Immunomodulatory Effects of Macrolide Antibiotics – Part 1: Biological Mechanisms

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Key Words

Diffuse panbronchiolitis · Macrolides · Quorum sensing

Abstract

Macrolide antibiotics are well known for their antibacterial and anti-inflammatory properties. This article provides an overview of the biological mechanisms through which macrolides exert this 'double effect'. Their antibacterial effect consists of the inhibition of bacterial protein synthesis, impaired bacterial biofilm synthesis, and the attenuation of other bacterial virulence factors. Apart from these direct antimicrobial effects, macrolides are known for their modulating effect on many components of the human immune system. By influencing the production of cytokines, they have a dampening effect on the proinflammatory response. Furthermore, the majority of cells involved in the immune response are, in one way or another, influenced when macrolide antibiotics are administered. Having such an obvious effect on the various aspects of the immune system, macrolides seem to be exceptionally suited for the treatment of chronic inflammatory diseases.

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Introduction

Since their discovery in 1952, many beneficial effects have been attributed to antibiotics belonging to the macrolide family, originally isolated from cultures of *Streptomyces erythraea*. Macrolide antibiotics were named after their main characteristic: a macrocyclic lactone ring which can contain up to 23 atoms [1]. The most commonly used macrolides have 14 (e.g. erythromycin, clarithromycin, and roxithromycin) or 15 (e.g. azithromycin) atoms attached to their macrocyclic rings and are therefore defined as 14- or 15-membered ring macrolides. Over the last decades macrolide antibiotics have been used as a treatment for common infectious diseases like pneumonia, bronchitis, pharyngitis, and skin infections, possessing a moderately broad spectrum of antibacterial activity.

An accumulating body of evidence has emerged indicating that 14- and 15-membered ring macrolides possess modes of action which are independent of their antimicrobial activity. This first became known in 1987 when Kudoh et al. [2] reported a spectacular decrease in symptoms and an increase in life expectancy in patients with diffuse panbronchiolitis (DPB) when they were treated with the macrolide erythromycin. Until then, DPB had been a rapidly progressive and debilitating inflammatory

airway disorder associated with a very poor prognosis. After 1987, when erythromycin was introduced as standard therapy for DPB, an impressive increase in 10-year survival was seen, i.e. from 10–20% to over 90% [3–6].

The unexpected success was attributed to a previously unknown anti-inflammatory effect of erythromycin. This theory was supported by the fact that serum levels of erythromycin in these DPB patients were well below minimal inhibitory concentrations (MIC) for the detected pathogens and by the known lack of susceptibility of most Gram-negative organisms to erythromycin.

In the last two decades, exhaustive evidence has shown that macrolides indeed have a direct antimicrobial effect but, more importantly, also modulate many components of the immune response. Because of this anti-inflammatory or ‘immune modulating’ effect, macrolide antibiotics have been widely used as maintenance treatment for various chronic inflammatory pulmonary diseases. Chronic inflammatory diseases generally feature a distorted inflammatory response. Instead of protecting the human body against exogenous attacks, the cascade of anti-inflammatory responses fails, damaging cells and making them more vulnerable to new attacks. In this article we aim to clarify the biological mechanisms through which macrolides exert their immune-modulating and antibacterial effect. These mechanisms are shown schematically in figure 1.

Effects on Host-Pathogen Interactions

Most macrolides are active against Gram-positive cocci (including anaerobes) and have limited Gram-negative activity. They inhibit bacterial protein synthesis by binding to the 50S subunit of the ribosome [1, 7, 8].

Biofilm

A biofilm is an aggregate of microorganisms immersed in a polysaccharide matrix adherent to each other and to the airway mucosa. Biofilm-forming bacteria are protected from phagocytosis, antimicrobial agents, and the ciliary action of the airway epithelial cells. Furthermore, microorganisms gathered in a biofilm develop significantly different genetic properties compared to planktonic species. Research on the biofilm effects of macrolides mainly focuses on *Pseudomonas aeruginosa*, which is one of the more virulent biofilm-forming microorganisms with a natural resistance to macrolides.

However, effects of macrolides were also demonstrated on biofilm formation in *Haemophilus influenzae* and

Staphylococcus epidermidis [9, 10]. Macrolides have been shown to alter the structure and architecture of the bacterial biofilm [11–13]. Results of Japanese in vitro studies indicate that azithromycin and clarithromycin change the structure of bacterial biofilms via the inhibition of polysaccharide synthesis [12, 14]. An insufficient biofilm allows for enhanced phagocytosis and clearance of bacteria by alveolar macrophages [11, 15].

Quorum Sensing

During infection, bacteria employ mutual communication [quorum sensing (QS)] to coordinate the expression of genes, for instance, genes encoding for tissue-damaging factors [16]. Through the production of autoinducer molecules, genes can be switched on or off depending on the local pathogen density. Furthermore, activation of the QS cascade is claimed to promote biofilm formation and to stimulate IL-8 production, causing an enhanced neutrophil influx at the site of infection [6]. Several authors suggest that suppression of QS systems through reduced transcription of QS genes is also one of the mechanisms of macrolide action [16–18].

Bacterial Adherence

In vitro and in vivo evidence suggests that *P. aeruginosa* bacilli, when cultured in the presence of low levels of macrolides, e.g. erythromycin, demonstrate decreased adherence to cells of the airway epithelium [19–21]. Since the adherence of bacteria to mucosal surfaces is an important initial event in the pathogenesis of most bacterial infectious diseases, this could help explain the clinical efficacy of low-dose macrolide therapy in patients colonized with PA.

Mobility

The effect of macrolides on *P. aeruginosa* is accompanied by an impairment of the mobility of this microorganism. *Pseudomonas* spp. are mobile thanks to 2 distinctive modalities: flagella, which are tail-like structures that project from the cell body and move in a whip-like manner, and type IV pili (fimbriae) that provide twitching motility.

Exposure to sub-MIC concentrations of macrolide antibiotics results in loss of mobility, partly due to the inhibition of flagellin production [22–24], the principal constituent of bacterial flagella, and partly because some macrolides alter the assembly of type IV pili [13, 25]. This loss of mobility facilitates easier phagocytosis and the killing of bacteria by alveolar macrophages.

Fig. 1. Cellular and noncellular effects of macrolides. 1 = Attenuation of biofilm function; 2 = suppression of bacterial quorum sensing; 3 = decrease in bacterial adherence; 4 = loss of flagellar mobility; 5 = reduced production of bacterial toxins; 6 = consolidation of epithelial tight junctions; 7 = increasing ciliary beat frequency; 8 = reduction of sputum quantity; 9 = diminished sputum viscosity; 10 = inhibition of the synthesis of proinflammatory

agents by bacteria, eosinophils, neutrophils, and epithelial cells; 11 = reduction of neutrophil chemotaxis; 12 = stimulation of neutrophil degranulation; 13 = acceleration of neutrophil apoptosis; 14 = downregulation of adhesion molecules; 15 = stimulation of phagocytosis by alveolar macrophages; 16 = reduction of T cell numbers and T cell migration; 17 = modulation of dendritic cell function.

Bacterial Toxins

Cytotoxic enzymes produced by bacteria when causing infection, including exotoxin A, alkaline protease, elastase, and phospholipase C, are important factors in bacterial virulence. Erythromycin and, more recently, azithromycin have been shown to suppress the production of those enzymes and, consequently, to diminish bacterial virulence [23, 26–28].

Intracellular Effects

Macrolides accumulate and show a prolonged retention in human cells after oral or intravenous administra-

tion, an effect that is augmented when macrolide treatment is given for a longer period of time [29–32]. In cystic fibrosis patients treated with azithromycin (500 mg daily) for at least 35 consecutive days, the concentration of azithromycin in neutrophils appeared to be up to 3,000 times higher as compared to the concentration in plasma [32]. Macrolides have also been shown to accumulate in alveolar macrophages [30, 33].

This suggests that tissue and intracellular concentrations may be more useful for assessing the antibacterial activity of azithromycin than serum concentrations [34, 35]. Because intracellular concentrations of macrolide

antibiotic often exceed the MIC of phagocytized pathogens, macrolides have also been demonstrated to be effective against microorganisms with in vitro macrolide resistance [35, 36]. The excellent intracellular penetration of macrolides also appears to explain their effectiveness against intracellular pathogens [34].

Effects on Airway Epithelial Cells and Mucus Properties

Besides inhibiting the production of proinflammatory cytokines by bronchial epithelial cells [37, 38], macrolides distinctly modulate features of the bronchial epithelium, making it better armed against exogenous attacks. The bronchial epithelium is critically important in lung defense. In addition to being a mechanical barrier, it regulates the electrolyte content of the airway surface liquid by means of its tight junctions between adjacent cells. In vitro studies demonstrate that azithromycin increases the transepithelial electrical resistance of human airway epithelium by changing the processing of tight junction proteins, thus preventing excess leakage of electrolytes and ameliorating mucus properties [39].

Furthermore, when airway epithelial cells are exposed to inflammatory mediators in vitro, macrolides display a protective effect against epithelial damage and ciliary dysfunction [40, 41]. This positive effect on ciliary beat frequency, however, was not confirmed in in vivo studies in patients with chronic rhinosinusitis or bronchiectasis [42, 43].

Airway mucus hypersecretion and the resulting excess sputum expectoration is an important characteristic of several chronic inflammatory pulmonary diseases. Mucus hypersecretion may lead to more exacerbations and poor health-related quality of life [44]. Macrolides have been shown not only to reduce the quantity of expectorated sputum in vivo, e.g. in bronchiectasis, but also to change the composition of mucus, thereby enhancing mucus clearance [45–51].

Effects on the Immune System

Innate Immunity

Cytokine and Chemokine Response

Cytokines are hormone-like proteins that enable immune cells to communicate, and they play an integral role in the initiation, perpetuation, and subsequent downregulation of the immune response. Chemokines are cyto-

kines with a particularly strong chemotactic capacity. Production of cytokines is effectuated by a variety of cell types, including alveolar macrophages, eosinophils, neutrophils, and bronchial epithelial cells. Proinflammatory cytokines [such as interleukin (IL)-1, IL-2, IL-4, IL-6, IFN- γ , TNF- α , and GM-CSF] and chemokines (such as IL-8 and RANTES) amplify the immune response through positive feedback loops. Anti-inflammatory cytokines, such as IL-10, prostaglandins, and transforming growth factor (TGF)- β , attenuate the immune response through a negative-feedback mechanism. In general, macrolides inhibit the synthesis and/or secretion of proinflammatory cytokines while increasing the release of anti-inflammatory cytokines [1]. Some recent research however, promotes a view in which macrolides can differentially modulate proinflammatory cytokine secretion [37]. Changes in cytokine and chemokine production are probably related to an effect of macrolides on the activation of transcription factors, i.e. nuclear factor (NF)- κ B and activator protein (AP)-1 [52]. Inhibition of the production of proinflammatory cytokines has been described in several in vivo studies in healthy subjects and in patients with CF, asthma, or chronic rhinosinusitis [53–59].

Alveolar Macrophages

Macrophages play a critical role in the phagocytosis of apoptotic cells and in the removal of exogenous particles, such as bacteria. Recent studies prove that macrolides promote the phagocytosis of apoptotic cells by alveolar macrophages, thus avoiding secondary necrosis and the release of cell contents that may induce further inflammation [60–62].

In addition, some authors propose that macrolides promote monocyte-to-macrophage differentiation, thereby increasing the number of active macrophages [63, 64]. Results of earlier research suggest that macrolide antibiotics also enhance other macrophage functions, including cytotoxic activity [65].

Neutrophils

Neutrophils are key players in the inflammatory response of patients with chronic airway disease [66]. They accumulate at the site of infection, responding to increased levels of chemokines and cytokines, primarily IL-8 and TNF- α . Macrolide antibiotics exert an influence on several domains of neutrophil function.

Reaction to Chemokines. Macrolide antibiotics cause a significant reduction in the chemotactic response of neutrophils to chemokines [67, 68]. Together with the previously described inhibition of chemoattractant genera-

tion, this results in markedly decreased airway neutrophilia in patients with various inflammatory pulmonary diseases [8, 51, 59, 69–72].

Degranulation. Upon activation, neutrophils release granules containing cytotoxic enzymes, such as elastase, a process called neutrophil degranulation or exocytosis. In general, macrolides seem to stimulate exocytosis, which may result in enhanced antibactericidal activity [1, 73–76].

Adhesion. Leukocyte adhesion is a hallmark of the inflammatory cascade, and cell adhesion molecules are the mediators of this event [1]. Cultured bronchial epithelial cells treated with erythromycin show reduced levels of intercellular adhesion molecule 1 [38, 77]. These findings suggest that reducing the release of adhesion molecules in bronchial epithelial cells is another anti-inflammatory effect of macrolide antibiotics.

Oxidative Burst. The production and release of reactive oxygen species by neutrophils to enhance their cytotoxic capability is referred to as the ‘oxidative’ or ‘respiratory’ burst, a process mediated by NADPH-dependent oxidase. Contradictory data have been reported with regard to the effect of macrolides on the oxidative burst. Initially, evidence was presented showing an attenuation of the oxidative burst capacity, but more recent studies have disclosed an opposite effect or no effect at all [74, 78–80].

Apoptosis. In the previous decade, it had already been proposed that apoptosis (programmed cell death) limits the ability of neutrophils to damage tissue while being involved in an inflammatory response [81, 82]. Since then, several in vitro studies have demonstrated that macrolides shorten neutrophil survival by accelerating neutrophil apoptosis [74, 79, 82–84].

Adaptive Immunity

The aforementioned research data indisputably show the existence of a direct modulating effect of macrolides on the innate immune system. Studies focusing on the effects of macrolide antibiotics on cellular immunity also clearly demonstrate an impact on T cell regulation and antigen presentation.

Long-term use of macrolide antibiotics reduces the elevated number of lymphocytes in the bronchoalveolar lavage fluid of DPB patients to subnormal levels [85, 86]. In addition, 14- and 15-membered ring macrolides appear to be involved in the augmentation of the apoptosis of activated lymphocytes, thus reducing inflammation [87]. Dendritic cells are the most important antigen-presenting cells and play a central role in the initiation and regu-

lation of immune responses. Sugiyama et al. [88] demonstrated that clarithromycin and azithromycin modulate the function of dendritic cells; each macrolide shows a different immune-dampening effect. In addition, macrolides appear to have a suppressive effect on the proinflammatory cytokine production of T cells [89, 90].

An early in vivo study in healthy volunteers showed a small but significant positive effect of azithromycin on the proliferative B cell response of stimulated lymphocytes [91]. However, a more recent study in patients with bronchiectasis failed to confirm this finding [92], while research in vitro demonstrated an opposite effect [93].

Conclusion

Macrolide antibiotics are well known for their antibacterial and anti-inflammatory properties. They clearly possess an antibacterial effect that consists of the inhibition of bacterial protein synthesis, impaired bacterial biofilm synthesis, and the attenuation of other bacterial virulence factors. Apart from these direct antimicrobial effects, macrolides are known for their modulating effect on many components of the human immune system. By influencing the production of cytokines, they have a dampening effect on the proinflammatory response. Furthermore, the majority of the cells involved in both the innate and adaptive immune responses are, in one way or another, influenced when macrolide antibiotics are administered. The most distinct effect of macrolides is found in neutrophils, the key players of the anti-inflammatory response. Among other things, neutrophil accumulation, adhesion, and apoptosis are clearly reduced, which results in markedly decreased airway neutrophilia. Studies focusing on the effects of macrolide antibiotics on cellular immunity also clearly demonstrate an impact on T cell regulation and antigen presentation.

Future Perspectives

In the near future, clinicians might add new immunomodulatory drugs of the macrolide family to their armamentarium. Immunomodulatory macrolide antibiotics devoid of anti-infective properties are developed by modifying the molecular structure of the atoms attached to the macrocyclic ring. These purely immunomodulatory macrolides would offer a way to circumvent bacterial resistance. This concept has been investigated in tetracyclines, another group of antibiotics which also have

anti-inflammatory properties. Chemically modified tetracyclines, with no antibacterial capacity, induce an anti-inflammatory response by modulating cytokine and matrix metalloproteinase secretion [94–98]. However, only in vitro and animal studies have been performed investigating the effect of chemically modified tetracyclines. To our knowledge, no phase 1 studies are yet available describing the efficacy and safety of purely immunomodulatory drugs.

Acknowledgement

We would like to thank Mr. Rene Blom for creating the illustration.

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