Long-term macrolides in diffuse interstitial lung diseases

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ABSTRACT In the present review we provide currently available evidence for the use of macrolides in the treatment of diffuse interstitial lung diseases (ILDs). Up to now, research on macrolides has mainly focused on three areas. First, macrolides have shown some promising results in cellular models and case reports as antifibrotic agents, by promoting autophagy and clearance of intracellular protein aggregates and acting as regulators of surfactant homeostasis. Secondly, macrolides have an immunomodulatory effect, which has been applied in some organising pneumonia cases. In particular, macrolides have been tested in association with systemic corticosteroids as steroid-sparing agents and alone as either first-line agents in mild cases or second-line agents where steroids were poorly tolerated or had failed. Thirdly, a recent area of research concerns the possible role of macrolides as modulators of lung microbiota and the host–microbiota interaction. This function has been particularly studied in idiopathic pulmonary fibrosis patients, in whom changes in microbiota have been proved to be associated with disease progression. However, the lack of high-quality studies makes the application of macrolide therapy in ILDs a field in which research should be conducted on a large scale.

Introduction

In recent years, the use of low-dose, long-term macrolides has been described in case reports and small case series of patients with diffuse interstitial lung diseases (ILDs) and pulmonary fibrosis. We conducted a literature search using the PubMed/MEDLINE and EMBASE databases. We used the terms "macrolide" OR "erythromycin" OR "clarithromycin" OR "azithromycin" OR "roxithromycin" OR "troleandomycin" OR "telithromycin" in combination with the terms "interstitial lung disease", "idiopathic pulmonary fibrosis", "organising pneumonia" and "extrinsic allergic alveolitis". We also conducted a search using the terms "microbioma" OR "microbiota" in combination with "interstitial lung disease". Our review included items published between 1990 and July 2017 in the English language.

From the available literature, there is increasing evidence of a possible regenerative effect of macrolides on respiratory epithelium that has been damaged [1]. Pulmonary structure remodelling, together with inappropriate epithelial regeneration, is a key factor in the genesis of pulmonary fibrosis [2]. Excessive stress of the endoplasmic reticulum is among the mechanisms involved in the pathogenesis of the disease [2].

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This intracellular organ is responsible for the synthesis of functional proteins and the recognition of altered proteins, which are then removed to be degraded *via* the autophagocytosis process. Autophagocytosis has a fundamental role in type II pneumocytes, which, among their many functions, produce the proteins that make up surfactant. These proteins have a complex structure and a high likelihood of structural changes leading to the formation of amyloid fibril aggregates. When these aggregates exceed the cell clearance capacity, the endoplasmic reticulum undergoes excessive stress, which, if not resolved, leads to apoptosis [3, 4].

Defects in the autophagocytosis mechanism have recently been found in many pathological conditions associated with tissue damage and inflammation [5]. Focusing on lung diseases, an example is provided by Hermansky–Pudlak syndrome, a rare autosomal-recessive transmission disorder characterised by multi-organ alterations, including the progressive development of ILD [6]. Pulmonary involvement in this syndrome appears to be caused by excessive stress of the endothelial reticulum and alteration of lysosomal traffic, leading to chronic damage and apoptosis of type II pneumocytes. In murine models, pulmonary fibrosis and type II pneumocytes containing giant lamellar bodies appear together [7]; these cellular alterations seem to be a direct expression of stress in the endothelial reticulum and lysosomes. In this scenario, macrolides appear to play a key role at various levels, primarily by promoting autophagy and clearance of intracellular protein aggregates [1]. Hence, there is a reduction in lysosomal and endothelial reticulum stress that can ultimately prevent cellular apoptosis. Rapamycin, a type of macrolide not used for antibiotic purposes, induces autophagy by inactivating mTOR (mammalian target of rapamycin), a protein kinase that has been shown to have an inhibitory effect on autophagy [8]. Other macrolides, including azithromycin, have also shown this effect, supporting the hypothesis that they protect against the toxic effect produced by excessive amounts of intracellular protein aggregates [9].

Macrolides may also have an action on lipid metabolism and surfactant homeostasis by interacting with regulating molecules of lipid homeostasis [1]. This role is of particular importance when considering that lipids account for almost 90% of the surfactant. In a case report published by Thouvenin *et al.* [10] regarding a 6-year-old child with diffuse ILD due to mutation of the *ABCA3* gene (ATP-binding cassette transporter of the A subfamily, member 3), the use of long-term azithromycin, three times a week, led to a rapid and significant radiological and functional improvement. In this condition, type II pneumocytes appear hyperplastic and filled with abnormal lamellar bodies consisting of phospholipids. *ABCA3* plays an important role in the transport of phospholipids, which is a key step in surfactant production. In this situation, macrolides could play a double role: reducing cellular stress by promoting autophagy of abnormal lamellar bodies, as well as controlling lipid homeostasis.

Macrolide intracellular action in lung epithelium is not the only one studied to investigate their possible role in preventing the progression of certain ILDs. Possible effects on lung microbiota will be discussed in the next sections.

Microbiota

The "microbiota" is the community of microbes (bacteria, fungi, viruses and non-fungal eukaryotes) associated with a determined environment [11]; the term "microbiome" refers to the totality of the microbes and their genetic material harboured in a given microbiota and their interactions with it [12]. Most of the microbial and microbiome studies conducted so far have focused on bacteria.

For many years, the lower airways were considered sterile by definition, and the frequent microbiological samples traditionally taken were considered contamination by bacteria originating from the upper respiratory tract/oral cavity and also as a consequence of chronic microspiration [13]. In addition, the bacterial burden available in traditional isolation was often considered insufficient. This problem was related to the method of bacterial research, which was a culture-dependent method with the need to isolate a single bacterial agent in culture [14].

Currently, in the study of microbiota, culture-independent techniques are used, whose aim is to isolate the nucleic acids of bacterial DNA. This has allowed identification of the potential pathogens present in samples that tested negative in classical cultures [15, 16]. With these new methods it has been possible to demonstrate that the lower airways of healthy individuals have low levels of oropharyngeal bacteria (*Prevotella* and *Veillonella* species), rendering the dogma of sterility of the lower airways invalid [17, 18]. There is still a controversy about the real vitality of these bacteria, as fragments of bacterial DNA, not actual bacteria, are identified using these culture-independent techniques. In any case, it is now clear that the airway microbiota participates in the immunological homeostasis of the lung epithelium mucosa.

The microbiota plays a key role in shaping the immune response of the airway mucosa during childhood, particularly with regard to the development of atopy and bronchial asthma [19, 20]. In other chronic adult lung diseases, the microbiota also plays an important role. In asthma it is one of the factors that control

the disease, while in chronic obstructive pulmonary disease (COPD), progression of the disease is characterised by an increase in bacterial load and by a loss of heterogeneity, with the prevalence of a single species [21, 22].

For ILDs, studies on the microbiota are few compared to the other diseases already mentioned. However, it is logical to hypothesise that microbial alteration may be associated with a worsening of the disease, linked to immune dysregulation, excessive inflammation and infection. ILDs other than idiopathic pulmonary fibrosis (IPF) are often treated with immunosuppressive therapy. On the one hand, this makes patients more susceptible to the development of bacterial infections. On the other hand, the reduced immune reserve can alter the characteristics of the microbiota [23].

The genesis of IPF is multifactorial, one factor being the environment [2]. A potential role for infections, both as a cofactor of initial development and as a cofactor of fibrosis progression, has been widely postulated [24]. The questions just expressed have stimulated studies capable of quantifying and characterising the airway microbiota of IPF patients, using culture-independent methods.

IPF and host–microbiota interactions, and potential effects of macrolides

IPF is a progressive pulmonary disease with poor prognosis in the short to medium term. The genesis of the disease, as mentioned earlier, is multifactorial, with environmental factors playing a role in determining damage to the epithelial integrity of the lung and repair mechanisms, which are also altered on a genetic and epigenetic basis, causing aberrant repairing with consequent development of fibrosis [2]. Respiratory infections are among the possible environmental factors that cause the development of both fibrosis and acute exacerbations (AEs) of the disease [24]. The new available methods, which detect the presence of bacterial DNA of species that cannot be cultured, have paved the way for the study of complex microbial communities.

In this regard, a case–control study published by Molyneaux *et al.* [25] analysed the bronchoalveolar lavage (BAL) of 65 patients with IPF and compared it with that of 17 patients with COPD and 27 subjects with normal respiratory function, both smokers and nonsmokers. By using metagenomic techniques, the authors observed that in the BAL of IPF patients the number of copies of bacterial 16S rRNA was more than double that of non-IPF patients, but there was no difference between subjects with normal respiratory function and those with COPD. In addition, by observing patients with IPF prospectively and dividing them into two groups, depending on whether they showed disease stability or progression (progression defined as worsening of forced vital capacity >10% in 6 months or death), the authors noticed a higher bacterial burden in patients with progressive disease. Regarding the bacterial taxonomy in patients with IPF as well as in controls, the most represented species were *Streptococcus* species followed by *Prevotella* and *Veillonella* species. However, IPF patients showed a lower taxonomic diversity among bacterial species than controls, with an increased burden of possible pathogenic species such as *Neisseria*, *Haemophilus* and *Fusobacterium*. Therefore, from these first observations it appears that patients with IPF exhibit a greater bacterial load and less taxonomic diversity than controls; moreover, a higher bacterial load is associated with progression of the disease. The mucin 5B (MUC5B) promoter gene polymorphism confers an increased risk of developing IPF, but, paradoxically, if possessed by a patient with IPF, it confers an advantage on survival [26, 27]. In the previously mentioned study by Molyneaux *et al.* [25], the bacterial load in BAL was independently related to a particular polymorphism of this gene. As MUC5B is directly implicated in alveolar macrophage homeostasis and in surfactant constitution, a direct relationship has been postulated between the microbial load and immune regulation.

Using the data from the COMET-IPF study (Correlating Outcomes with Biochemical Markers to Estimate Time-progression in Idiopathic Pulmonary Fibrosis), Han *et al.* [28] retrospectively evaluated the role of the microbiota in disease progression. Although the most frequently isolated bacterial species (*Prevotella*, *Veillonella* and *Escherichia*) coincided in part with those identified by Molyneaux *et al.* [25], the authors also observed a new element: a strong association between the presence of specific species of *Streptococcus* and *Staphylococcus* and disease progression [28].

Other authors have recently tried to take a step forward in the understanding of the pathogenesis of IPF by assessing the interaction between the host and lung microbiota [29, 30]. Molyneaux *et al.* [25] performed a longitudinal evaluation of host gene expression in peripheral blood cells from 60 IPF patients and 20 controls, from their prior contribution, and explored the potential association with BAL microbiota collected at baseline [29]. In this preliminary study, the authors demonstrated that in IPF patients, when compared to controls, there were changes in the peripheral blood expression profile associated with the presence of an altered or more abundant microbiome. Huang *et al.* [30], once again using the data from the COMET-IPF study, showed that host defence, as assessed by immune pathway gene expression, could be modulated by variations in the lower airway microbiome. In particular, up- and downregulation of

immune-response-relevant pathways was associated with changes in the abundance of specific microbial operational taxonomic units, which had an impact on disease progression. Furthermore, the authors demonstrated an even more direct contribution of the host–microbiome interaction to the pathogenesis of the disease by influencing immune-mediated fibroblast responsiveness and the composition of circulating leukocytes [30].

Even when evaluating rapid progressions of the disease, such as AEs, recent studies have demonstrated a possible role of the microbiota [31]. In a comparison of 20 patients with AE to 15 matched controls with stable IPF, Molyneaux *et al.* [31] found an increased BAL bacterial burden and a shift in the composition of the microbiota, favouring bacteria usually confined to the gastrointestinal tract, such as *Campylobacter* species.

Given these assumptions about the role of bacterial burden and different species in host response and IPF progression, the next step will be to investigate the role played by antibiotic molecules administered for long periods on the natural history of the disease [32–36]. A few studies have explored the role of doxycycline and co-trimoxazole in IPF. The former has been studied for its inhibitor effect on matrix metalloproteinases, which play a role in the pathogenesis of IPF [34–36], and the latter has been demonstrated in a randomised controlled trial (RCT) (the TIPAC trial) to improve quality of life and, in the per-protocol analysis, to reduce all-cause mortality in association with a reduction in the frequency of respiratory tract infections, but without an effect on pulmonary function [32]. However, the generalisability of the data is limited, because not all patients had a diagnosis of IPF, nearly one-third of those receiving co-trimoxazole withdrew due to side-effects (mostly rash and nausea) and a consistent proportion was on immunosuppressive therapy. Because the available evidence led to conflicting results, an ongoing RCT (CleanUp-IPF trial; NCT02759120) is investigating the role of double-strength co-trimoxazole or doxycycline administered daily for 12–42 months plus standard of care, *versus* standard of care alone, in reducing non-elective, respiratory hospitalisation or all-cause mortality in patients with IPF.

Despite the promising data on the rationale to use macrolides in IPF, in particular because, due to their combined antibiotic and immunomodulator effects, they may act simultaneously on both microbial modulation and host-immune regulation, there are still very few data available. In a recent retrospective monocentric study, Kuse *et al.* [33] noted that the addition of macrolides to conventional IPF therapy seems to reduce the incidence of AEs. However, these preliminary data need to be considered with great caution, not only because of their retrospective nature, but also because they refer to the Japanese population alone and were collected over a period of time (2003–2008) in which the "conventional therapy" for IPF has changed profoundly. For example, 52% of the patients included in this study (27 out of 52) were subjected to corticosteroid therapy or other immunosuppressive agents, both of which are no longer recommended in IPF treatment [37].

All of this undoubtedly leads to the need to further investigate the role of macrolides in IPF patients. A clinical trial (NCT02173145) on the use of azithromycin at a dose of 500 mg·day^{-1} three times per week for 12 weeks in IPF is currently under way. The primary outcome is a reduction in coughing and, as secondary outcomes, modification of pulmonary function tests and the composition of oropharyngeal flora are being considered.

We can therefore conclude that the currently available evidence seems to identify a key role for the microbiota and its modifications regarding the mode and progression of IPF, even outside AEs. Macrolides could participate in the "modulation" of the microbiota itself, but their role is still largely to be investigated.

Macrolides in organising pneumonia

Organising pneumonia (OP) is caused by an insult that produces lung damage to which the alveolar epithelium reacts, giving rise to granulation tissue [38]. Excessive inflammatory tissue fills the alveoli and spreads to the alveolar ducts and terminal bronchioles, resembling typical endolumenal gemstones of granular tissue [38]. These alterations are associated with interstitial inflammatory infiltrates, which is why OP is classified among the ILDs [38]. The disease can be either idiopathic (cryptogenic OP (COP)) or associated with infections, drug toxicity, vasculitis, connective tissue diseases, haematological tumours, radiotherapy, *etc.* [39].

Although in OP, as well as in IPF, the predominant pathogenetic mechanism is excessive production of repair tissue, the two diseases show fundamental differences [40]. First, in IPF, the fibro-proliferative process results in a subversion of alveolar structure with irreversible scarring, while in OP the pathological process is usually reversible with restoration of normal pulmonary structure [40]. Secondly, corticosteroid therapy, which has proved to be ineffective in IPF, is the first-choice treatment in OP [39].

As reported by many studies, pro-inflammatory mediators are hyperactivated in both COP and OP and this seems to be the main reason for the efficacy of anti-inflammatory therapies in this disease [41–44].

However, for steroid treatment to be effective, it must be administered at a medium to high dose (0.5–1 $mg \cdot kg^{-1} \cdot day^{-1}$ in the initial stages) and for prolonged periods due to the risk of early recurrence after suspension. This obviously implies an increase in the risk of steroid-related side-effects [39]. Such observations led, in the 1990s, to an attempt to use steroid-sparing agents, including macrolides, in OP treatment.

Some studies have used macrolides as an alternative to corticosteroids [45, 46]. One of the first of these studies, by Ichikawa *et al.* [45] in 1993, used erythromycin at 600 $mg \cdot day^{-1}$ for 3–4 months in six patients with a diagnosis of bronchiolitis obliterans OP confirmed on histological examination. The clinical and radiological response was poor in the first 2–4 weeks of treatment, but it became complete in all patients after 3 months. In addition, although no significant side-effects were reported during treatment, the relapse time was rather short (3.6±1.8 months). By comparing BAL cellularity before and after treatment, normalisation of both neutrophils and lymphocytes was observed. These latter data regarding the cellularity of BAL are particularly significant; in fact, a mixed alveolitis with increase in lymphocytes (from 20% to 40% of total cellularity), neutrophils (up to 10%) and eosinophils (up to 5%) is usually observed in OP [47]. In addition, during OP there is an increase in cytotoxic T-lymphocytes with a CD4/CD8 reduction [48].

In this scenario, the immunomodulatory effect of macrolides could act at various levels. For example, macrolides could reduce the degree of inflammation linked to polymorphonucleated leukocytes and their products. In a study in 1996 on biopsy-proven COP patients who showed neutrophilia on BAL, Hotta [49] observed a reduction in interleukin (IL)-8 and neutrophilic chemotactic activity in BAL after 600 $mg \cdot day^{-1}$ erythromycin for 2–3 months, suggesting a beneficial effect of low-dose macrolides on neutrophil-mediated inflammation. Radzikowska *et al.* [44] reported a decrease in serum concentration of pro-inflammatory cytokines, including IL-6, IL-8 and transforming growth factor-β1, and in the BAL concentration of IL-6 in patients with biopsy-proven COP who responded to clarithromycin 500 mg twice daily for 3 months. Moreover, Aoki and Kao [50] showed that the anti-inflammatory effect of erythromycin is also expressed at the level of cytotoxic T-lymphocytes. A recent study published by Cai *et al.* [41] reported that alveolar macrophages of both COP and secondary OP patients (diagnosed through lung biopsy) also show aberrant pro-inflammatory cytokines production compared to non-OP control subjects. In this study, exposure of alveolar macrophages to macrolides inhibited the release of pro-inflammatory cytokines and, for this purpose, clarithromycin was proven to be more effective than azithromycin.

In most studies, macrolides were not used alone as a first choice, but in combination with corticosteroids to reduce the dosage or to replace them in the case of ineffectiveness or when intolerable steroid-related side-effects occurred [46, 51–53]. An example is the case reported by Chang *et al.* [52], where clarithromycin therapy (500 $mg \cdot day^{-1}$) produced beneficial results in a patient with biopsy-proven

TABLE 1 Summary of the main areas of application of low-dose, long-term macrolides in diffuse interstitial lung diseases (ILDs)

Macrolide uses and functions	Evidence type	First author [ref.]
In ILDs, including idiopathic pulmonary fibrosis		
Macrolides may play an antifibrotic role	Still to be demonstrated in human models	
Promoters of autophagocytosis and clearance of intracellular protein aggregates	Only cellular models	Guillot [1] Thomson [8] Stamatiou [9]
Regulators of surfactant homeostasis	One case report	Thouvenin [10]
Macrolides may act as host–microbiota interaction modulators and/or host immune response modulators	Still to be demonstrated in human models	
Reduction of acute exacerbations rate	Retrospective monocentric study	Kuse [33]
In organising pneumonia		
Macrolides may exert an anti-inflammatory/immunomodulatory action	Case reports and case series	
As adjuvants to steroid therapy and steroid-sparing agents	One case report	Ding [51]
As first-line agents as an alternative to steroids	Case reports and case series	Ichikawa [45] Stover [46] Hotta [49] Radzikowska [44]
As second-line agents when steroids are not tolerated or failed	Case reports and case series	Chang [52] Stover [46] Pathak [53]

bronchiolar COP in whom steroids (1 mg·kg^{-1}·day^{-1}) and azathioprine had proven to be ineffective and related to side-effects. In the cases available so far, side-effects associated with chronic use of macrolides have been rare and not severe (one case of skin rash associated with clarithromycin) [46].

It is noticeable how the evidence available so far regarding the use of low-dose, long-term macrolides in COP and secondary OP is, in reality, limited to case series. Moreover, in many published cases, macrolides were not used alone but in association with steroids or other immunosuppressive agents, making it impossible to evaluate their actual efficacy [51]. In conclusion, given the lack of large-scale prospective studies, at present there is no definitive data on the efficacy of macrolide therapy either as a first-line or as an adjuvant agent.

Conclusions and future perspectives

The experience gained so far has allowed the identification of many intracellular pathways on which macrolides act, but much remains to be investigated, especially with regard to the role of the lung microbiota and host–microbiota interaction in the genesis and evolution of ILDs and the action that macrolides can play on the latter. The main areas of application known to date are summarised in table 1. In addition, although macrolide use has been assessed in OP and IPF patients, other ILDs might potentially benefit from it. In this regard, the use of macrolides has been tested in animal models or anecdotally in case reports of patients with extrinsic allergic alveolitis and desquamative interstitial pneumonia [54, 55]. It is important to remember that the limited evidence available derives almost exclusively from studies in animal models, case series and retrospective studies. Before being actually able to support the utility of low-dose, long-term macrolides in the treatment of ILD patients, RCTs are certainly needed.

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