonary disease (COPD) has recently increased due to an increase in the number of older people, the habit of smoking and the inhalation of toxic particles. COPD exacerbations are caused by airway bacterial and viral infections, as well as the inhalation of oxidative substrates. COPD exacerbations are associated with the worsening of symptoms and quality of life, as well as an increased mortality rate. Several drugs, including long-acting anti-cholinergic agents, long-acting β_2 agonists and inhaled corticosteroids, have been developed to improve symptoms in COPD patients and to prevent COPD exacerbations. Treatment with macrolide antibiotics has been reported to prevent COPD exacerbations and improve patient quality of life and symptoms, especially in those patients who have frequent exacerbations. In addition to their antimicrobial effects, macrolides have a variety of physiological functions such as anti-inflammatory and anti-viral effects, reduced sputum production, the inhibition of biofilm formation and the inhibition of bacterial virulence factor production. These unique activities may relate to the prevention of exacerbations in the COPD patients who receive macrolides. Here, we review the inhibitory effects that macrolides have on COPD exacerbations and explore the possible mechanisms of these effects.

Keywords: anti-inflammatory effects, anti-viral effects, biofilm, COPD exacerbation, mucus secretion, pro-inflammatory cytokines

INTRODUCTION

Patients with COPD are treated with various types of drugs to improve their symptoms and quality of life (QOL), prevent exacerbations and reduce the mortality rate. These drugs include long-acting anti-cholinergic agents, long-acting β_2 agonists, inhaled corticosteroids, and mucolytic agents [1-12]. Macrolides have a variety of physiological activities other than antimicrobial effects, including anti-inflammatory and anti-viral effects [13-26], reduced sputum production [27-30], and antimicrobial peptide production [31], in addition to inhibiting biofilm formation and reducing the bacterial production of virulence factors [32, 33]. These unique properties of macrolides have been suggested to contribute to the clinical benefits of macrolides in patients with diffuse panbronchiolitis (DPB) [34-36] and cystic fibrosis (CF) [37-40]. COPD exacerbations are caused by airway bacterial and viral infections [41-46], as well as the inhalation of oxidative substrates that induce airway inflammation [47, 48]. Based on the novel physiological activities of macrolides, we sought to investigate the clinical effects that macrolides have on the prevention of COPD exacerbations [49, 50]. Other reports have also shown the effects that macrolides have on preventing COPD exacerbations and improving QOL in these patients [51-56]. Here, we review the clinical effects of macrolides in COPD patients, including the prevention of exacerbations. Furthermore, we introduce the physiological activities of macrolides (such as their anti-inflammatory effects) to explore the mechanisms by which macrolides may contribute to the clinical effects observed in COPD patients.

MACROLIDE EFFECTS ON COPD EXACERBATIONS

Prospective randomized study in Japan

Suzuki et al. were the first to perform a prospective study on the clinical benefits that erythromycin has in preventing COPD exacerbations [49] (Table 1). The authors performed a prospective, randomized, controlled (but not blinded) trial. A total of 109 patients with COPD was enrolled and randomly assigned to erythromycin therapy or no active treatment. The mean forced expiratory volume in one second (FEV_1) values were 1.30 l/s in the control group and 1.47 l/s in the erythromycin group. Those COPD patients who exhibited bronchiectasis and DPB complications were excluded from the study. The patients in the erythromycin group received erythromycin (200 to 400 mg/day) daily, and the patients in the control group received riboflavin in addition to sustained-release theophylline and inhaled anti-cholinergic agents. The observation period was 12 months. The authors did not examine the bacteria in the sputum in this study. The mean number of exacerbations and the mean number of common colds in the patients in the erythromycin group were lower than those in the control group. Furthermore, more patients were hospitalized due to exacerbations in the control group than in the erythromycin group. These findings suggest that erythromycin has beneficial clinical effects in moderate to severe COPD patients with frequent exacerbations due to upper respiratory tract infections. One patient in the erythromycin group had anorexia and diarrhea and was excluded from the study, whereas the rest of the patients in the erythromycin group did not have any apparent adverse effects from erythromycin therapy during the study period. Based on these findings, the authors concluded that erythromycin therapy has beneficial effects on the prevention of COPD exacerbations. However, the authors also concluded that this intervention should be restricted to patients who are at high risk for COPD exacerbations because of the potential risk of the emergence of erythromycin-resistant pathogens.

Multicenter retrospective study in Japan

Because the prospective study by Suzuki et al. [49] was performed in the hospitals in one prefecture in Japan, we performed a multicenter study in Japan [50]. We analyzed the records of 123 patients in seven Japanese university hospitals and one Japanese general hospital. Of this group, 45 patients received macrolide therapy (macrolide group) and 78 patients did not (control group). The proportion of macrolide-receiving patients with symptoms of sputum (84%, $p<0.03$) was larger than that in the control group (68%). The macrolide-receiving patients were distributed among the classification of global initiative for chronic obstructive lung disease (GOLD) stages as follows: 9% of the patients were in GOLD stage I, 67% were in stage II, 18% were in stage III and 7% were in stage IV. The severity of the GOLD stages in the macrolide group did not differ from the stage severity in the control group. The mean observation period was 42.9 months. The COPD patients participating in this study were treated with inhaled anti-cholinergic agents, long-acting β_2 agonists, and sustained-release theophylline or inhaled steroids. The patients in the macrolide group received clarithromycin (200 to 800 mg/day) or erythromycin (200 to 1200 mg/day) daily for the observation period. A total of 41 patients was treated with clarithromycin or erythromycin alone, and another 4 patients were treated with clarithromycin and erythromycin at different times. The number of patients with an exacerbation frequency of 1.5 times per year or more was lower in the macrolide group than in the control group. Likewise, the number of patients with a hospitalization frequency of 0.75 times per year or more was lower in the macrolide group than in the control group. The patients did not have any apparent adverse effects from erythromycin or clarithromycin treatment. This retrospective multicenter clinical study suggested that macrolide treatment may reduce the frequency of exacerbations and hospitalization due to the exacerbations in Japanese patients with moderate to severe COPD who exhibit frequent exacerbations and hospitalization and complained of sputum.

Prospective, randomized, double-blind, placebo-controlled study

Based on the report by Suzuki et al. [49], Seemungal et al. designed and performed a randomized, double-blind, placebo-controlled study to examine the effects of long-term erythromycin therapy on the prevention of COPD exacerbations [51]. The authors randomized 109 outpatients and observed the number of exacerbations for 12 months. Patients were included in the study if they had moderate to severe COPD with FEV_1 between 30% and 70% predicted. The mean FEV_1 and FEV_1 % predicted values of the enrolled patients were 1.32 l/s and 50%, respectively. Of these, 38 patients had 3 or more exacerbations in the year prior to recruitment. The patients received inhaled steroids (78%), long-acting β_2 agonists (63%), long-acting anti-cholinergic agents (33%), or theophylline (11%). Furthermore, 53 patients in the erythromycin group received erythromycin (250 mg twice daily). There was a total of 206 moderate to severe exacerbations in 109 of the patients who were enrolled, with 125 exacerbations occurring in the 56 patients in the placebo group. The frequency of moderate to severe exacerbations in the erythromycin group was lower than that in the placebo group. The duration of exacerbations in the erythromycin group was shorter than that in the placebo group, and the time to first exacerbation was longer for the erythromycin group than for the placebo group. There was no significant difference in adverse effects between the erythromycin and placebo groups. The frequency of adverse effects was low in both groups. In some cases, more than one adverse effect occurred in the same patient; for example, an upper gastrointestinal symptom occurred in a patient who also had tinnitus.

There was no difference in the microorganism detection rate in the spontaneously produced sputum of the two study groups at any of the follow-up time points over the 1-year study period. Of the samples taken during stable conditions, 27% tested positive for *Haemophilus influenzae* (*H. influenzae*), as did 40% of the samples taken during an exacerbation. The distribution for *Streptococcus pneumoniae* (*S. pneumoniae*) was 7% during stable conditions and 10% during exacerbation. Furthermore, pathogens were

detected in 17 of the 43 sputum samples at the 12-month follow-up visit and included *H. influenzae* (3 samples from the placebo group and 1 sample from the macrolide group), *S. pneumoniae* (2 samples from the placebo group and 1 sample from the macrolide group) and *Moraxella catarrhalis* (*M. catarrhalis*) (2 samples from the placebo group). Sensitivity tests showed that all 4 of the *H. influenzae* samples were resistant to erythromycin and 1 *S. pneumoniae* sample from the macrolide group was also resistant to erythromycin. Based on the findings of the sensitivity test, the authors suggested that there were no differences between the macrolide and placebo groups in terms of the bacterial flora in the sputum [51]. The authors concluded that macrolide therapy may be useful in decreasing the excessive disease burden in patients with COPD. Macrolide therapy may have clinical benefits in moderate to severe COPD patients with frequent exacerbations.

He et al. performed a randomized, placebo-controlled, double-blind erythromycin trial for a 6-month period [52]. In this study, 36 COPD patients were randomized to treatment with oral erythromycin (n=18, 125 mg three times per day) or placebo (n=18) daily. A total of 31 patients completed the study (16 patients in the erythromycin group and 15 patients in the placebo group). The mean FEV_1 value was 1.1 l/s in both groups, and the FEV_1 % predicted value was 42% in the placebo group and 44% in the erythromycin group. These values suggest that primarily severe COPD patients were enrolled in the study. There were a total of 31 moderate-severe exacerbations over the 6-month treatment time, of which 20 occurred in the placebo group and 11 in the erythromycin group. Erythromycin therapy reduced the mean exacerbation rate and delayed the time to the first exacerbation. The neutrophil counts and neutrophil elastase levels in the sputum were decreased in the patients in the erythromycin group. Two patients discontinued their participation in the study. One patient had abdominal pain after erythromycin treatment, and another experienced left heart failure. A bacterial examination test showed that there was no difference in the detection rates for the three

main microorganisms (*S. pneumoniae, H. influenzae and M. catarrhalis*) between the two groups at baseline or after 6 months of treatment. The three main pathogens that were detected at the 6-month time point were *S. pneumoniae* (3 samples from the placebo group and 2 samples from the erythromycin group), *H. influenzae* (2 samples from the placebo group and 2 samples from the erythromycin group) and *M. catarrhalis* (2 samples from the placebo group and 1 sample from the erythromycin group). The authors concluded that erythromycin treatment in COPD patients can reduce airway inflammation and decrease exacerbations, and may therefore be useful in the management of COPD. Erythromycin therapy may reduce the frequency of exacerbations and airway inflammation in severe COPD patients who experience frequent exacerbations.

Banerjee et al. performed a controlled study in 67 patients with COPD [13]. The patients were randomized to 3 months of clarithromycin (500 mg daily) or placebo and were observed for 3 months. Overall, 26 patients receiving clarithromycin and 34 receiving placebo completed the trial. The mean FEV_1 % predicted value was 44% in the placebo group and 43% in the clarithromycin group. These values suggest that primarily severe GOLD stage COPD patients were enrolled in the study. One patient from the clarithromycin group had gastrointestinal symptoms and withdrew from the study. The pathogen growth rate in the sputum of the clarithromycin group was similar before treatment (55%) and after 3 months (58%) of treatment. However, the frequency of exacerbations in the clarithromycin group (n = 5) did not differ from that of the control group (n = 2). The exacerbation frequency was smaller in this study than in other reports [49-53]. Macrolide therapy may not be necessary in patients who do not have frequent exacerbations.

Randomized trial investigating the effects of azithromycin

To examine the effects that macrolide therapy has on COPD exacerbations, Albert et al. performed a prospective, parallel-group, placebo-controlled study, for which 1577 subjects were screened [53]. Moderate to very severe GOLD stage COPD patients were enrolled in this study. None of the COPD patients were at the mild GOLD stage. The mean FEV_1 value was 1.1 l/s in both the placebo and azithromycin groups, and the mean FEV_1 % predicted value was 40% in the placebo group and 39% in the azithromycin group. The patients were either using continuous supplemental oxygen or had received systemic glucocorticoids within the previous year [53]. The patients had visited an emergency room or had been hospitalized for an acute COPD exacerbation, but had not experienced an acute COPD exacerbation for at least 4 weeks before enrollment [53]. A total of 1142 (72%) patients were randomly assigned to receive 250 mg azithromycin (570 participants) or placebo (572 participants) daily for 1 year (in addition to their usual care). In addition to azithromycin or placebo, the patients received inhaled steroids (76%), long-acting β_2 agonists (73%), long-acting anti-cholinergic agents (62%), or no medications (9%) as their usual care. The time to the first exacerbation was longer in the azithromycin group than the placebo group, and the COPD exacerbation frequency in the azithromycin group was lower than that in the placebo group. The rate of COPD exacerbations per patient per year was 1.48 in the azithromycin group and 1.83 in the placebo group, and the exacerbation rates in this study were higher than those in the other studies which we reported previously [49, 50]. The total St. George's Respiratory Questionnaire (SGRQ) scores decreased in the azithromycin group, showing an improvement in patient QOL.

No significant differences were observed in the frequency of serious adverse events or adverse events leading to the discontinuation of the study drug. However, hearing decrements were more common in the azithromycin group (n=142, 25%) than in the placebo group (n=110, 20%, p=0.04). Although all of these COPD patients should have

had their study drug discontinued, the drug was discontinued in only 61 patients in the azithromycin group (76%) and 37 in the placebo group (82%), owing to protocol errors.

At the time of enrollment, the rate of selected respiratory pathogen colonization in the nasopharyngeal swabs from the azithromycin group (14%) did not differ from that in the placebo group (15%). However, more patients in the placebo group (31%) than the azithromycin group (12%) became colonized during the course of the study. Susceptibility testing revealed that the prevalence of respiratory pathogen resistance to macrolides in the azithromycin group (52%) did not differ from that in the placebo group (57%) ($p=0.64$) at the time of enrollment. In contrast, the test investigating the pathogens from the COPD patients who were not colonized at the time of enrollment but became colonized during the course of the study revealed that the incidence of resistance to macrolides was higher (81%) in the azithromycin group than the placebo group (41%, $p<0.001$). Thus, COPD patients receiving azithromycin were less likely to become colonized with respiratory pathogens but were more likely to become colonized with macrolide-resistant organisms [53].

Based on these findings, the authors concluded that azithromycin treatment decreased COPD exacerbations and improved QOL, but caused hearing decrements in a small percentage of subjects [53]. This study suggested that treatment with azithromycin has clinical benefits in COPD patients with severe conditions.

Studies using other designs

The efficacy of treatment with 500 mg of azithromycin three days per week was evaluated for 6 months in a multicenter, randomized, uncontrolled pilot study of tracheostomized COPD patients [54]. Ten patients (46%) received long-term oxygen therapy. An average number of exacerbations in the previous year was 3.1 (standard care group) and 3.0 (azithromycin group). These values suggest the severe conditions of the patients who were enrolled. A total of 22 patients were randomized, and the

cumulative number of exacerbations and hospitalizations was lower for the patients in the azithromycin group than the patients in the standard care group. Azithromycin also improved the patients' QOL in comparison to standard care. No serious adverse events were reported in the azithromycin group. Four patients experienced mild adverse events [diarrhea (3 patients) and stomachache (3 patients)]. A total of 6 of the 11 patients in the azithromycin group and 5 of the 11 patients in the standard care group were colonized with *P. aeruginosa* at baseline. Two of the azithromycin-treated patients who were initially colonized with *P. aeruginosa* exhibited reduced colony counts after 3 months of treatment, and the microorganism was eradicated in one patient. In contrast, *P. aeruginosa* became resistant to ceftazidime, and an erythromycin-resistant *S. pneumoniae* strain was identified in one patient after 6 months of treatment with azithromycin. The authors concluded that long-term azithromycin treatment seems to be safe and effective in severe COPD outpatients with tracheostomy in reducing exacerbations, hospitalizations, as well as in improving QOL.

Gomez et al. examined the effects of azithromycin in 54 COPD patients in a prospective, randomized study [56]. The patients were treated with 500 mg azithromycin daily for 3 days every 21 days during the winter months [56] and were compared to the control group of patients who did not receive treatment. The authors reported that the frequency of acute infectious episodes and hospitalizations was lower in the azithromycin group than in the control group.

Macrolide effects on bronchiectasis

The presence of bronchiectasis is suggested to be important for the pathogenesis of COPD exacerbations [57]. A recent report demonstrated that more than 50% of COPD patients were shown to have bronchiectasis with severe airflow obstruction and at least one hospitalization for COPD exacerbation in the previous year [58]. Macrolide therapy improves sputum purulence, airway hypersensitivity [59], lung function and

sputum volume [27, 60-63], and symptom scores in patients with bronchiectasis, in addition to reducing the frequency of exacerbations [63, 64].

Treatment with clarithromycin or erythromycin did not change the total amount of bacteria, the bacterial flora or the pathogenic densities in the sputum of patients with bronchiectasis [27, 61]. Likewise, a recent study by Serisier et al. demonstrated that 12 months of erythromycin therapy reduced the frequency of exacerbations in patients with bronchiectasis, and that neither new respiratory pathogens nor erythromycin resistant organisms was detected in the sputum during the study [65].

These findings suggest the possibility that macrolides may have clinical benefits in COPD patients with bronchiectasis, although small numbers of patients were studied.

ADVERSE EFFECTS OF LONG-TERM MACROLIDE THERAPY IN COPD

Long-term treatment with macrolides has been suggested to have several adverse effects, with the most common adverse effects related to the gastrointestinal tract [66, 67]. These effects are caused by stimulating gastrointestinal motility through motilin-like activity [66, 67]. Gastrointestinal symptoms, including anorexia, diarrhea, abdominal pain and stomachache, were also reported in COPD patients treated with erythromycin [13, 49, 51, 52, 54]. Those patients with gastrointestinal symptoms did not complete the course of the study [13, 49, 51, 52, 54]. Albert et al. [53] also reported hearing decrements in COPD patients treated with azithromycin by, which has also been reported in patients with other diseases [68, 69]. As a result, azithromycin treatment was discontinued in those patients experiencing hearing decrements [53]. Erythromycin and clarithromycin are also associated with ototoxicity such as vertigo, tinnitus, deafness and hearing loss [67], and one COPD patient withdrew from the Seemungal et al. study because of tinnitus caused by erythromycin therapy [51]. Macrolide-induced allergic reactions, skin eruptions, hepatotoxicity and cardiac arrhythmias, including QTc

prolongation on an electrocardiogram (ECG) [66, 67], were not reported in the COPD patients in the studies cited here [13, 49-54].

BACTERIOLOGY IN LONG-TERM MACROLIDE THERAPY FOR COPD

Seemungal et al. [51] demonstrated that pathogens were detected in 17 of 43 sputum samples from COPD patients and that 3 samples from the placebo group and 2 samples from the erythromycin group were resistant to erythromycin at the 12-month follow-up visit. The authors concluded that there were no differences in the bacterial flora of the macrolide and placebo groups. He et al. showed that there was no difference in the bacterial detection rate of the erythromycin group and the placebo groups, but they did not describe the detection rate for macrolide-resistant bacteria [52]. Albert et al. showed that the rate of macrolide resistance in the azithromycin group did not differ from that in the placebo group at the time of enrollment [53]. However, more patients in the placebo group than the azithromycin group became colonized during the course of the study. In contrast, the susceptibility test investigating the pathogens from the COPD patients who were not colonized at the time of enrollment but became colonized during the course of the study revealed that the incidence of resistance to macrolides was higher in the azithromycin group than the placebo group. The authors concluded that COPD patients receiving azithromycin were less likely to become colonized with respiratory pathogens but azithromycin therapy could change microbial resistance patterns [53]. These findings suggest that azithromycin therapy in COPD patients might increase the rate of macrolide resistance. However, neither the bacterial flora nor the pathogen density in the sputum of macrolide-treated patients experiencing either DPB or bronchiectasis changed after treatment with clarithromycin, erythromycin or azithromycin [27, 61, 64]. Likewise, a recent study by Serisier et al. demonstrated that neither new respiratory pathogens nor erythromycin resistant organisms were detected in the sputum of

bronchiectasis patients during 2 months of erythromycin therapy [65]. Although there have not been any reports of patients who were treated with long-term macrolides and experienced a life-threatening infection with macrolide-resistant bacteria, attention must be paid to the induction of macrolide-resistant pathogens [36].

DOSAGE AND EFFECTS OF LONG-TERM MACROLIDE THERAPY IN COPD

The COPD patients in five of eight studies cited here [13, 49-52] were treated with 14-member class macrolides, including erythromycin and/or clarithromycin. The macrolide dosage was between 200 and 500 mg/day for erythromycin [49, 51, 52], 200 and 1200 mg/day for erythromycin [50] or 200 and 800 mg/day for clarithromycin [50]. The macrolide dosage was similar to that used in a report of DPB patients who had been treated with a low-dose of erythromycin (400-600 mg/day) [34, 35]. Furthermore, Blasi et al. [54] treated COPD patients with azithromycin, a 16-member class macrolide, 3 days per week (500 mg), and Gomez et al. [56] treated patients with 500 mg azithromycin daily for 3 days out of every 21 days. In contrast, the COPD patients in the Albert et al. study [53] ingested azithromycin (250 mg/day) daily. This low dose of azithromycin was the same dose that Walter et al. used for younger CF patients [38]. As reported here, macrolide therapy had clinical effects on COPD patients with frequent exacerbations [49-54], suggesting that macrolide therapy has clinical benefits in a frequent-exacerbation COPD phenotype [70]. However, one study showed that macrolide therapy had no effects on COPD patients who do not have frequent exacerbations [13]. These findings suggest that only patients who experience frequent exacerbations may benefit from macrolide therapy. Macrolide therapy also has clinical benefits in COPD patients who have severe disease accompanied by frequent exacerbations, including those patients who receive long-term oxygen or have a tracheostomy or symptoms such as sputum. Adverse effects were reported in a small

number of COPD patients who were treated with erythromycin [49, 51, 52], clarithromycin [13] or azithromycin [54], whereas hearing decrements were reported in patients who received ingested azithromycin daily [53]. However, Walter et al. reported that non of the younger CF patients experienced hearing loss after treatment with azithromycin [38]. Erythromycin ototoxicity (such as hearing loss and deafness) has also been reported previously [67]. Thus, the prevalence of ototoxicity due to long-term macrolide therapy may differ between erythromycin, clarithromycin and azithromycin, or between younger and older patients.

COPD EXACERBATION MECHANISMS

Bacteria such as *H. Influenzae*, *M. catarrhalis* and *S. pneumonia* have been detected in 50-60% of the COPD patients who experience exacerbations [41-43]. These bacteria can enhance mucus secretion, inhibit the ciliary beat frequency, and cause airway epithelial injury, thereby impairing mucociliary clearance [71, 72] (Figure 1). Infection with these bacteria also stimulates the cells in the airways and the lung parenchyma to induce pro-inflammatory substrates, interleukin (IL)-8 and leukotriene (LT) B_4 [15, 30 47, 73, 74] (Figure 1). These substrates induce inflammation by causing neutrophils to infiltrate the airways and alveoli and by activating neutrophils to release cytotoxic products and neutrophil elastase [47, 75]. Neutrophil elastase causes airway smooth muscle contraction, mucosal edema and mucin secretion, thereby resulting in the limitation of airflow and gas exchange in the airways and the alveoli, respectively [47, 76-80] (Figure 1).

Various species of viruses, such as rhinovirus (RV), influenza virus and respiratory syncytial virus (RSV) have also been detected in the induced sputum and nasal samples or the blood of 23-56% of patients experiencing COPD exacerbations [44-46]. The production and secretion of a variety of mediators, including IL-6, IL-8 and

interferon-gamma-inducible protein (IP)-10, are stimulated by RV, influenza virus and RSV in airway epithelial cells and the blood [81-87]. Viral infection-induced mediator release may also stimulate neutrophils and induce airway inflammation and mucin secretion through similar mechanisms that are induced by bacterial infection [47, 88]. In fact, RV infection induces the infiltration of neutrophils, lymphocytes and eosinophils into the nasal and bronchial mucosa [89, 90]. Furthermore, RV infection can stimulate mucin secretion from airway surface epithelial cells and submucosal gland cells [91] and airway smooth-muscle contraction through IL-1 and IL-5 [92] (Figure 1). In contrast to the weak cytotoxic effects caused by RV, the influenza virus causes epithelial cell damage in the airways and lung parenchyma [93] and may affect the barrier function of the endothelium [94]. These mechanisms induce fluid accumulation and epithelial detachment, as well as subsequent airflow limitation and exudative alveolar destruction [93]. Secondary bacterial infections in the respiratory tract and lung parenchyma that are followed by influenza virus and RV infection may also be associated with COPD exacerbations [95, 96]. These mechanisms may cause airway inflammation and subsequent COPD exacerbations following bacterial and viral infections [35, 47, 48, 88, 97] (Figure 1).

MACROLIDE CHARACTERISTICS

Macrolides are macrocyclic lactones consisting of ≥8-membered rings. This very large class (>2000 compounds) comprises both natural substances isolated from fungi and other organisms and synthetic molecules with similar structures [67]. The most common agents used in the clinic are semi-synthetic 14-, 15-, or 16-membered ring antibiotics related to erythromycin. These agents include erythromycin, clarithromycin and roxithromycin as members of the 14-member class and azithromycin as the prototypical 15-member compound [98]. Macrolide antibiotics bind to the 50*S*

ribosomes of both prokaryotes and eukaryotes, inhibiting the transpeptidation or translocation of nascent peptides. Macrolides accumulate in many tissues, such as the fluid of the epithelial lining, and easily enter host defense cells such as macrophages and polymorphonuclear leukocytes. The macrolide concentrations in respiratory tract tissues and extracellular fluids are higher than those in the serum (especially after the ingestion of clarithromycin), making them useful for airway and alveolar infections [67].

MECHANISMS FOR THE MACROLIDE-MEDIATED PREVENTION OF COPD EXACERBATIONS

Anti-inflammatory effects of macrolides

Kadota et al. demonstrated that the high percentage of neutrophils in the bronchoalveolar lavage fluid from patients with DPB decreased after erythromycin treatment [14]. The previously elevated neutrophil chemotactic activities were also reduced after erythromycin treatment [14, 15] (Table 2).

Takizawa et al. and Desaki et al. demonstrated that macrolides have effects on bronchial epithelial cells, including suppressing mRNA levels and the release of IL-8 [16, 18] through the activation of nuclear factor-kappa B (NF-κB) and activator protein (AP)-1 [18]. The anti-inflammatory effects of macrolide antibiotics were also demonstrated in human peripheral blood monocytes and polymorphonuclear neutrophils [15, 99, 100].

Several other macrolide-mediated anti-inflammatory effects have also been reported in the sputum of patients with COPD, including decreases in the total cell counts, neutrophil chemotaxis, and the levels of IL-8 and tumor necrosis factor (TNF)-α [13, 19].

Inhibitory effects on mucus secretion

Tamaoki et al. [27] conducted a parallel, double-blind, placebo-controlled study to determine the effects that long-term clarithromycin administration has on the amount of sputum in patients with clinical conditions that are associated with excessive airway secretions. A total of 31 patients was divided into two groups: a clarithromycin group (100 mg, twice a day, daily) and a placebo group, in which 16 patients had chronic bronchitis and 7 patients had bronchiectasis. The authors reported that treatment with clarithromycin decreased sputum production [27]. Tagaya et al. also demonstrated that treatment with clarithromycin decreased the sputum volume in 16 patients with chronic bronchitis (n=5) or bronchiectasis (n=11) [62].

Shimizu et al. examined the effects that macrolide antibiotics have on mucus hypersecretion *in vivo*, reporting that clarithromycin inhibited ovalbumin (OVA)- and lipopolysaccharide (LPS)-induced mucus production induced by the intranasal instillation of OVA in OVA-sensitized rats and intranasal LPS instillation [28].

In vitro studies have demonstrated that macrolide antibiotics such as erythromycin [28, 29], clarithromycin [28] and azithromycin [30] have inhibitory effects on mucin or MUC5AC production or secretion after stimulation with TNF-α [28], RV infection [29] or extract of *H. influenzae* [30] in airway epithelial cells.

Inhibitory effects on bacterial virulence and biofilms

Anderson et al. demonstrated that clarithromycin reduces the production of pneumolysin, a key virulence factor in the infection of *S. pneumoniae* [101]. Macrolides reduce the production of pro-inflammatory cytokines, soluble intercellular adhesion molecule (ICAM)-1 and mucin in cells such as airway epithelial cells in response to endotoxin and extract of *H. influenzae* [15, 30, 102, 103]. Azithromycin also maintains the integrity of airway epithelial cells during *P. aeruginosa* infection [104]. These findings suggest that macrolides may inhibit virulence factor production

and the inflammation caused by the bacteria that induce COPD exacerbations. Furthermore, clarithromycin inhibits the twitching motility of *P. aeruginosa* [33], and azithromycin inhibits the quorum-sensing circuitry of *P. aeruginosa,* which relates to virulence factor production [32, 105]. In addition, incubating *P. aeruginosa* with clarithromycin altered the structure and architecture of the biofilm [33]. These findings suggest that macrolides may modulate the virulence of bacteria at the early and late stages of COPD (Table 2).

Inhibitory effects on virus infection

Sato et al. [20] and Tsurita et al. [21] reported that erythromycin and clarithromycin increased the survival rate of mice infected with a lethal dose of influenza virus. They also suggested that the reduction in lung injury and the severity of pneumonia were associated with the reduced production of nitric oxide, reactive oxygen species and interferon (IFN)-γ [20] and the elevated IL-12 levels [21] induced by erythromycin and clarithromycin. Kido et al. [23] and Miyamoto et al. [24] also demonstrated that clarithromycin suppresses the growth of the influenza virus and its release in mouse airways and epithelial cells, respectively. Furthermore, Yamaya et al. [26] reported that clarithromycin decreased the release of viruses and cytokines into supernatant fluids in human tracheal epithelial cells that were infected with seasonal type A influenza (H_3N_2) by reducing the expression of the viral receptor and inhibiting viral RNA entry.

Suzuki et al. [22] showed that erythromycin inhibited RV infection by reducing the levels of ICAM-1, an RV receptor, and/or by blocking RV RNA entry and that erythromycin reduces the production of pro-inflammatory cytokines in the human tracheal epithelial cells. Gielen et al. showed that IFNs were induced in human bronchial epithelial cells when the cells were pretreated with azithromycin and infected with RV [106] and that this effect may relate to the shorter duration of exacerbations in COPD patients who are treated with macrolides. Asada et al. have also reported that

macrolides have inhibitory effects on RSV virus infection in human airway epithelial cells [25].

These anti-viral and anti-inflammatory effects of macrolides may be associated with the inhibition of viral infection-induced COPD exacerbations, although there is no evidence that macrolides reduce the frequency of viral infection in COPD exacerbations.

Summary of the possible mechanisms by which macrolides exert *clinical benefits on COPD exacerbations*

As described above and in other reports, macrolides have been found to have various functions other than antimicrobial effects, including anti-inflammatory effects [13-19], reduced mucus secretion [27-30], inhibitory effects on bacterial virulence and biofilm formation [15, 30, 32, 33, 102-105], enhanced production of antimicrobial peptides and human β-defensins [31] and anti-viral effects [20-26, 106]. The physiological functions of macrolides, including those reported by other authors [17, 100, 107-112], are shown in Figure 1 and Table 2. Previous studies have suggested that the clinical effects that erythromycin has on DPB are associated with immunomodulatory and physiological activities other than antimicrobial effects [36]. Based on these findings, the clinical benefits that macrolides have on the prevention of COPD exacerbations are also considered to be mediated by these immunomodulatory and other physiological activities [49].

CONCLUSIONS

The recent reports that have studied the beneficial effects that macrolides have on the attenuation of acute COPD exacerbations are summarized in this review. Interactions between the host immune system and bacterial and viral infections are in COPD exacerbations, and macrolides might attenuate the overall risk of mortality. The

contribution that macrolides make to COPD exacerbations may carry a benefit for human survival, despite the worry of an increase in the number of macrolide-resistent bacterial strains.

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FIGURE LEGENDS

FIGURE 1. Bacterial and viral infection-induced COPD exacerbation mechanisms (left side), macrolide contributions to the prevention of COPD exacerbations (right side) and the relationships between them. IL-8: interleukin-8, LTB_4: leukotriene B_4.

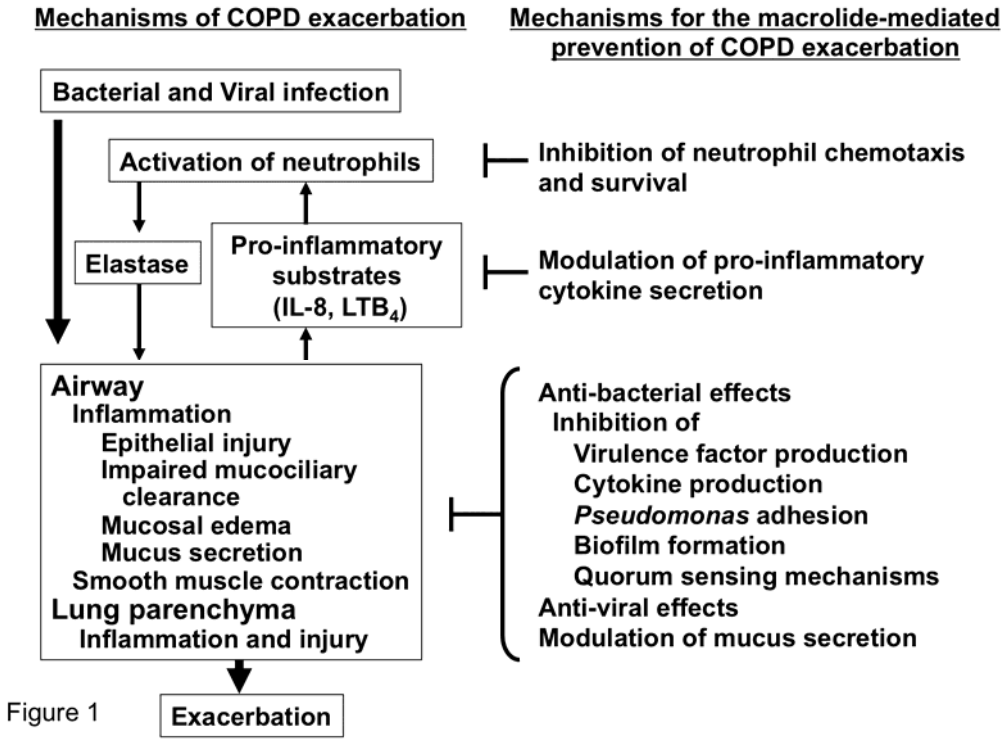

Figure 1

Table 1. Clinical benefits that macrolides have on COPD patients

Author and publication year	Number of subjects	Drug(s)	Effects
Suzuki et al. 2001	109	EM	Reduction in the number of exacerbations and common colds
Banerjee et al. 2004	67	CAM	No difference in the frequency of exacerbations
Yamaya et al. 2008	123	EM or CAM	Reduction in the number of patients with exacerbations and the number of patients experiencing hospitalization
Seemungal et al. 2008	109	EM	Reduction in the frequency of exacerbations Shorter duration of exacerbation Longer time to the first exacerbation
He et al. 2010	36	EM	Reduction in the exacerbation rate Delay in the time to the first exacerbation
Blasi et al. 2010	22	AZM	Reduction in the number of exacerbations Reduction in the number of hospitalizations Improvement in QOL
Albert et al. 2011	1142	AZM	Reduction in the frequency of exacerbations Longer time to first exacerbation Improvement in QOL

EM: erythromycin, CAM: clarithromycin, AZM: azithromycin, QOL: quality of life.

Table 2. Anti-inflammatory and physiological effects of macrolides.

Author, publication year and reference number	Effects
Oishi et al. 1994 [107]; Khair et al. 1995 [15]; Takizawa et al. 1997 [16]; Suzaki, et al. 1999 [17]; Khan et al. 1999 [99]; Desaki, et al. 2000 [18]; Suzuki et al. 2002 [22]; Kikuchi et al. 2002 [108]; Banerjee et al. 2004 [13]; Basyigit et al. 2004 [19]; Reato et al. 2004 [100].	Modulation of pro-inflammatory cytokine secretion
Tamaoki et al. 1995 [27]; Rubin et al. 1997 [109]; Tagaya et al. 2002 [62]; Shimizu et al. 2003 [28]; Inoue et al. 2008 [91]; Araki et al. 2010 [30].	Modulation of mucus secretion
Kadota et al. 1993 [14]; Oda et al. 1994 [110]; Khair et al. 1995 [15]; Villagrasa et al. 1997 [111].	Inhibition of neutrophil chemotaxis
Yamasawa, et al. 2004 [112].	Inhibition of neutrophil survival
Ishizawa et al. 2005 [31].	Stimulation of defensin secretion
Khair et al. 1995 [15]; Tateda et al. 2001 [32]; Comte et al. 2001 [105]; Takaki et al. 2003 [102]; Wozniak, et al. 2004 [33]; Anderson 2007 [101]; Ishida et al. 2007 [103]; Araki et al. 2010 [30]; Halldorsson et al. 2010 [104].	Anti-bacterial effects
Sato et al. 1998 [20]; Tsurita et al. 2001 [21]; Suzuki et al. 2002 [22]; Miyamoto et al. 2008 [24]; Kido et al. 2004 [23]; Asada et al. 2009 [25]; Gielen et al. 2010 [106]; Yamaya et al. 2010 [26].	Anti-viral effects