Non-antimicrobial activity of macrolides: therapeutic potential in chronic inflammatory airway disorders*

C Feldman, R Anderson

Charles Feldman, Division of Pulmonology, Department of Medicine, Charlotte Maxeke Johannesburg Academic Hospital and Faculty of Health Sciences, University of the Witwatersrand, South Africa.
Ronald Anderson, Medical Research Council Unit for Inflammation and Immunity, Department of Immunology, Faculty of Health Sciences, University of Pretoria, and Tshwane Academic Division of the National Health Laboratory Service, South Africa.
Correspondence to: Prof Charles Feldman, Department of Medicine, 7 York Road, Parktown, 2193, Johannesburg, South Africa.
E-mail: charles.feldman@medicine.wits.ac.za

* This article was originally published in Italian [Feldman C, Anderson R. Non-antimicrobial activity of macrolides. *Clinical Drug Investigation* (Special Issue 1) 2007; 27: 27-35] and has been reproduced and updated with permission from Wolters Kluwer Health/Adis (Copyright Adis Data Information BV 2007. All rights reserved.)

In addition to their conventional antimicrobial activity, the macrolide group of antibiotics are known to have a number of anti-inflammatory/immunomodulatory activities, which may be of benefit to patients with chronic obstructive pulmonary disease (COPD), both in the stable state and during acute exacerbations. These activities involve interactions of the macrolides with the various components of inflammation in patients with COPD, namely the ciliated airway epithelium, the immune and phagocytic cells of the host and their proinflammatory mediators, as well as with the microbes themselves. There are a number of factors that have been shown to cause injury to the bronchial epithelium and its mucociliary mechanism, including bacterial, chemical, mechanical and host-derived factors. The macrolide, azalide, ketolide group of antibiotics has been shown in many studies to be cytoprotective of human ciliated epithelium in vitro*, protecting against both chemical mediator- and bacterial-mediated injury. Mucus hypersecretion is an important and characteristic feature of many respiratory diseases, including COPD, and increases the morbidity and mortality of these diseases especially as a consequence of pulmonary infection. Chronic inflammation causes not only airway damage, but also goblet cell hyperplasia, which leads to the mucus hypersecretion. Mediators of this inflammation include cytokines, chemokines and oxygen radicals. Both* in vitro *and* in vivo *studies have shown that macrolides reduce mucus and sputum secretion, which may also contribute to their beneficial effects on airway inflammation. Among many other anti-inflammatory actions, clarithromycin (and other macrolides) has been shown to interfere with the generation of neutrophil-selective chemoattractants by bronchial epithelial cells, eosinophils, monocytes, fibroblasts, and airway smooth muscle cells by mechanisms which appear to involve inhibition of intracellular signalling mechanisms. Macrolides also appear to selectively down-regulate exuberant inflammatory responses which result from the interaction of viruses and Gram-negative bacteria with toll-like receptors, TLR3 and TLR4, respectively, while preserving the interaction of Gram-positive bacteria with TLR2. In addition to their standard antimicrobial activity, the macrolide group of antibiotics has been documented to have additional effects against bacteria which are not associated with inhibition of bacterial proliferation, and occur even in microorganisms that are totally resistant to their anti-proliferative actions. This has been most well studied in the case of* Pseudomonas aeruginosa*, a microorganism against which macrolides have no anti-proliferative activity. Macrolides have the ability to interfere with a number of virulence factors produced by* P. aeruginosa*. We have recently reported that the production of pneumolysin, a major protein virulence factor of* Streptococcus pneumoniae*, which promotes extra-pulmonary dissemination of this microorganism, is attenuated by exposure of the bacteria to sub-inhibitory concentrations of clarithromycin. Interestingly, clarithromycin-mediated inhibition of the production of penumolysin was observed not only with macrolide-susceptible strains of the pneumococcus, but also with macrolide-resistant strains which harboured either the ribosomal methylase (erm(B) gene)- or efflux pump (mef gene)-based mechanisms of macrolide resistance. Taken together, the evidence presented in this review, supports the contention that macrolides possess a seemingly unique profile of complementary therapeutic activities.*

J Epidemiol Infect 2009;24(4):21-26

Introduction

In addition to their standard antimicrobial activity, the macrolide group of antibiotics, and in particular clarithromycin, is known to have a number of anti-inflammatory/immunomodulatory activities, that may be of benefit to patients with chronic obstructive pulmonary disease (COPD), both in the stable state and during acute exacerbations.[1-3] These activities involve interactions of the macrolides with the various components of inflammation in patients with COPD, namely the ciliated airway epithelium, the host immune and phagocytic cells and their proinflammatory mediators, as well as with the microbes themselves,[1-8] and are found to occur with the 14-membered macrolides, such as erythromycin, dirithromycin, clarithromycin and roxithromycin, the 15-membered azalide, azithromycin, and the ketolide, telithromycin,[1,7] but not with the 16-membered macrolides, such as josamycin and spiramycin. These secondary, immunomodulatory activities of macrolides may underpin the beneficial anti-inflammatory effects of these agents in chronic inflammatory conditions, of both infective and non-infective origin, especially, but not limited to, those of the airways. Conditions responsive to the anti-inflammatory actions of macrolides are listed in Table 1 and include, most convincingly, cystic fibrosis and diffuse panbronchiolitis.[4,9-19]

The clinical efficacy of macrolides in the treatment of inflammatory diseases has sparked the design and development of a novel class of dissociated macrolides, termed immunolides, which are attenuated in respect of antimicrobial activity in the setting of retention of anti-inflammatory properties.[20,21] However, for reasons described in detail in the following sections, we believe that it is the combination

Table 1: Conditions which may respond to the anti-inflammatory effects of macrolides.

Conditions	Agents	References
Cystic fibrosis	Azithromycin, clarithromycin	9, 10
Diffuse panbronchiolitis	Clarithromycin, erythromycin	11, 12
Bronchiectasis	Erythromycin	13
Chronic obstructive pulmonary disease	Clarithromycin	5
Acute and chronic sinusitis	Macrolides generally	4, 14, 18
Chronic bronchitis	Macrolides generally	4, 14
Bronchial asthma	Macrolides generally	14, 15, 17
Bronchiolitis obliterans syndrome after lung transplantation	Azithromycin	16
Undifferentiated connective	Clarithromycin	19

of immunomodulatory and antimicrobial activities which is most likely to confer optimum anti-inflammatory activity on the macrolide/azalide/ketolide group of antimicrobial agents.

In the case of macrolide-susceptible microbial pathogens, the anti-inflammatory properties of macrolides are attributable to two distinct, albeit complementary, mechanisms. These are: i) interference with the growth of microbial pathogens by conventional ribosomal-targeted antimicrobial mechanisms, which in turn effectively eliminates the stimulus for protracted, ineffective inflammatory responses and ii) suppression of the activities of various types of inflammatory cells, including granulocytes, monocytes/macrophages, and dendritic cells, as well as epithelial cells. More recently, however, it has been speculated that macrolides, in addition to possessing antimicrobial activity, also have immunomodulatory activity, which may explain their therapeutic activity against ostensibly macrolide-resistant microorganisms such as *Pseudomonas aeruginosa* and some strains of *Streptococcus pneumoniae.* In this latter setting, macrolides appear to target microbial virulence factors and quorum sensing mechanisms.

The contribution of the secondary immunomodulatory properties of macrolides to therapeutic efficacy remains to be conclusively established, and is complicated by their activity against atypical microbial pathogens. Interestingly, in a retrospective study designed to address these issues by excluding patients with primary atypical infection, Metersky *et al* have recently reported that initial antibiotic treatment strategies which included a macrolide, as opposed to a fluoroquinolone or tetracycline, were associated with improved outcome in patients hospitalised with bacteraemic pneumonia.[22] As stated by the authors, these results have implications in respect of the mechanisms by which the use of a macrolide for treatment of pneumonia is associated with improved outcome, and appear to support the involvement of the beneficial, secondary immunomodulatory/anti-inflammatory properties in complementing the antimicrobial activity of the macrolides.

Effects on human ciliated respiratory epithelium

Both in the upper and the lower respiratory tract, the human airway is lined by a specialised epithelium which consists of a number of different cell types, of which the specialised ciliated columnar epithelial cells are particularly important.[23] The bronchial epithelium is the primary barrier between the environment and the conducting airways and the cilia acting in concert with the mucus layer secreted above the epithelium, constitute the mucociliary escalator. This is the first-line host defence mechanism of the airway which serves to keep the lower respiratory tract normally sterile. There are a number of factors that have been shown to cause injury to the epithelium and its mucociliary mechanism, including bacterial, chemical, mechanical and host-derived factors.[23] For example, cigarette smoke affects ciliary beating, although the ciliary slowing that may occur is reversible provided exposure is stopped before ciliostasis occurs. The consequences of attenuation of mucociliary fuction may include chronic inflammation, mucosal injury, and persistent bacterial colonisation, as well as bacterial invasion that occur in a variety of chronic airway disorders, including COPD.[23]

In contrast to this is a number of factors that protect the epithelium from these harmful effects. The macrolide, azalide, ketolide group of antibiotics has been shown in studies to be cytoprotective of human ciliated epithelium *in vitro*.[7] This may serve to protect the epithelium from both chemical- and bacterial-mediated injury. The effects appear to be mediated both directly and indirectly.[7] In the first instance, it has been noted that macrolides stimulate ciliary beat frequency and improve mucociliary clearance. This is in contrast to the actions of several bacteria, including *Haemophilus influenzae,* one of the most important pathogens associated with infective exacerbations of COPD. This microorganism has been shown to cause ciliary slowing and damage to respiratory epithelium.[7,24] However, incubation of nasal epithelial tissue with sub-minimum inhibitory concentrations (MICs) of dirithromycin significantly reduced these effects caused by *H. influenzae,* and in particular the epithelial damage. The authors of that study suggested these effects may be direct and possibly associated with elevations in cyclic adenosine monophosphate (AMP).[24]

Furthermore, erythromycin in sub-MIC levels has been shown to inhibit the adherence to human respiratory cells of another important respiratory pathogen encountered in patients with COPD exacerbations, namely *S. pneumoniae*.[25] The mechanism may be related to inhibition of the microorganism's ability to produce one of its most important virulence factors, namely its thiol-activated, cytolytic, protein toxin pneumolysin.[25] Pneumolysin has been shown to slow ciliary beating and to cause damage to human ciliated epithelium. It is thought that these effects may aid in the initial colonisation and the subsequent invasion of the respiratory epithelium by the pneumococcus. Similarly, *P. aeruginosa,* which is a microorganism that may be associated with infective exacerbations in patients with severe COPD, particularly occurring in association with structural airway changes and/or bronchiectasis of the airways, causes ciliary slowing and disruption of the structural integrity of respiratory epithelium. However, macrolides are able to decrease its production of various virulence toxins, thereby protecting the epithelium directly, as well as indirectly by inhibiting neutrophil-associated cytotoxicity.[7] The effects of the macrolide group of antibiotics on microorganisms are discussed more fully below.

Effects on mucus secretion

Mucus hypersecretion is an important and characteristic feature of many respiratory diseases, including COPD, and increases the morbidity and mortality of these diseases especially as a

consequence of pulmonary infection.[26] Chronic inflammation causes not only airway damage, but also goblet cell hyperplasia, which leads to mucus hypersecretion.[26] Mediators of this inflammation include cytokines, chemokines and oxygen radicals. Both *in vitro* and *in vivo* studies have shown that macrolides, and in particular clarithromycin, reduce mucus and sputum secretion[26] which may also contribute to their beneficial effects on airway inflammation.

P. aeruginosa has been shown to stimulate NCI-H292 epithelial cells to produce the mucin core protein, muc5ac.[27] The mechanism appears to be related to stimulation of these cells by the pseudomonal virulence factor, N-(3-oxododecanoyl) homoserine lactone (30-C12-HSL), which is a component of the microorganism's quorum sensing mechanism.[27] In contrast, clarithromycin, inhibits muc5ac gene expression. Azithromycin also inhibited muc5ac production in an ERK 1/2-dependent manner.[26,27] Other macrolides have also been shown to have effects on additional mucus secreting pathways. Macrolides may also decrease sputum production by decreasing water secretion into the airway by inhibiting chloride secretion.[26] Furthermore, in one study of patients with purulent rhinitis,[28] clarithromycin-treated patients showed a reduction in mucus volume and improvement in the quality of nasal mucus, such as viscoelasticity, cohesion, hydration, and transportability. In addition to decreasing the volume of secretions and increasing the mucus clearance by 30%, the rheology, hydration, cohesion and transportability of mucus in patients were similar to controls.[28]

Some of these effects may be due to inhibition of neutrophil accumulation and function.[26] Erythromycin, clarithromycin and azithromycin inhibited tumour necrosis factor (TNF)-induced release of granulocyte-monocyte colony-stimulating factor (GM-CSF) from A549 human airway epithelial cells, thereby inhibiting the ability of these cells to sustain neutrophil survival *in vitro.*[29] Furthermore, anti-GM-CSF antibodies reduced epithelial cell-conditioned medium-mediated neutrophil survival. These combined inhibitory effects of macrolides on both inflammatory cells and mucus secretion were confirmed in an experimental rat model in which mucus production and neutrophil infiltration induced by intranasal ovalbumin and lipopolysaccharide were inhibited.[30] These combined inhibitory effects are also thought to underlie the longstanding beneficial clinical effects seen with macrolides in the treatment of diffuse pan-bronchiolitis and other chronic inflammatory disorders of the airways.[1,2,4-6,31]

Effects on neutrophil and macrophage function

As previously described, the inhibitory effects of macrolides on inflammatory cells are achieved by several mechanisms, including interference with the synthesis and expression of the adhesion molecules ICAM-1 and VCAM-1 on vascular endothelium, as well as with the upregulated expression of β_2-integrins on activated neutrophils.[7] Most importantly, however, clarithromycin (and other macrolides) interfere with the generation of neutrophil-selective chemoattractants by bronchial epithelial cells, eosinophils, monocytes, fibroblasts, and airway smooth muscle cells[11,32] by mechanisms which appear to involve inhibition of intracellular signalling mechanisms, specifically the activities of mitogen-activated protein kinases,[32] and extracellular signal-regulated kinases 1/2,[33] as well as nuclear translocation of the transcription factors nuclear factor kappa B, activator protein-1, and specificity protein 1.[7,33,34] Interference with the synthesis of IL-8, and possibly TNF,[35] appears to be key events in macrolide-mediated attenuation of neutrophil influx into the airway.

Phagocytic clearance of apoptotic cells is impaired in COPD and aggravates and perpetuates the chronic inflammation found in this condition as a consequence of secondary necrosis.[36,37] However, clarithromycin and azithromycin have been shown to increase the phagocytosis of apoptotic epithelial cells and neutrophils by alveolar macrophages.[3,38] More recently Hodge and colleagues[39] have demonstrated a decrease in alveolar macrophage mannose receptor expression in patients with COPD, which increased by 50% following azithromycin therapy, concomitant with both improvement in alveolar macrophage phagocytic ability and reduced inflammatory markers in the peripheral blood. These investigators suggested that the findings implicated the mannose receptor abnormality in the defective phagocytic function, which appeared to be amenable to azithromycin therapy.

Macrolides and toll-like receptors

Toll-like receptors (TLRs) are the prototype pattern recognition molecules which function primarily as the sentinels of the innate immune system. Although they perform key roles in host defence against microbial and viral pathogens, TLRs have also been identified as potential targets in the control of infection-associated harmful acute/hyperacute and chronic inflammatory responses. Interestingly, clarithromycin has recently been reported to down-regulate the expression of TLR4 on monocytes infected with *Helicobacter pylori,*[40] while treatment of monocyte-derived dendritic cells with erythromycin caused differential modulation of expression of TLRs, resulting in up-regulation of TLR2, down-regulation of TLR3, and no effect on expression of TLR4.[41] Activation of dendritic cells with peptidoglycan, polyriboinosinic: polyribocytidylic acid [poly (I:C)], or lipopolysaccharide (LPS), agonists of TLR2, 3 and 4, respectively, resulted in up-regulation of expression of costimulatory molecules and cytokine production. These pro-inflammatory activities of dendritic cells were effectively attenuated by treatment with erythromycin when the cells were activated with poly (I:C) or LPS, but not with peptidoglycan.[41] In the case of poly (I:C), erythromycin-mediated inhibition of pro-inflammatory activity was associated with decreases in both the activation of NF–B and levels of TLR3 mRNA transcripts, while the mechanism by which the macrolide attenuated TLR4 signalling was unclear.[41] These findings suggest that macrolides may selectively down-regulate exuberant inflammatory responses which result from the interaction of viruses and Gram-negative bacteria with TLR3 and TLR4, respectively, while preserving the interaction of Gram-positive bacteria with TLR2.[41]

Effects of macrolides on dendritic cells

In one recent study, Sugiyama *et al*[42] reported that cytokine production by LPS-activated murine dendritic cells is differentially modulated by 14-(clarithromycin) and 15-membered (azithromycin) macrolides, but is unaffected by a 16-membered macrolide (midecamycin). Clarithromycin was found to suppress the production of IL-6 by LPS-activated dendritic cells, and to render these cells less efficient inducers of IL-2 production by naïve T lymphocytes, while

azithromycin augmented the production of the anti-inflammatory cytokine, IL-10.[42] Taken together with the inhibitory effects of macrolides on TLR3/TLR4-mediated dendritic cells,[41] it is clear that in addition to neutrophils, eosinophils, monocytes/macrophages, and epithelial cells, dendritic cells also represent potentially important targets for the anti-inflammatory actions of macrolides. The direct anti-inflammatory actions of macrolides are summarised in Table 2.

Table 2: Direct anti-inflammatory activities of macrolides

Anti-inflammatory activity	Mechanisms	References
Inhibition of neutrophil influx	Interference with: i) synthesis and release of IL-8 by various types of cells ii) synthesis of ICAM-1 and VCAM-1 iii) upregulation of β2-integrins	7* 7* 7*
Inhibition of generation of reactive oxidant species	Interference with: i) activity of NADPH oxidase ii) synthesis of type II nitric oxide synthase (macrophages)	7* 7*
Inhibition of cytokine production	Interference with: i) activation of TLRs and intracellular signalling mechanisms ii) dendritic cell activation and function	32-35,40,41 41, 42
Pro-apoptotic	i) inhibition of synthesis of IL-8 (anti-apoptotic for neutrophils) ii) induction of apoptosis iii) accelerated clearance of apoptotic neutrophils by macrophages	7* 7* 7*

*recently reviewed by Feldman et al

(Table 2 modified from Feldman C, Anderson R. The cytoprotective interactions of antibiotics with human ciliated epithelium. *In:* Rubin BK, Tamaoki J (eds.). *Antibiotics as Anti-inflammatory and Immunomodulatory Agents.* Basel: Birkhauser Verlag, 2005 (Table 2 page 57) with permission).

Additional effects of macrolides against bacteria

In addition to their conventional antimicrobial activity, the macrolide group of antibiotics has been documented to have additional effects against bacteria, which are not associated with inhibition of bacterial proliferation, and occur even in microorganisms that are totally resistant to their anti-proliferative actions.[43-50] This has been most well studied in the case of *P. aeruginosa,* a microorganism against which macrolides have no antiproliferative activity.[43,44] Macrolides have the ability to interfere with a number of virulence factors produced by *P. aeruginosa*.[43,44] In addition to inhibiting virulence toxin production by this microorganism, clarithromycin also inhibits their ability to undergo 'twitching motility'.[44] The latter is mediated by the *P. aeruginosa* type IV pilus and mediates cell surface translocation. Type IV pili act as adhesins for binding to host cells in the initial stages of infection. This, together with alginate production, contributes to the virulence of the organism.[44] Alginate is an exopolysaccharide capsule of the organism, which is inhibited by clarithromycin in a dose-dependent manner, which occurs even at very low, sub-MIC concentrations of the antibiotic[44] By inhibiting alginate, macrolides decrease the viscocity of media-containing *P. aeruginosa,* which is conversely markedly increased if alginate is present.

Another important virulence factor is biofilm, which is a self-generated polymer matrix[44-47] Biofilms have the ability to bind cells and organic and inorganic materials to each other and to various other substrates.[46] They have a tightly formed structure which reduces antimicrobial activity, promotes bacterial adherence to lung epithelium and prevents bacterial dehydration.[46] These are important mechanisms for chronic pseudomonal persistence in the airways of patients with conditions such as diffuse panbronchiolitis, cystic fibrosis, bronchiectasis and even COPD. Quorum sensing is a mechanism of cell-to-cell communication utilising diffusible signalling molecules which enables bacteria to detect and regulate their population density. Importantly, quorum sensing is necessary for biofilm formation, which in turn enhances microbial virulence by creating a milieu which not only favours persistence, but also protects microbial pathogens againt antibiotics. Indeed, conventional *in vitro* antibiotic sensitivity testing procedures do not reflect the responses of bacteria which are encased in biofilm. Clarithromycin does not inhibit initiation of biofilm formation, but rather the quality of biofilm is impaired.[44] It has also been shown that incubation of biofilm-grown *P. aeruginosa* with clarithromycin altered the structure and architecture of the biofilm.[44] It is thought that the reason macrolides have been shown to be effective in chronic airway disorders, such as diffuse panbronchiolitis and cystic fibrosis, even in cases colonised with *P. aeruginosa,* is due to their ability to inhibit these various pseudomonal virulence factors, which are essential for bacterial survival.

These effects of macrolides have been much less well studied in other microorganisms, such as *S. pneumoniae.* We have recently reported that the production of pneumolysin, a major protein virulence factor of *S. pneumoniae,* which promotes extra-pulmonary dissemination, is attenuated by exposure of the bacteria to sub-inhibitory concentrations of clarithromycin. Interestingly, clarithromycin-mediated inhibition of the production of pneumolysin was observed not only with macrolide-susceptible strains of the pneumococcus, but also with macrolide-resistant strains which harboured either the ribosomal methylase (*erm*(B) gene)- or efflux pump (*mef* gene)-based mechanisms of macrolide resistance.[49] These observations are in agreement with those of Lagrou *et al,*[25] as well as those of Fukuda *et al*[50] who reported that erythromycin, or clarithromycin/azithromycin inhibited the production of pneumolysin by macrolide-resistant strains of *S. pneumoniae in vitro*, as well as in the lungs of experimentally infected mice. In a recent study, we reported that it is only macrolides (clarithromycin, erythromycin),

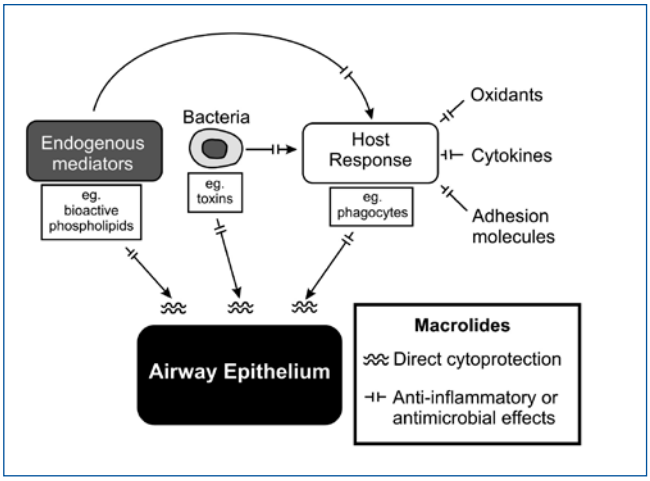

Figure 1: The cytoprotective and anti-inflammatory activities of macrolides and their relationship to protection of airway epithelium. The cytoprotective activity enables the epithelium to resist the direct damaging actions of microbial- and host-derived cytotoxins (≈). The indirect effects are targeted at inhibiting one or more of synthesis, release or activity of bacterial toxins or mediators of host inflammatory responses (⊣).

(Reproduced from Feldman C, Anderson R. The cytoprotective interactions of antibiotics with human ciliated epithelium. *In:* Rubin BK, Tamaoki J, eds. *Antibiotics as Anti-inflammatory and Immunomodulatory Agents.* Base: Birkhauser Verlag, 2005 (Figure 1, page58) with permission).

ketolides (telithromycin), clindamycin, and to a lesser extent azalides (azithromycin), which, at sub-MIC concentrations, attenuate the production of pneumolysin by both macrolide-susceptible and macrolide-resistant strains of the penumococcus, while other types of antimicrobial agent commonly used in the treatment of infections caused by this microbial pathogen, including several which also target microbial protein synthesis, were largely without effect. The other antibiotics tested were amoxicillin, ceftriaxone, ciprofloxacin, moxifloxacin, and tobramycin, while doxycycline showed modest effects.[51]

Importantly, these mechanisms of macrolide-mediated antimicrobial activities counteract microbial persistence and perpetuation of harmful, ineffectual inflammatory responses. The various mechanisms by which macrolides suppress harmful inflammatory responses are summarised in Figure 1.

Experimental studies with macrolides

In one experimental model in which mice were exposed to cigarette smoke daily for six months, those treated for six months with clarithromycin had decreased airspace enlargement and destruction of the alveolar walls and impaired accumulation of macrophages in the lung (demonstrated on bronchoalveolar lavage) in a dose-dependent manner. The authors concluded that at clinically achievable dosages, clarithromycin was able to prevent cigarette smoke-induced emphysema in this animal model by modulating lung inflammation.[52]

In another very recent study, administration of azithromycin at a dose of 20 mg/kg 24 hours after experimental infection of cystic fibrosis mice with an alginate-overproducing strain of *P. aeruginosa* was associated with significantly improved survival, reductions in bacterial load, decreased lung inflammation and increased levels of interferon-gamma.[53] The authors concluded that azithromycin enhances bacterial clearance and reduces lung inflammation by improving innate immune defence mechanisms in cystic fibrosis mice.

Use of macrolides in patients with COPD

Antibiotics are often used in patients with acute exacerbations of chronic obstructive pulmonary disease, which are now recognized to be due to bacterial infection in many cases. The indications for their use and the choice of agent, which may include a macrolide, are reviewed elsewhere.[4,5,31] Many studies investigating the efficacy of macrolides in patients with exacerbations of COPD, as well as comparator studies against other classes of antibiotics, have confirmed them to be very effective.[2,4] However, studies have also been undertaken with macrolides in patients with COPD in which they have been used for their anti-inflammatory activities as well as their effects on mucus secretion.[2,4,5,26] For example, in a double-blind, placebo-controlled parallel group trial of 31 patients with chronic bronchitis, bronchiectasis or diffuse panbronchiolitis, clarithromycin 100 mg twice daily for eight weeks reduced sputum production from 51 g/day to 24 g/day and also increased sputum elasticity.[54] Treatment with clarithromycin was associated with improved hypoxia, hypercapnia, pulmonary function and quality of life. In addition, in a study of patients with chronic bronchitis or bronchiectasis, seven days of treatment with clarithromycin (400 mg/day) decreased sputum production.[55] The percentage of patients in whom it decreased >30% (so-called responders) was 38%. During treatment with clarithromycin, the sputum solid composition increased, while chloride concentration decreased in responders, but not in non-responders.

In one double-blind study, 27 patients with COPD were randomised 2:1 to either azithromycin 500 mg once daily for three days or to placebo.[56] In patients randomised to azithromycin, there were significant changes in serum cytokine values and indices of degranulation as well as oxidative burst of neutrophils, that lasted for 15 days after the end of treatment. Decreases in IL-8, lactoferrin and beta$_2$-microglobulin levels were documented.

Preliminary data also suggest that improvements in clinical end-points and/or in lung function may occur in patients with COPD who are treated with a macrolide. In one study, 67 patients with moderate to severe stable COPD were randomised to either three months of clarithromycin 500 mg daily or placebo and numerous markers of airway inflammation, bacterial colonisation, lung function, exercise tolerance and overall health status were evaluated.[57] At the end of the time period there were no changes in sputum total cell count, inflammatory cytokine levels, or pathogen count in the clarithromycin group. It has been suggested by others that this may be due to the fact that the investigators only included patients on inhaled corticosteroids, since benefit on inflammatory markers has been shown in placebo-controlled studies in patients not on corticosteroids (see below).[6] The clarithromycin group did have a small reduction in the neutrophil count and neutrophil chemotaxis. While total cell count and absolute neutrophil count did not correlate with lung function or walk test, they did correlate with health status score, respiratory symptoms and activity scores in the clarithromycin-treated group ($p<0.03$). The same authors were not able to show an effect of clarithromycin on health status, sputum bacterial numbers or exacerbation rates.[58]

Shorter term studies have also noted benefit from clarithromycin therapy. Nixon and colleagues documented improvement in spirometry, as well as clinical benefit, in their study of patients with COPD.[59] In that study, 25 patients with COPD experiencing an exacerbation were given clarithromycin 500 mg daily for two weeks. FEV_1 increased from 1.12 L to 1.34 L ($p=0.003$), and the mean total scores for signs and symptoms of COPD decreased. Another group investigated the effects of clarithromycin on inflammatory markers in 30 men with mild to moderate COPD, who were not on corticosteroids, in a double-blind, placebo-controlled study.[60] They documented significant decreases in sputum total cell count, IL-8 and TNF levels, with similar decreases in serum inflammatory markers.

More recently, in a randomised, controlled trial, investigators administered erythromycin (250 mg twice daily) or placebo to 109 patients with moderate to severe COPD for one year.[2] The primary end-point was frequency of exacerbations requiring antibiotics. The frequency of exacerbations in the erythromycin group was decreased by 35% and there was an increased median time to first exacerbation from 89-271 days (both significantly different from control). There were no significant differences in any of the secondary endpoints including lung function and selected sputum and serum inflammatory markers.

Conclusions

Taken together, the evidence presented in this review, supports the contention that macrolides possess a seemingly unique profile of complementary therapeutic activities. Notwithstanding their classical ribosome-targeted mechanism of antimicrobial activity, macrolides possess broad-spectrum anti-inflammatory properties affecting a range of inflammatory cell types and their mediators, as well as additional mechanisms of antimicrobial activity which selectively target virulence mechanisms involved in microbial persistence and perpetuation of harmful inflammatory responses, without affecting proliferation.

References

1. Amsden GW. Anti-inflammatory effects of macrolides – an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions? *J Antimicrob Chemother* 2005; **55**: 10-21
2. Seemungal TAR, Wilkinson TMA, Hurst JR, Perera WR, Sapsford RJ, Wedzicha JA. Long term erythromycin therapy is associated with decreased COPD exacerbations. *Am J Respir Crit Care Med* 2008; **178**: 1139-1147
3. Lopez-Boado YS, Rubin B. Macrolides as immunomodulatory medications for the therapy of chronic lung disease. *Curr Opin Pharmacol* 2008; **8:** 286-291
4. Bishai WR. Macrolide immunomodulatory effects and symptoms resolution in acute exacerbation of chronic bronchitis and acute maxillary sinusitis: a focus on clarithromycin. *Expert Rev Anti Infect Ther* 2006; **4**(3): 405-416
5. Swords WE, Rubin BK. Macrolide antibiotics, bacterial populations and inflammatory airway disease. *J Med* 2003; **61**: 242-248
6. Parnham MJ. Immunomodulatory effects of antimicrobials in the therapy of respiratory tract infections. *Curr Opinion Infect Dis* 2005; **18**: 125-131
7. Feldman C, Anderson R. The cytoprotective interactions of antibiotics with human ciliated airway epithelium. *In*: Rubin BK, Tamaoki J, eds. *Antibiotics as anti-inflammatory and immunomodulatory agents.* Basel: Birkhauser Verlag, 2005: 49-63
8. Giarmarellos-Bourboulis EJ. Macrolides beyond the conventional antimicrobials: a class of potent immunomodulators. *Int J Antimicrob Agents* 2008; **31**: 12-20
9. Pukhalsky AL, Shmarina GV, Kapranov NI, Kokarovtseva SN, Pukhalskaya D, Kashirskaja NJ. Anti-inflammatory and immunomodulatory effects of clarithromycin in patients with cystic fibrosis lung disease. *Mediators Inflamm* 2004; **13**: 111-117
10. Smyth A. Update on treatment of pulmonary exacerbations in cystic fibrosis. *Curr Opin Pulm Med* 2006; **12:** 440-444
11. Keicho N, Kudoh S. Diffuse panbronchiolitis: role of macrolides in therapy. *Am J Respir Med* 2002; **1**: 119-131
12. Kadota J, Mukae H, Ishii H, *et al.* Long term efficacy and safety of clarithromycin treatment in patients with diffuse panbronchiolitis. *Respir Med* 2003; **97:** 844-850
13. Tsang KW, Ho PI, Chan KN, *et al.* A pilot study of low-dose erythromycin in bronchiectasis. *Eur Respir J* 1999; **13**: 361-364
14. Healy DP. Macrolide immunomodulation of chronic respiratory diseases. *Curr Infect Dis Resp* 2007; **9**: 7-13
15. Fonseca-Aten M, Okada PJ, Bowlware KL, *et al.* Effect of clarithromycin on cytokines and chemokines in children with an acute exacerbation of recurrent wheezing: a double-blind, randomized, placebo-controlled trial. *Ann Allergy Asthma Immunol* 2006; **97**: 457-463
16. Verleden GM, Vandaudenaerde BM, Dupont LJ, Van Raemdonck DE. Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* 2006; **174**: 566-570
17. Simpson JL, Powell H, Boyle MJ, *et al.* Clarithromycin targets neutrophilic airway inflammation in refractory asthma. *Am J Respir Crit Care Med* 2008; **177**: 148-155
18. Cervin A, Wallworte B, Mackay-Sim A, Coman WB, Greiff L. Effects of long-term clarithromycin treatment on lavage fluid markers of inflammation in chronic rhinosinusitis. *Clin Physiol Funct Imaging* 2009; **29**: 136-142
19. Moskowitz RW, Lesko M, Hooper M. Open-label study of clarithromycin in patients with undifferentiated connective tissue disease. *Semin Arthritis Rheum* 2006; **36**: 82-87
20. Fecik RA, Nguyen PL, Venkatraman L. Approaches to the synthesis of immunolides: selective immunomodulatory macrolides for cystic fibrosis. *Curr Opin Drug Discov Devel* 2005; **8**: 741-747
21. Mereu A, Moriggi E, Napoletano M, *et al.* Design, synthesis and in vivo activity of 9-(S)-dihydroerythromycin derivatives as potent anti-inflammatory agents. *Bioorg Med Chem Lett* 2006; **16**: 58001-58004
22. Metersky ML, Ma A, Houck PM, Bratzler DW. Antibiotics for bacteremic pneumonia: improved outcomes with macrolides but not fluoroquinolones. *Chest* 2007; **131**: 466-473
23. Feldman C. Nonspecific host defenses: Mucociliary clearance and cough. *In*: Niederman MS, Sarosi GA, Glassroth J, eds. *Respiratory infections.* Philadelphia: Lippincott Williams & Wilkins, 2001: 13-26
24. Rutman A, Dowling R, Wills P, *et al.* Effect of dirithromycin on *Haemophilus influenzae* infection of the respiratory mucosa. *Antimicrob Agents Chemother* 1998; **42**: 772-778
25. Lagrou K, Peetermans WA, Jorrisen M, *et al.* Subinhibitory concentrations of erythromycin reduce pneumococcal adherence to respiratory epithelial cells *in vitro. J Antimicrob Chemother* 2000; **46**: 717-723
26. Tamaoki J, Kadota J, Takizawa H. Clinical implications of the immunomodulatory effects of macrolides. *Am J Med* 2004; **117**(9A): 5S-11S
27. Imamura Y, Yamagihara K, Mizuta Y, *et al.* Azithromycin inhibits MUC5AC production induced by the *Pseudomonas aeruginosa* autoinducer N- 3- oxododecanoyl homoserine lactone in NCI-H292 cells. *Antimicrob Agents Chemother* 2004; **48**: 3457-3461
28. Rubin BK, Druce H, Ramirez OE, *et al.* Effect of clarithromycin on nasal mucus properties in healthy subjects and in patients with purulent rhinitis. *Am J Respir Crit Care Med* 1997; **155**: 2018-2023
29. Yamasawa H, Osikawa O, Ohno S, *et al.* Macrolides inhibit epithelial cell-mediated neutrophil survival by modulating granulocyte macrophage colony-stimulating factor release. *Am J Respir Cell Mol Biol* 2004; **30**: 569-575
30. Shimizu T, Shimizu S, Hattori R, *et al.* In vivo and in vitro effects of macrolide antibiotics on mucus secretion in airway epithelial cells. *Am J Respir Crit Care Med* 2003; **168**: 581-587
31. Gotfield MH. Macrolides for the treatment of chronic sinusitis, asthma, and COPD. *Chest* 2004; **125**: 52S-61S
32. Vanaudenaerde BM, Wuyts WA, Geudens N, *et al.* Macrolides inhibit IL17-induced IL8 and 8-isoprostane release from human airway smooth muscle cells. *Am J Transplant* 2007; **7**: 76-82
33. Shinkai M, Tamaoki J, Kobayashi H, *et al.* Clarithromycin delays progression of bronchial epithelial cells from Gl phase to S phase and delays cell growth via extracellular signal-regulated protein kinase suppression. *Antimicrob Agents Chemother* 2006; **50**: 1738-1744
34. Cigana C, Nicolis E, Pasetto M, Assael BM, Melotti P. Anti-inflammatory effects of azithromycin in cystic fibrosis airway epithelial cells. *Biochem Biophys Res Commun* 2006; **350**: 977-982
35. Cigana C, Assael BM, Melotti P. Azithromycin selectively reduces tumor necrosis factor in cystic fibrosis airway epithelial cells. *Antimicrob Agents Chemother* 2007; **51**: 975-981
36. Hodge S, Hodge G, Scicchitano R, Reynolds PN, Holmes M. Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. *Immunol Cell Biol* 2003; **81**: 289-296
37. Hodge S, Hodge G, Ahern J, Jersmann H, Holmes M. Reynolds PN. Smoking alters alveolar macrophage recognition and phagocytic ability: implications in COPD. *Am J Respir Cell Mol Biol* 2007; **37:** 748-755
38. Hodge S, Hodge G, Brozyna S, Jersmann H, Holmes M, Reynolds PN. Azithromycin increases phagocytosis of apoptotic bronchial epithelial cells by alveolar macrophages. *Eur Respir J* 2006; **28:** 486-495
39. Hodge S, Hodge G, Jersmann H, *et al.* Azithromycin improves phagocytic function and expression of mannose receptor in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2008; **178:** 139-148
40. Park JY, Kim HY, Lee JY, *et al.* Macrolide-affected Toll-like receptor 4 expression from *Helicobacter pylori*-infected monocytes does not modify interleukin-8 production. *FEMS Immunol Med Microbiol* 2005; **44:** 171-176
41. Yasutomi M, Ohshima Y, Omata N, *et al.* Erythromycin differentially inhibits lipopolysaccharide- or poly(I:C)-induced but not peptidoglycan-induced activation of human monocyte-derived dendritic cells. *J Immunol* 2005; **175**: 8069-8076
42. Sugiyama K, Shirai R, Mukae H, *et al.* Differing effects of clarithromycin and azithromycin on cytokine production by murine dendritic cells. *Clin Exp Immunol* 2007; **147**: 540-546
43. Tateda K, Standiford TJ, Pechere JC, *et al.* Regulatory effects of macrolides on bacterial virulence: potential role as quorum-sensing inhibitors. *Current Pharmaceutical Designs* 2004; **10**: 3055-3065
44. Wozniak DJ, Keyser R. Effects of subinhibitory concentrations of macrolide antibiotics on *Pseudomonas aeruginosa. Chest* 2004; **125:** 62S-69S
45. Costerton W, Veeh R, Shirtliff M, *et al.* The application of biofilm science to the study and control of chronic bacterial infections. *J Clin Invest* 2003; **112**: 1466-1477
46. Fux CA, Costeron JW, Stewart PS, *et al.* Survival strategies of infectious biofilms. *TRENDS in Microbiology* 2005; **13**(1): 35-40
47. Ichimiya T, Yamasaki T, Nasu M. In-vitro effects of antimicrobial agents on *Pseudomonas aeruginosa* biofilm formation. *J Antimicrob Chemother* 1994; **34:** 331-341
48. Naka Y, Jansch L, Bredenbruch F, Geffers R, Buer J, Haussler S. Quorum-sensing antagonistic activities of azithromycin in *Pseudomonas aeruginosa* PA01: a global approach. *Antimicrob Agents Chemother* 2006; **50:** 1680-1688
49. Anderson R, Steel HC, Cockeran R, *et al.* Clarithromycin alone and in combination with ceftriaxone inhibits the production of pneumolysin by both macrolide-susceptible and macrolide-resistant strains of *Streptococcus pneumoniae. J Antimicrob Chemother* 2007; **59**: 224-229
50. Fukuda Y, Yanagihara K, Higashiyama Y, *et al.* Effects of macrolides against pneumolysin of macrolide-resistant *Streptococcus pneumoniae*. Eur Respir J 2006; 27: 1020-1025
51. Anderson R, Steel HC, Cockeran R, *et al.* Comparison of the effects of macrolides, amoxicillin, ceftriaxone, doxycycline, tobramycin and fluoroquinolones on the production of pneumolysin by *Streptococcus pneumoniae in vitro. J Antimicrob Chemother* 2007; **60**: 1155-1158
52. Nakanishi Y, Kobayashi D, Asano Y, *et al.* Clarithromycin prevents smoke-induced emphysema in mice. *Am J Respir Crit Care Med* 2009; **179**: 271-278
53. Tsai WC, Hershenson MB, Zhou Y, Sajjan U. Azithromycin increases survival and reduces lung inflammation in cystic fibrosis mice. *Inflamm Res* 2009 [Epub ahead of print].
54. Tamaoki J, Takeyama K, Tagaya E, *et al.* Effect of clarithromycin on sputum production and its rheological properties in chronic respiratory tract infections. *Antimicrob Agents Chemother* 1995; **39**: 1688-1690
55. Tagaya E, Tamaoki J, Kondo M, *et al.* Effect of a short course of clarithromycin therapy on sputum production in patients with chronic airway hypersecretion. *Chest* 2002; **122**: 213-218
56. Parnham MJ, Culic O, Eracovic, *et al.* Modulation of neutrophil and inflammation markers in chronic obstructive pulmonary disease by short-term azithromycin treatment. *Eur J Pharmacol* 2005; **517**: 132-143
57. Banerjee D, Honeybourne D, Khair OA. The effect of oral clarithromycin on bronchial airway inflammation in moderate-to-severe stable COPD: a randomized controlled trial. *Treat Respir Med* 2004; **3**(1): 59-65
58. Banerjee D, Khair OA. Honeybourne D. The effect of oral clarithromycin on health status and sputum bacteriology in stable COPD. *Respir Med* 2005; **99**(2): 208-215
59. Nixon LS. Boorman J, Papagiannis AJ, *et al.* Circulation and airways inflammatory markers in chronic obstructive pulmonary disease (COPD) during an exacerbation (abstract). Available at: www.abstracts-on-line.com/abstracts/ATSALL. Accessed January 12, 2004
60. Basyigit I, Yildiz F, Ozkara SK, *et al.* The effect of clarithromycin on inflammatory markers in chronic obstructive pulmonary disease: preliminary data. *Ann Pharmacother* 2004; **38**(9): 1400-1405