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REVIEW

Macrolide antibiotics, bacterial populations and inflammatory airway disease

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) and other inflammatory airway conditions are major causes of morbidity and mortality worldwide. Antibiotics are used to treat acute infectious exacerbations of airway disease. However, for the macrolides, a significant and growing body of evidence indicates that anti-inflammatory effects of these antibiotics, which may be independent of their antibacterial effects, are at least partially responsible for their beneficial effect. In this review, we describe current thinking on the means whereby anti-inflammatory effects of macrolides impact chronic airway disease.

The current data indicate that some macrolides have immunomodulatory activity, mediated at least in part by effects on the activation of gene transcription mediated by NF-κβ activation that may be separable from their antimicrobial activities, and could explain their surprising efficacy in asthma and viral infections for which the role of bacteria is not established. Other, provocative work indicates that subclinical doses of macrolides may also affect signalling within and between bacterial communities, and thus impact developmental processes such as biofilm formation that are important in the establishment and persistence of chronic infections. The current data clearly suggest that activities beyond antimicrobial effects contribute significantly to the beneficial effect of macrolide therapy on inflammatory conditions.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), asthma, and chronic sinusitis are among the most common causes of morbidity and mortality worldwide.[1] Environmental and host factors play a significant role in all of these diseases, but infection by opportunistic bacterial and viral pathogens is thought to lead to a more rapid progression and worsening of disease.[2] Most of these persistent infections are caused by organisms that normally reside in the upper airways as benign commensals.[3,4] The chronically infected airway is host to an abundant microbial community.[5] This is generally asymptomatic during most of the time of carriage, although a chronic state of low-level inflammation usually occurs, and data suggest that adaptations among the bacterial population result in a modulation of the innate response to bacteria and bacterial components.[6] Periodic exacerbations of airway disease occur that are characterised by a robust inflammatory response with the production of copious amounts of mucus, and release of cytokines and other signalling molecules.[7] Inflammation is the hallmark of chronic airway disease, and treatments that eliminate or reduce the inflammatory response are beneficial.[8] Treatment with antimicrobials reduces the severity of infection and inflammatory episodes.[9] In addition, a growing body of work has clearly demonstrated that some antimicrobial agents, especially the macrolides, can also act by reducing the host inflammatory response.

MACROLIDE ANTIBIOTICS

The macrolides are a class of antimicrobials that feature one or more deoxy- or amino-sugar bound to a 14-, 15- or 16-membered macrocyclic lactone ring. Erythromycin, a 14-member macrolide, was first isolated by McGuire and

colleagues in 1952 from *Streptomyces erythreus* found in a soil sample in the Philippines. The antimicrobial activity of macrolides is due to inhibition of protein synthesis by binding to the junction of the 30S and 50S subunits of the prokaryotic ribosome, probably by means of the ribosomal L16 protein.[10] Most macrolides are bacteriostatic although they can also be bacteriocidal at higher concentrations. Bacterial resistance occurs by mutations that affect permeability and accessibility of the drug, and by alterations in ribosomal proteins. Their efficacy is typically greater for Gram-positive bacteria than for Gram-negatives.

In addition to their antimicrobial activity, macrolide antibiotics have peptide hormone (motilin receptor stimulation) activities and immunomodulatory (anti-inflammatory) activity.[11-19] These effects are independent of antibacterial properties, as the macrolide clarithromycin reduces mucin secretion and cytokine release from host cells challenged with lipopolysaccharide (LPS). Treatment with erythromycin or other macrolides significantly reduces mucus secretion and other hallmarks of inflammation independently of any antimicrobial effect, although the molecular details of the mechanism are not presently clear.[20] The mechanism for clarithromycin's anti-inflammatory activity appears to be inhibition of the activation of nuclear transcription factors NF-κβ, and AP-1 which results in diminished transcriptional activation of a host of genes associated with the inflammatory response.[19,21-25] This effect is manifested in airway epithelial cells,[19,25] as well as phagocytes.[21,22] Goswami *et al.* studied nasal mucus glycoconjugate secretion from healthy nonsmoking adults before and after treatment with erythromycin, penicillin, ampicillin, tetracycline or cephalosporins.[26] Subclinical doses of erythromycin reduced nasal secretion by 35% in both the resting state and when the nose was stimulated with methacholine or histamine. The other antibiotics had no effect on glycoconjugate secretion.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema, affects an estimated 5 to 15% of all adults in industrialised countries.[27] COPD is the fourth leading cause of death among adults in the Western world,[28,29] the sixth leading cause of death in all countries and is predicted to be among the top three causes of death around the world by 2020.[30-32] The primary risk factor for COPD is cigarette smoking, although other cofactors are certainly involved. COPD patients are heavily colonised by a variety of bacteria, including *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis.* The bacterial community extends into the lower airways, which are not usually colonised by bacteria. *H. influenzae* strains, predominantly acapsular (nontypeable) strains, account for 34% of all bacterial infections in patients with COPD.[33] The resistance of bacterial isolates to commonly used antimicrobials is increasing, as has been recognised for some time.[33]

A significant controversy regarding the progression and severity of COPD relates to the role of the colonising bacterial community in the genesis of inflammatory exacerbations. Studies using a protected-specimen brush sampling method have clearly demonstrated that bacterial counts are increased during exacerbations of COPD to levels consistent with the clinical definition of pneumonia,[34,35] but whether shifts in the bacterial population initiate the host response in an exacerbation or whether other factors are involved is a subject of intense debate.[7,36,37] The most clear evidence in support of a bacterial aetiology for inflammatory exacerbations of COPD was provided by two recent independent studies, both of which demonstrated that *H. influenzae* isolates from patients during an exacerbation are genetically and phenotypically distinct from those in asymptomatic carriage.[38,39]

Macrolides are a recommended choice for antimicrobial therapy in patients with acute exacerbations of COPD.[40] These data provide support for a bacterial aetiology for at least some exacerbations of COPD, other data have clearly demonstrated that macrolide therapy is beneficial even in exacerbations elicited by viral infection.[41] In this study, 109 patients with COPD were given prophylactic treatment with erythromycin or placebo, and the incidence of colds and number and severity of exacerbations between the two groups were compared. A majority (41/54, 76%) of the patients in the control group were diagnosed with a cold or experienced an acute exacerbation (30/54, 56%), whereas a significantly lesser number (7/55, 13%) of the patients given erythromycin had colds or exacerbations (6/55, 11%). The interpretation of this study is somewhat difficult, as the frequency and load of bacterial carriage was not evaluated. A possible interpretation is that viral infection disrupts a normally benign relationship between host and commensal bacteria.

Koh gave subtherapeutic doses of roxithromycin to 25 children with chronic bronchitis, and observed decreases in sputum production and airway hyperresponsiveness.[42] In a similar study, Tsang and colleagues tested the effect of sub-bacteriocidal levels of erythromycin on lung function and mucus production in patients with COPD, and the data clearly demonstrated a beneficial effect.[43] In a more recent study, low-level roxithromycin decreased airway levels of IL-8, neutrophil elastase, and C5a, and reduced neutrophil recruitment into the lung.[44] The results of these studies provide more convincing evidence for an immunomodulatory effect, as in all cases, the level of antibiotic is significantly below the MIC.

CYSTIC FIBROSIS

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The primary cause of morbidity and mortality in patients with CF is opportunistic bacterial infections. There is a clear successional hierarchy in the bacterial inhabitants in the CF lung, beginning with *H. influenzae* and *Staphylococcus aureus* in infancy. These are gradually supplanted by *Pseudomonas aeruginosa* and, to a lesser extent, *Burkholderia cepacia* and other pseudomonads.[45] In general, the onset and severity of *P. aeruginosa* colonisation is a marker for worsening of CF disease. Like many organisms at mucosal surfaces, *P. aeruginosa* forms complex, differentiated bacterial communities known as biofilms. Biofilms are defined as multicellular bacterial communities that form upon a solid biotic or abiotic surface within a polysaccharide matrix. *P. aeruginosa* isolates from CF patients typically produce copious amounts of the extracellular polysaccharide alginate, which is composed of mannuronic and guluronic acids.[46] Alginate plays a role in late-stage biofilm structure and organisation,[47] but alginate-deficient mutants do form biofilms (D. Wozniak, personal communication). Biofilm formation is a complex process that involves multiple steps that are largely coordinated by quorum signalling by means of released homoserine lactone signal molecules.[48-50]
The primary defect in CF that leads to increased bacterial colonisation is a subject of intense current debate and study.[51-55] Smith and colleagues have demonstrated that the increased chloride levels found in the CF airway secretions are inhibitory for the antimicrobial activity of defensins and other peptides that are important in the innate immune defences.[51] Other work has suggested that the CFTR protein mediates bacterial uptake and killing by epithelial cells, and that mutant CFTR alleles found in CF patients are less efficient in mediating bacteriocidal uptake.[53,54] The properties of the mucus secretions in the CF lung may be different, and some have suggested that the adhesivity of mucus may have a detrimental effect on the function of the mucociliary defences.[56-62]
Antimicrobial therapy for CF has been largely credited with extending the average lifespan of CF patients during the past 20 years. The aminoglycoside antibiotics are the primary drugs used in these patients. As with the macrolides, the antimicrobial activity of the aminoglycosides is only partially responsible for their therapeutic effect. Some aminoglycosides can also diminish translational fidelity, leading to read-through of premature stop codons in mutant CFTR alleles, and thus remedy the basic defect.[63-67]
The inherent resistance of *P. aeruginosa* to most antibiotics, including the macrolides, is relatively high, and certainly is higher than the doses that are usually used in CF patients.[68] Therefore, most clinical *P. aeruginosa* strains can be considered to be effectively resistant to erythromycin and other macrolides. Equi *et al.* tested the effect of prolonged azithromycin treatment in a set of 41 patients with cystic fibrosis over the course of 15 months.[69] The lung capacity, as measured by forced expiratory volume, was significantly increased, and the presence of bacteria, IL-8 and neutrophil elastase in sputum was decreased. Wolter *et al.* reported similar results in a randomised trial comparing 60 adult patients with cystic fibrosis given azithromycin or placebo over a three-month study period.[70] In this study, forced expiratory volume (FEV) declined significantly in the control group, whereas it was unchanged in those receiving azithromycin. The levels of C-reactive protein in serum were compared as a general index of inflammation, and declined in the treatment group and remained elevated in the control group. However, Ordonez and colleagues reported no effect on FEV or sputum production in a smaller patient group given clarithromycin for a shorter period of time (six weeks).[71] The results of the former two studies suggest that long-term therapy with macrolides decreases inflammation in CF patients. Possible insights into the mechanism behind these observations were provided by work showing that sub-lethal doses of macrolides can affect *P. aeruginosa* adherence[72] and production of proteases and other virulence factors.[73] Macrolides with 14-member ring or 15-member ring structures also inhibit alginate production, whereas 16-member ring macrolides do not.[74-77]

SINUSITIS

Sinusitis is a chronic, recurrent inflammatory condition that is perhaps best viewed as a 'vicious circle' in which inflammation leads to oedema and blockage of normal sinus drainage, which allows for increased colonisation by a number of different bacterial species. Although many of the same airway symbionts that cause opportunistic infections in other chronic inflammatory conditions (pneumococcus, *H. influenzae*, *M. catarrhalis*) are often isolated from patients with sinusitis, recent work has indicated that anaerobic bacteria constitute the majority of the bacterial load from cases of chronic recurrent sinusitis as compared with facultative anaerobes or aerobes.[78]
Macrolide therapy has been recognised for some time to significantly decrease mucus secretion in patients with sinusitis, and to significantly improve outcomes.[26,79] More recent work has demonstrated that macrolide therapy significantly reduces the release of IL-8 in nasal secretions and reduces the incidence of nasal polyps.[80]

DIFFUSE PANBRONCHIOLITIS

Diffuse panbronchiolitis (DPB) is a progressive lung disorder similar to CF in clinical presentation that is found primarily

in persons from East Asia. Patients with DPB typically have chronic bronchiectasis, with coughing, excess sputum production, and a reduction in airway conductivity. Most patients also have chronic sinusitis. Unlike COPD, there is not a correlation between DPB and smoking. As in patients with CF, chronic infections with mucoid strains of *P. aeroginosa* are common in DPB.[81,82]

Macrolide therapy provides significant benefit for patients with DPB. The first evidence of this was provided by the work of Kudoh *et al.* who demonstrated that erythromycin therapy significantly improves the long-term survival of DPB patients.[83] There has been a significant body of work suggesting that clarithromycin and other macrolides significantly inhibit biofilm formation by *P. aeruginosa*, perhaps by inhibition of the production of alginate and other extracellular polysaccharides.[84-88] Because there is still significant controversy regarding the role for alginate in biofilm formation[47] relative to other potential matrix components,[89] it is not entirely certain how the inhibition of alginate production would be expected to affect bacterial biofilm formation or persistence in the lung.

ASTHMA

As in the preceding inflammatory diseases, macrolide therapy also provides benefit for patients with bronchial asthma. Some have interpreted these results as indicative of a bacterial aetiology for some asthmatic episodes. Amayasu *et al.* evaluated the effect of clarithromycin treatment on 17 patients with bronchial asthma, and demonstrated a significant reduction in inflammatory episodes and general markers of inflammation, such as blood and sputum neutrophil counts.[90] Kamoi *et al.* evaluated the impact of roxithromycin on bronchial hyperreactivity and neutrophil activation in ten asthmatic patients over the course of three months' treatment, and observed a significant reduction in both the release of superoxide and airway reactivity as compared with untreated controls. No statistical benefit was observed in this study earlier than two months into the treatment regimen. Konno *et al.* evaluated the effect of roxithromycin therapy on cytokine secretion by peripheral blood leucocytes isolated from patients with asthma, and observed lower levels of IL-3, IL-4, IL-5 and tumour necrosis factor alpha in lung lavages as compared with controls, and an overall decrease in bronchial responsiveness.[91] Shimuzu and colleagues performed two trials testing the effect of roxithromycin on the progression and severity of disease in children with asthma, and in both cases saw a significant beneficial effect.[92,93] As in the preceding sections, the exact mechanism whereby macrolides exert this effect is not presently clear.

POSSIBLE MECHANISMS FOR ANTI-INFLAMMATORY EFFECTS OF MACROLIDES: AVENUES FOR FUTURE WORK

Translational effects

As noted above, while aminoglycoside antibiotics are not generally toxic to host cells, there is some degree of modification of host translational machinery. For the CF patient, this can be of benefit by leading to production of a less defective CFTR allele. It may be worthy of note that the macrolides have a similar mode of action to the aminoglycosides, involving binding to and modulation of the prokaryotic ribosome. Therefore, an intriguing possibility for future study would be whether macrolide treatment suppresses premature stop mutations in CFTR and other host genes. Because the host defects associated with COPD and other chronic airway conditions are less defined, and probably multifactorial, it is less clear how such a translational mechanism would affect these disorders.

Bacterial signal inhibition

Signal transduction among bacterial communities mediated by homoserine and acylhomoserine lactone molecules is a common theme among many bacterial species, especially those that form biofilms. The most compelling evidence for biofilm formation by *P. aeruginosa* within the cystic fibrosis lung was provided by the work of Singh and colleagues, who demonstrated that CF isolates had quorum signalling profiles consistent with a biofilm mode of growth.[94] In the normal lung, the formation of biofilms is inhibited by a variety of factors, including the sequestering of iron by lactoferrin.[95] Some reports have indicated that low-level macrolide treatment can inhibit the formation of biofilms by *P. aeruginosa*.[84-88] The basic structure of most macrolides is similar to that of homoserine lactone and acylhomoserine lactone quorum signal molecules. Therefore, one could envisage a model in which macrolides interfere with normal bacterial population signalling, and thus eliminate a source of inflammation without killing bacteria.

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