MiniReview

Macrolide antibiotics and pulmonary inflammation

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Abstract

Many clinically effective therapeutic agents can exhibit localized and systemic effects that are manifestly different from their intended primary pharmacological mode of action. Macrolide antibiotics such as erythromycin and its derivatives are no exception. In addition to their antibacterial action, this class of antibiotics exhibits anti-inflammatory activity in a variety of airway diseases such as asthma and diffuse panbronchiolitis that is separate and distinct from a direct antibacterial action. A variety of erythromycin derivatives have been shown to be clinically beneficial in these airway diseases. The anti-inflammatory activities of these macrolide antibiotics are becoming a research topic of intense interest. Recent work in this field has led to the understanding of the various physiological, cellular and molecular processes of the inflammatory response that are inhibited or suppressed by these compounds. This review presents a brief summary of the fascinating recent work in this active research area. ß 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.

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1. Introduction

This brief review covers recent findings in the research conducted on the use of macrolide antibiotics in the treatment of pulmonary inflammation. Most chronic airway disorders are the result of a lung injury that results in the infiltration of inflammatory cells such as neutrophils, macrophages, lymphocytes, eosinophils, or mast cells. The nature of the injury (e.g. infectious, smoke inhalation or allergy) will determine the pattern of cell infiltration, as will the time elapsed since the onset of the injury [1]. The infiltration of these inflammatory cells depends on the local generation of soluble chemotactic factors such as cytokines, complement fragments, or lipid products, which attract inflammatory cells into the lung.

Macrolide or polyketide antibacterial compounds are a multitude of polyoxygenated fungal and bacterial secondary metabolites which are assembled from C3 units [2]. That is, propionyl-S-CoA is used as a starter piece and successive additions of methylmalonyl-S-CoA are used to elongate the growing macrolide chain. Most of these metabolites possess large ring structures, hence the name macrocyclic or macrolide.

The macrolide family of antibiotic therapeutic agents is capable of more than one pharmacological mode of action. They exhibit separate and distinct antibacterial and anti-inflammatory actions. Macrolide antibiotics, which have been known to reduce inflammation associated with a variety of airway diseases since the early 1970s [3], include erythromycin, azithromycin, clarithromycin and roxithromycin, as well as many other compounds (Fig. 1). They are well known antibacterial agents and are widely used in the chemotherapeutic treatment of a variety of infectious diseases. The broad spectrum antibacterial activity as well as good tissue penetration have led to the widespread use of these macrolide antibiotics for many years.

In addition to their antibacterial effects, increasing evidence suggests that macrolides may play a role in the modulation of the immune responses in a variety of airway diseases. Effective modulation of the inflammatory response by macrolide antibiotics has been shown in airway diseases such as asthma [4,5], diffuse panbronchiolitis [6,7] and in a variety of bacterial and viral airway infections caused by Streptococcus pneumoniae [8], Pseudomo-
nas aeruginosa [9], Haemophilus influenzae [10], and influenza virus [11].

The anti-inflammatory effects of macrolides influence a wide, diverse series of cellular events [12] and exert their anti-inflammatory effects at a variety of different sites. The interaction between macrolides, epithelial cells and leukocytes may play an important regulatory role in inflammation. Macrolides can accumulate in leukocytes, reaching concentrations that are significantly higher than therapeutic concentrations in serum [13]. This might contribute to their anti-inflammatory effects and account for alteration of other cellular functions in leukocytes. Consistent with their accumulation in leukocytes, effects on leukocyte synthesis of several cytokines, oxidant molecules such as hydrogen peroxide, nitric oxide and superoxide, adhesion molecules as well as granulation of phagolysosomes and chemotaxis have been reported suggesting an intracellular target(s) for macrolide anti-inflammatory action [14–16].

Several different macrolides have been evaluated for differing abilities to mediate a variety of inflammation processes. Differences in anti-inflammatory activity have been reported between different macrolides [13,17]. The number of atoms in the macrolide ring system may be a significant factor in the anti-inflammatory activity of a given macrolide. Macrolides having 14- and, to a lesser extent, 15-member rings display substantial anti-inflammatory activity [18]. However, the anti-inflammatory effect of the macrolide antibiotics with 16-member rings such as josamycin [14,19] and spiramycin [20–22] is highly variable and controversial.

2. Macrolides and pulmonary inflammation

Clinical studies have documented the efficacy of macrolide therapy in the reduction of airway hyperresponsiveness that is observed in asthma and other pulmonary disorders [4]. However, the mechanism(s) of action are not well understood.

Konno et al. [23] demonstrated that pretreatment of mitogen-activated human peripheral blood leukocytes with roxithromycin suppressed interleukins (IL) 2–4 and tumor necrosis factor alpha (TNF-α) secretion. Additional work demonstrated that roxithromycin reduced bronchial responsiveness to methacholine after cytokine induction by intratracheal injection of lipopolysaccharide (LPS). Furthermore, a single 5 mg kg⁻¹ daily dose of this macrolide significantly inhibited the appearance of IL-3, IL-4, IL-5 and TNF-α. Clarithromycin has been shown to suppress bronchial hyperresponsiveness associated with eosinophilic inflammation in asthma patients [24]. Eosinophil and eosinophilic cationic protein levels were significantly decreased in blood and sputum samples after macrolide treatment.

The airway disease diffuse panbronchiolitis is characterized by severe chronic inflammation localized in the respiratory bronchioles [6]. Although the pathophysiology is unknown, P. aeruginosa pulmonary infection likely plays a role [6]. Before the advent of erythromycin therapy, the survival rate of patients was 12.4–21.9% but after erythromycin survival it is greater than 90% despite erythromycin having neither bacteriostatic nor bacteriocidal activity against Pseudomonas sp. [6,25]. This suggests that macrolides likely exert their effect by reducing inflammation rather than by an antimicrobial effect. Consistent with this concept, erythromycin suppresses Pseudomonad-induced IL-8 production, and in addition, erythromycin-treated patients with diffuse panbronchiolitis exhibit decreased levels of IL-1β and TNF-α [6]. Increased percentages of CD8+, CD4+ and CD3+ lymphocytes were found in peripheral blood from patients with diffuse panbronchiolitis and treatment with erythromycin resulted in a decrease in these percentages [6].

Other than panbronchiolitis, macrolides are not commonly used as anti-inflammatory agents in airway disorders. Although there is evidence that macrolides are effective in asthma [3,4], the drugs are not commonly used for their anti-inflammatory effect and the NIH asthma guidelines do not recommend macrolides as anti-inflammatory agents [26]. For other lung disorders the evidence of clinical improvement is either unknown or weak. Nevertheless, several in vitro studies have suggested the potential for beneficial actions in inflammatory airway disorders. However, a 10-patient pilot study with clarithromycin showed no effect on forced expiratory volume in 1 s and no significant effect on airway indices of inflammation in cystic fibrosis such as neutrophils or IL-8. Larger trials in cystic fibrosis and asthma are currently ongoing and may provide more definitive answers to the clinical utility of macrolides in these disorders.

3. Macrolide structure and inflammation

The chemical structures of several macrolides have been examined with respect to their anti-inflammatory properties. Erythromycin A derivatives that contain a 14- or 15-member ring structure, including clarithromycin, roxithromycin, dirithromycin, HMR 3004 and azithromycin (Fig. 1), exhibit anti-inflammatory activity. Bronchial hyperresponsiveness in adult asthmatics, endothelin-1, neutrophil oxidant burst, neutrophil chemotaxis and cytokine production [18], IL-8 release [27] and phagocytes, reactive oxygen species and cytokine levels [12] have been reported to be affected by these macrolide antibiotics. As noted above, macrolides containing a 16-member ring, such as spiramycin and josamycin (Fig. 1), have been reported as having either no or potent anti-inflammatory effects. Similarly, the immunosuppressant FK506 (tacrolimus, 23-member ring), which blocks T cell signal transduction by inhibiting calcineurin [28], has been purported to have
variable anti-inflammatory activity [18]. Some studies have found a potent anti-inflammatory effect of FK506, particularly in attenuating inflammatory cell responses to LPS [29–33]. A similar macrolide immunosuppressant, rapamycin, which has a 29-member ring and blocks signal transduction of TOR kinases [34], appears to be beneficial in the control of inflammation associated with chronic asthma [35,36]. Neither FK506 nor rapamycin is a true antibacterial macrolide and, as such, detailed comments are beyond the scope of this brief review.

Variation of anti-inflammatory activity is also seen within the class of 14-member ring macrolides. Using a rat carrageenin paw edema model, Scaglione and Rossoni [17] demonstrated that roxithromycin at a therapeutic dose suppressed carrageenin-induced edema with results almost equal to that seen with non-steroidal anti-inflammatory drugs. In contrast, azithromycin and clarithromycin exhibited only slight anti-inflammatory effects in this model. Ianaro et al. [13] examined the effects of four macrolide antibiotics on acute inflammation using a rat carra-
geenin pleurisy model. TNF-α, nitrite plus nitrate (oxidation products of nitric oxide), prostaglandin E$_2$, IL-1β, IL-6 and 6-keto-prostaglandin F$_{1α}$ levels were reduced by macrolide treatment. Reduction in prostanoid and NO$_x$ levels could not be attributed to inhibition of inducible nitric oxide synthase (iNOS) or cyclooxygenase-2 since macrolides did not inhibit either enzyme. Specifically, roxithromycin appeared to be more effective than erythromycin and clarithromycin at promoting these inhibitory effects. Azithromycin appeared to only slightly affect the inflammatory response.

The anti-inflammatory activities of these macrolide antibiotics appear to depend to a significant extent on the nature of the sugar moieties that are attached to the macrocyclic ring system. Abdelghafar et al. [37] demonstrated that erythromycin A derivatives that are characterized by the sugar 1-cladinose at position 3 of the macrocyclic lactone ring possess anti-inflammatory properties. These 1-cladinose-containing macrolides include erythromycin A, clarithromycin, roxithromycin, dirithromycin and azithromycin [37] (Fig. 1). The importance of the 1-cladinose moiety in modulation of inflammation is clear although its precise mode of action is still under investigation.

Production of biofilms by pathogenic Pseudomonads renders these infections difficult to treat with a regimen of antibiotics [38]. Kobayashi [38] investigated the inhibition of *P. aeruginosa* alginate biofilm synthesis by a variety of macrolides. Guanosine diphospho-mannose dehydrogenase (GPMH), a key enzyme in alginate biosynthesis, was found to be inhibited by 14- or 15-membered ring macrolides such as erythromycin, clarithromycin, roxithromycin and azithromycin but not by the 16-membered ring macrolide midecamycin. When the arrangement of the sugar moieties at position 5 of the macrocyclic ring were examined it was found that the 14- and 15-membered rings contained a monosaccharide, desosaminose, while the 16-membered ring of midecamycin contained a disaccharide of mycaminose and mycarose. Removal of the terminal sugar, mycarose, from midecamycin produced an inhibitor of GPMH.

### 4. Cellular targets of macrolide antibiotics

A variety of cells found in lungs such as macrophages, polymorphonuclear leukocytes and bronchial epithelial cells have been examined as possible cellular targets for macrolide anti-inflammatory action in airway disease. A wide variety of physiological mechanisms and biochemical components have also been studied to determine the molecular mechanisms by which macrolide antibiotics exert their anti-inflammatory effects.

Endogenous production of nitric oxide (NO) by iNOS may be involved in the regulation of normal airway function but may exert deleterious effects when it is inappropriately generated or overproduced [14]. Tamaoki et al. [39] examined the protective effect of macrolides on immune complex-induced lung injury and NO production by rat macrophages. Treatment of macrophages with erythromycin A decreased production of both NO and iNOS mRNA. Immune complex-induced release of the cytokines IL-1β and TNF-α was inhibited by erythromycin A treatment. Erythromycin A treatment was also found to decrease the lung wet-to-dry ratio, suggesting that fluid infiltration was decreased. These findings may suggest that macrolides' inhibition of immune complex-mediated NO production may be derived from inhibition of cytokine production [39]. This beneficial effect is not confined to erythromycin A alone. Treatment with a variety of other macrolides such as clarithromycin and josamycin has been reported to reduce iNOS-catalyzed NO production and iNOS mRNA transcription [14].

Macrolides have been implicated in altering the production of a wide variety of molecules and parameters that influence the inflammatory response. Among those influenced are the cytokines (including IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, granulocyte-macrophage colony stimulating factor (GM-CSF), interferon-γ, TNF-α), oxidant production, chemotaxis and granulocytosis [12]. The influence of the macrolides on the immune system can result in either an increase or a decrease in a particular function leading to complex and somewhat confusing results [12].

Macrolide therapy often appears to decrease the transcription of mRNA for a variety of cytokines. Kawasaki et al. [40] examined the effect of the macrolide roxithromycin on cytokine production in epithelial cells. Using Bet-1A human bronchial epithelial cells with or without stimulation by IL-1β, they found roxithromycin inhibited IL-8 release and decreased amounts of IL-8 mRNA. Inhibition of IL-6 and GM-CSF was also seen. Takizawa et al. [41] used BEAS-2B human bronchial epithelial cells to examine the effect of erythromycin A on levels of the proinflammatory cytokine IL-6. In this study, erythromycin A exhibited a dose-dependent suppressive effect on IL-6 release. Northern blot analysis revealed that erythromycin A treatment produced a decrease in the amount of IL-6 mRNA in a dose-dependent manner. Sato et al. [42] examined the effect of erythromycin on eosinophil chemotactic cytokines produced by human lung fibroblasts. Treatment of these cells in vitro with either IL-1β or TNF-α attenuated the release of eosinophil attractants eotaxin, GM-CSF and RANTES. The effect was most pronounced with eotaxin and erythromycin treatment also significantly suppressed eotaxin mRNA.

However, post-translational control of cytokine activity may also be influenced by macrolides. Long-term effects of erythromycin A on production of some cytokines and chemokines have been examined in rat alveolar macrophages by Sugiyma et al. [43]. TNF-α, cytokine-induced neutrophil chemoattractant-1 (CINC-1, the rat counterpart of human IL-8) and CINC-2 (the rat counterpart of human...
macrophage inflammatory peptide-2) were examined after LPS treatment in the presence or absence of erythromycin A. Production of all three cytokines was found to be significantly lower in macrophages obtained from animals that received low-dose, long-term erythromycin A treatment compared to untreated controls. mRNA levels for these cytokines were not decreased during erythromycin A treatment but were slightly increased, suggesting a possible translational mode of action.

Adhesion of neutrophils to the surface of epithelial cells is an important step in the inflammatory process and expression of the surface proteins responsible for this phenomenon has been found to be influenced by treatment with various macrolides. Lin et al. [44] examined the effect of erythromycin A treatment on β2-integrins (CD11b/CD18) (cell surface adhesion molecules) by LPS-treated neutrophils. Flow cytometric analysis revealed that expression of these cell surface adhesion molecules was decreased following erythromycin A treatment. Interestingly, this decrease was observed in the presence or absence of LPS stimulation of the neutrophils. Kawasaki et al. [40] also examined the effect of the roxithromycin on neutrophil attachment to Bet-1A human bronchial epithelial cells. They found roxithromycin inhibited neutrophil adhesion to Bet-1A bronchial epithelial cells and decreased expression of intracellular adhesion molecule-1 (ICAM-1), with or without stimulation by IL-1β. Macrolide treatment decreased the number of neutrophils in bronchoalveolar lavage fluids from patients with airway inflammation. Decreased expression of ICAM-1 may explain, in part, the attenuating effects of macrolides on local neutrophil recruitment.

Polymorphonuclear neutrophils (PMN) are an initial defense against pathogens. Their function depends on degradative enzyme release (degranulation), phagocytosis and the production of various oxidant molecules [45]. However, an exaggerated response by PMNs can lead to an increased inflammatory response and host tissue injury [45]. Initial work showed that degranulation of PMNs is variably affected by macrolides [37]. Dirithromycin was found to induce release of the enzymes lysozyme, lactoferrin and β-galactosidase to a greater extent than either erythromycin or erythromycylamine. Schultz et al. [46] also demonstrated that erythromycin A causes a decrease in the production of CXC chemokines, such as IL-8 and epithelial cell-derived neutrophil attractant 78, while increasing the degranulation of PMN azurophilic and specific granules in whole blood. Extending this work Abdelghaffar et al. [45] determined that the presence of the L-cladinose moiety at position 3 of erythromycin A derivatives was required to inhibit PMN oxidant production and for the induction of PMN degranulation while removal of the L-cladinose moiety did not affect PMN degranulation. L-Cladinose-containing erythromycin A derivatives such as roxithromycin have been reported to inhibit the phospholipase D (PLD)-phosphatidate phosphohydrolase (PPH) signal transduction pathway in PMN [45]. This pathway operates in PMN upon activation by particulate or soluble stimuli. Roxithromycin was found to stimulate the PLD activity portion of this pathway leading to an accumulation of the PLD product phosphatidic acid (PA). In contrast, the product of the PPH reaction, diacylglycerol, never increased relative to controls. PA appears to be a key messenger in PMN degranulation [47] and the increase in PA that is seen with roxithromycin treatment correlates well with increased PMN degranulation [45].

Macrolides target a wide variety of molecules and responses that control the inflammatory response. Several of these have been briefly discussed in this review. It will be of great interest to follow research in this area and to discover the details of how all of these intricate processes will be influenced by macrolide compounds.

5. Regulation of cytokine transcription

The decrease in cytokine mRNA transcription, which has been repeatedly demonstrated, as briefly discussed in the previous section, has led to examination of the mechanisms of this transcriptional control.

Aoki and Kao [48] were the first to demonstrate that erythromycin A downregulates IL-8 cytokine gene expression in Jurkat T cells by inhibiting transcriptional activation of nuclear factor-κB (NF-κB) through interference with non-calcineurin-dependent signaling pathways. In this respect the inhibitory effect of antibacterial macrolides on transcription of cytokine genes resembles the action of the immunosuppressant rapamycin [34] rather than that of the calcineurin-inhibiting immunosuppressant FK506 [28]. Expanding on the work of Aoki and Kao [48], Ichiyama et al. [49] used Western blots to demonstrate that clarithromycin suppresses production of proinflammatory cytokines via inhibition of NF-κB activation and that this inhibition occurs without being linked to preservation of the 1kB regulatory protein. Clarithromycin was found to modulate TNF-α-induced NF-κB activation in T cells, monocytes/macrophages and pulmonary epithelial cells although the precise mechanism of this inhibition remains to be elucidated [49].

In contrast, Abe et al. [50] examined the role of activator protein-1 (AP-1) in the repression of the IL-8 gene by clarithromycin in human bronchial epithelial cells. Using Northern blot analysis clarithromycin was found to inhibit TNF-α-induced IL-8 gene expression. Transfection studies with Bet-1A cells demonstrated significant promoter activity in a 174-bp segment of DNA (−130 to +44 bp relative to the transcription start site). This segment was found to contain both AP-1- and NF-κB-like regulatory sites and exhibited significant promoter repression by clarithromycin. Removal of the AP-1 site did not show a significant repression of TNF-α-induced promoter activity by clarithromycin. Similar results were obtained when the AP-1
site was mutated. These results indicate that macrolesides such as clarithromycin repress IL-8 gene transcription mainly via the AP-1 binding site. The repression of IL-8 gene expression by clarithromycin requires a long incubation period. This would seem to suggest that clarithromycin does not act directly at the AP-1 regulatory site but instead induces regulatory factors to inhibit AP-1 binding to the IL-8 gene promoter.

6. Conclusions

The role of various macroleide antibiotics in the treatment of pulmonary inflammation appears to be well established. Recent research involving the use of erythromycin and its derivative macroleide antibiotics has shown great promise in treating inflammation associated with a variety of airway diseases. A wide range of anatomical, physiological, immunological, molecular biological and biochemical processes are known to be affected by these macroleide treatments. The expression and function of cell surface adhesion factors, prostanoids, NO, oxidants such as hydrogen peroxide, many cytokines and other regulatory proteins are beneficially affected. Mechanisms for transcriptional regulation and regulation of signal transduction networks that are responsible for modulation of pulmonary inflammation are being examined. Further exploration of these areas will greatly aid the understanding of the mode of action of these macrolides in decreasing inflammation in many pulmonary diseases.

Current research has given much valuable information about the macroleide anti-inflammatory mode of action but in many cases the precise, exact, complex molecular and biochemical mechanisms have yet to be deciphered in this fascinating and extremely important area of health care research.

References


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