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Review article

# Macrolides. Antiinflammatory and immunomodulatory effects. Use in respiratory illnesses

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## RESUMEN

Se revisan las implicaciones básicas y clínicas inmunomoduladoras de los macrólidos en diferentes enfermedades respiratorias. Además de sus propiedades antimicrobianas, algunos poseen capacidades antinflamatorias e inmunomoduladoras (miembros con 14 átomos de carbono en su anillo de macrolactona). Estos macrólidos se han prescrito con éxito a pacientes con panbronquiolitis difusa (enfermedad progresiva inflamatoria) y están indicados en el tratamiento de: asma, bronquitis crónica, sinusitis crónica, poliposis nasal, fibrosis quística y bronquiectasias. Se comentan algunos resultados de la investigación básica y clínica que respaldan que puedan prescribirse. Su eficacia a largo plazo y sus efectos secundarios sólo podrán determinarlos los resultados de estudios clínicos, al igual que su efecto en pacientes con enfermedades crónicas inflamatorias de la vía respiratoria.

**Palabras clave**: macrólidos, agentes antinflamatorios, inmunomoduladores, panbronquiolitis difusa, moco, asma, bronquitis crónica, sinusitis crónica, pólipos nasales, otitis media con derrame, fibrosis quística, bronquiectasias.


## ABSTRACT

This article reviews the basics and the clinical implications of the immunomodulatory effects of macrolides in different respiratory diseases. In addition to their antiinfective properties, some macrolides possess immunomodulatory effects (14 member ring). These macrolides have been used successfully to treat diffuse panbronchiolitis, a progressive inflamatory disease and may be very useful in the treatment of asthma, chronic bronchitis, chronic sinusitis, nasal polyps, otitis media with effusion, cystic fibrosis and bronchiectasis. We present the basic and clinical work supporting its chronic use. We will need future double blind controlled trials to determine the long term efficacy of macrolides for the treatment of chronic inflammatory airway diseases, as well as development of resistance, how to improve side effects ratio and the duration of effects after cessation of treatment.

**Key words**: macrolides, antiinflammatory drugs, immunomodulator agents, diffuse panbronchiolitis, mucus, asthma, chronic bronchitis, chronic sinusitis, nasal polyps, otitis media with effusion, cystic fibrosis, bronchiectasis.

In 1952, erythromycin A was commercialized for the alternative treatment of beta-lactamic agents of gram-positive cocci infections. In 1990, medications such as clarithromycin and roxithromycin were introduced; in 1994 azithromycin was used and in 2004, telithromycin (ketolides). In comparison with erythromycin, these medications have a greater activity spectrum, less frequent doses and collateral gastrointestinal effects are lesser. Antimicrobial macrolides agents have a macrolactone ring which could have from 8 to 62 members. Macrolides antibiotics generally have rings of 14, 15 or 16 carbon atoms[1] (figure 1).

Interest in the immunomodulatory effects of macrolides commenced in 1960, when it was observed that troleandomycin (carbon 14 antibiotic) permitted reduction and spacing of the dose of corticoids in the treatment of patients with severe asthma.[2] In Japan, for 20 years the immunomodulatory effects of macrolides have been used for the treatment of diffuse panbronchiolitis ,[3] which became the treatment of choice.[4,5] Based on these findings, erythromycin and clarithromycin are frequently used in Japan for the treatment of sinusitis and chronic obstructive pulmonary disease.[6]

In 1980, erythromycin was used as an immunomodulatory agent for the treatment of panbronchiolitis. In the last three years, clarithromycin and azithromycin have been used as immunomodulators in the treatment of cystic fibrosis; in Europe and the United States bron-

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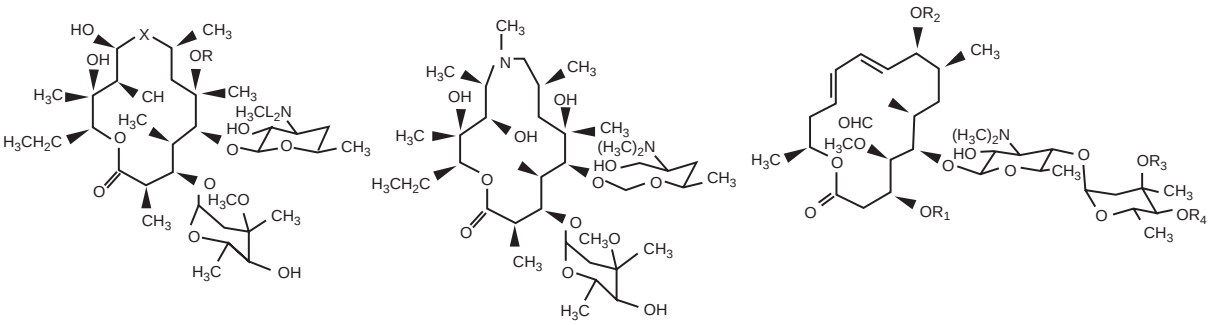

Erythromycin
Roxithromycin
Clarithromycin
Clarithromycin

Azithromycin

Josamycin
Spiramycin
Miocamycin

**Figure 1.** Chemical structure of macrolides.

chiectasis have been used. Currently, several research projects are evaluating these agents in other inflammatory illnesses of the respiratory tract, such as: asthma, sinusitis and chronic bronchitis and their effects in the hypersecretion of mucous. Bacterial resistance does not seem to have any effect on immunomodulatory activity. Macrolides reduce: neutrophil migration, processes of oxidation in phagocytes, cytokine expression through inhibition of transcription factors, eosinophilic inflammation and adhesion molecule expression.These also inhibit expression of some of the mucine genes and the formation of biofilms from *Pseudomonas aeruginosa*, a common cause of infection in chronic inflammatory illnesses of the respiratory tract.

There is not sufficient information to demonstrate the existence of significant clinical differences in the efficacy of macrolides antibiotics as immunomodulatory agents, with the exception of 16 carbon atom macrolides, such as: josamycin, spiramycin or miocamycin, which lack mucoregulatory and immunomodulatory effects.[7] There is less information pertaining to the use of azithromycin in the treatment of panbronchiolitis, given that it was introduced in Japan in 2000; however, studies of cystic fibrosis suggest that it has similar efficacy to clarithromycin. In the last 8 and 10 years these properties were investigated outside of East Asia and turned out to be of high interest for the treatment of pulmonary disease in cystic fibrosis, similar to that observed in the treatment of panbronchiolitis.[8]

Fourteen carbon atom macrolides include: erythromycin, roxithromycin, clarithromycin, dirithromycin and troleandomycin. Erythromycin is a macrolide derived from *Streptomyces erythreus* which contains a lactone ring with two sugars: desosamine and cladinose. As an antibiotic, erythromycin inhibits RNA protein synthesis which depends, upon reversible bonding, on a microorganism susceptible to the 50S ribosomal subunit. Erythromycin is the medication of choice for *Mycoplasma pneumoniae* infections and *Chlamydia trachomatis* pneumonia. The recommended oral dose for adults is from 250 mg to 1 gram every six hours. Clarithromycin and roxithromycin are commonly used in clinical practice, given that they are stable in gastric juice and bind in lower proportion to motilin receptors; resulting in lesser gastrointestinal effects than with erythromycin. The dose of clarithromycin is frequently from 250 to 500 mg orally, two times a day and for 7 to 15 days. Azithromycin is a 15 carbon atom macrolide and its pharmacokinetics provides prolonged tissue concentrations and allows for shorter treatment (3 to 5 days) in the majority of infections.[9] A new derived semisynthetic antimicrobial group of 14 carbon atom macrolides, known as ketolides, was

recently brought to market. With lateral chain modifications, ketolides have a group 3-keto instead of the sugar molecule L-cladinose. These disorders allow greater ability to overcome macrolides resistance (figure 2). Telithromycin is the first of the antimicrobial ketolides agents and was approved in 2004 by the FDA. More than 30% is metabolized in the liver, 50% has cytochrome P450 3A4 and 50% of the independent cytochrome P 450. Ketolides have excellent activity against erythromycin-resistant gram positive pathogens.[10] Azithromycin, clarithromycin and ketolides show greater activity against *Haemophilus influenzae* than erythromycin. All these agents have high volumes of distribution with excellent penetration in respiratory tissue[11] (figure 3).

## NON BACTERICIDAL ACTIONS OF MACROLIDES

They have prolonged use, with a dose lower than the minimal inhibitory concentration for 90% of the bacterial inoculate and provoke: antiinflammatory effects and effects in the mucous of the respiratory tract (reduction of volume and increased clearing), reduction of hyperreactivity of the respiratory tract, protection of the epithelium of the respiratory tract from bioactive phospholipids and reduction of the development of biofilms by microorganisms. These effects are apparently limited to 14 or 15 carbon atom ring macrolides (table 1).

## ANTIINFLAMMATORY EFFECTS (FIGURE 4)

### Release of cytokines and chemokines

One of the early steps in the inflammatory process is transduction of signals in cells affected by cytokines and proinflammatory chemokines. Low-dose, prolonged macrolides treatment suppresses these molecules. In patients with panbronchiolitis, erythromycin diminishes concentrations of interleukin 1B (IL-1b) and IL-8 in the broncheoalveolar lavage liquid.[12-14] Erythromycin and clarithromycin *in vitro* suppress expression of RNAm for IL-8 and protein concentrations in bronchial epithelial cells of normal individuals and in transformed bronchial epithelial cells.[15] It is suggested that the mechanism is found in nuclear transcription and suppresses the binding sites for AP-1 and the nuclear k-B factor.[16-19] Given that IL-8 is a greater chemoattractant for neutrophils, reduction of IL-8 reduces the number of neutrophils in the respiratory tract.

Erythromycin inhibits the release of eosinophil chemotactic cytokines (eotaxin, stimulation factor of colonies of granulocytes and monocytes [GM-CSF]), RANTES (normal and secreted expressed T cells, liberated in activation) and chemotactic activity of

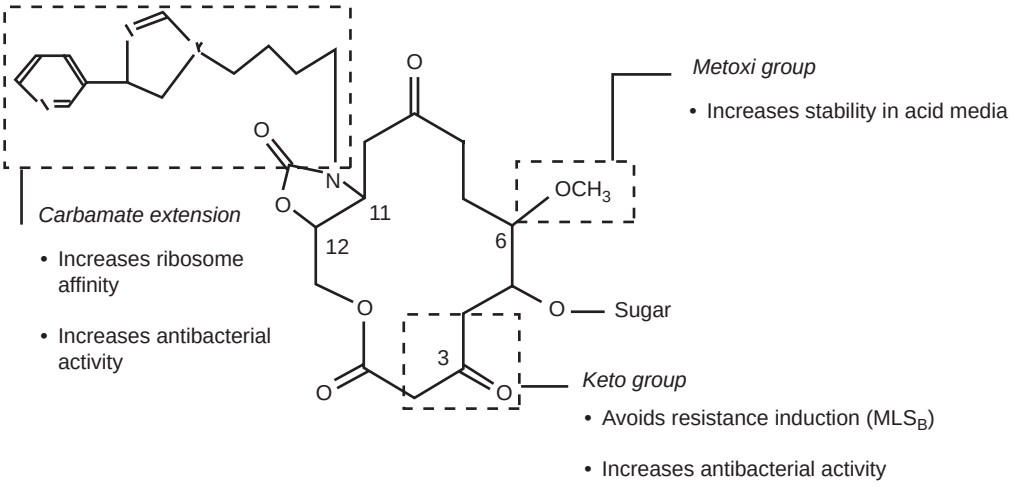


**Figure 2.** Ketolides-telithromycin group.

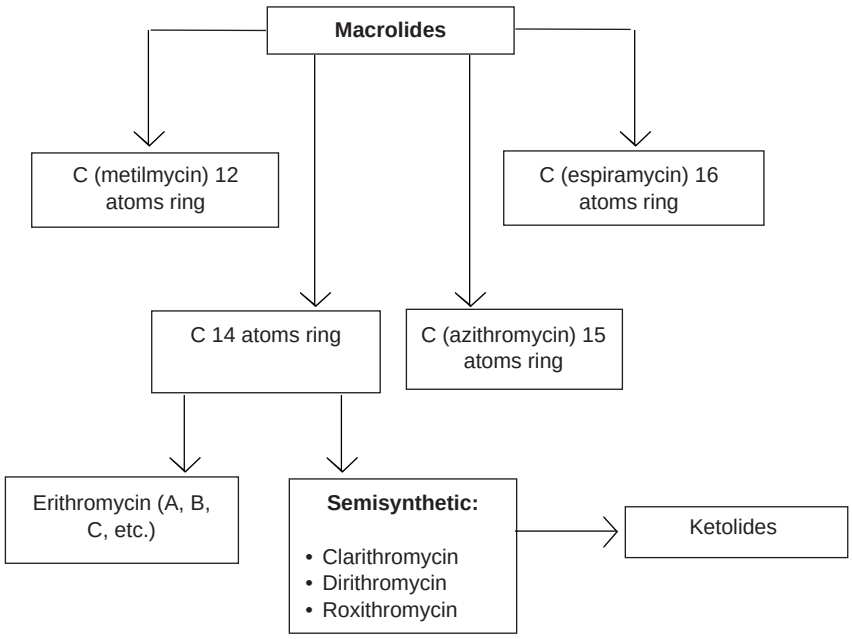


**Figure 3.** Macrolides are characterized by a macrolactone ring with 12 to 16 carbon atoms. Of the group of 15, carbon 9 changes permitted the creation of azalides. Some important structural modifications in 14 carbon atom ring macrolides led to the creation of a new group called ketolides.
Bryskier A. Macrolides. Chemistry. Pharmacology and clinical uses. Oxford: Blackwell Scientific Publications, 1993.

eosinophils in *in vitro* human pulmonary fibroblasts. The suppressor effect of eotaxin was the most pronounced.[20] Erythromycin also suppressed eotaxin RNAm.

**Table 1.** Antiinflammatory effects of macrolides

| | |
|---|---|
| Nitric oxide | Increases the release of nitric oxide influenced by cNOS<br>Suppression of nitric oxide influenced by iNOS |
| Respiratory tract mucous | Reduction of volume and hypersecretion<br>Greater clearance and ciliary movement |
| Hyperreactivity of the respiratory tract | Reduction of HRB to methacholine<br>Reduction of endothelin-1 protein |
| Bioactive phospholipids and epithelial damage | Protection of ciliary epithelium of the respiratory tract and of reactive oxidants |
| Bacterial adherence | Reduction of bacterial adherence to epithelium (*Pseudomonas aeruginosa*) |
| Biofilm development | Reduction of biofilm formation |
| *Pseudomonas aeruginosa* virulence factors | Reduction of *P. aeruginosa* virulence |

Roxithromycin lacks effects in production of IL-2 or of interferon γ by T cells, but significantly inhibits IL-4 and IL-5 as dose dependent.[21]

Macrolides suppress the following cytokines:

- IL-1B and TNFα in monocytes.22
- IL-1B, IL-6, TNFα and GM-CSF in mast cells.23
- IL- 8, ENA-78 (attractant of neutrophils derived from epithelial cells) and MIP-1 (macrophage inhibitory protein) in macrophages and leukocytes.24

### Adhesion molecule expression

The inflammatory process provokes the entrance of neutrophils and other inflammatory cells from the blood circulation to the respiratory tract. This process is initiated by activation of adhesion molecules in circulating polymorphonuclear leukocytes and vascular endothelial cells. Erythromycin reduces SICAM-1 (soluble intercellular adhesion molecule 1), which secretes cultivated bronchial cells[25] and reduces expression of B2 integrins (CD 11b/CD 18) by neutrophils.[26] Roxithromycin inhibits expression

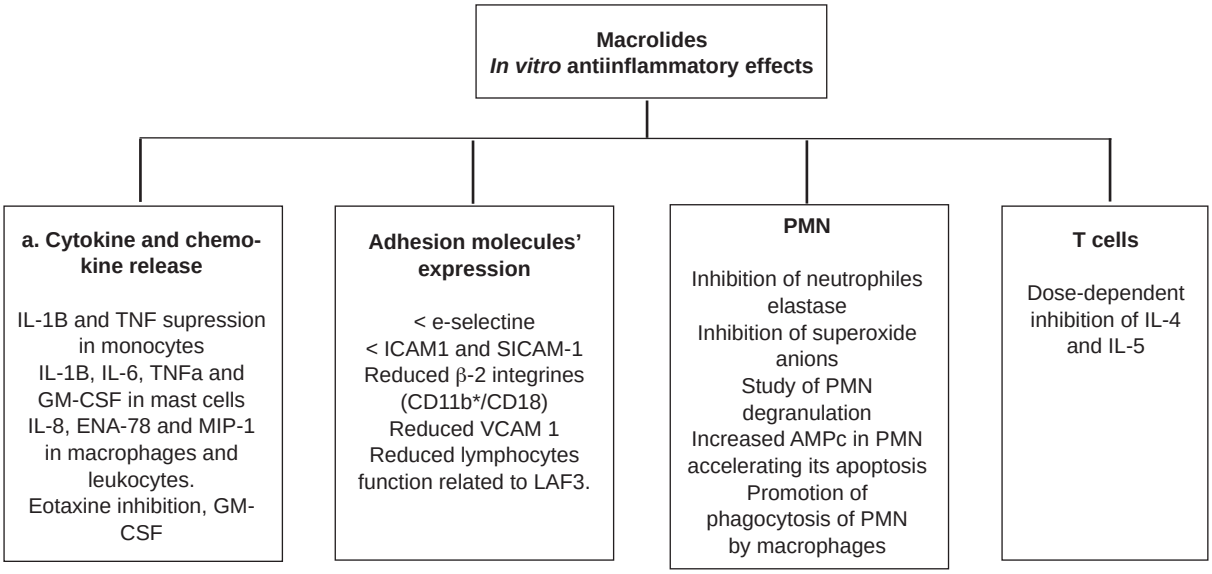


**Figure 4.**

of E-selectine and ICAM-1 in the endothelial cells.[27] Clarithromycin reduces the function of stimulated lymphocytes related to the antigen 3 (LAF3) and the vascular adhesion molecule 1 (VCAM-1) expressed in the synovial fibroblasts.[28] The reduced expression of these adhesion molecules contributes to attenuation of recruitment of inflammatory cells in the target organ.

### Neutrophils

Chronic inflammation is characterized by polymorphonuclear recruitment and accumulation with the release of lysosomal enzymes and generation of oxygen radicals, which increases with polymorphonuclear necrosis. These enzymes and oxygen radicals also damage the respiratory epithelium and recruit more polymorphonuclears. There are two proven mechanisms that facilitate recruitment of polymorphonuclear: increase of adhesion molecule expression, such as E-selectin and ICAM-1, and chemoattractants such as IL-8.[29] Both mechanisms are inhibited by macrolides.

The *in vitro* effects of erythromycin and flurithromycin (14 carbon atom 8-fluoro macrolid) on the activity of neutrophil elastase have been studied. Erythromycin inhibits neutrophil elastase and flurithromycin inactivates them irreversibly.[30] Erythromycin inhibits production of superoxide anions in neutrophils stimulated with N-formyl-methionyl-leucyl-phenilalanine.[2,31] Azithromycin rapidly stimulates polymorphonuclear degranulation and the oxidative chain related to phagocytosis, which contribute to the antibacterial activity of azithromycin. Twenty-eight days after the last dose of azithromycin there is persistent inhibition of the oxidative chain of neutrophils, of blood myeloperoxidase and serum IL-6.[32]

Erythromycin increases AMPc in polymorphostimulant leukocytes and accelerates its apoptosis.[33] Macrolides also promote phagocytosis of polymorphostimulant leukocytes through alveolar macrophages dependent on the phosphatidylserine receptor.[34] Therefore, macrolides reduce the ability of neutrophils to respond to chemotactic signals, reduce the release of potentially toxic mediators and may promote apoptosis of polymorphostimulant leukocytes in the respiratory tract.

## EFFECTS ON NITRIC OXIDE

Nitric oxide is generated by the synthetase enzyme, which exists in two isoforms: constitutive (cNOS)

and inducible (iNOS). cNOS appears in the vascular, nervous, and epithelial cells of the respiratory tract.[35] In the respiratory tract, nitric oxide intervenes in the regulation of muscular tone, vascular tone, neural signaling-communication and host defense.[36] However, inflammatory cells can produce nitric oxide, which favors inflammation and epithelial damage.[37] Erythromycin increases the release of nitric oxide influenced by cNOS of the endothelial cells through the action of the A kinase protein.[38] Production of nitrite oxide influenced by iNOS is suppressed *in vitro* by macrolides (erythromycin, clarithromycin, roxithromycin and azithromycin).[39-41]

## EFFECT ON RESPIRATORY TRACT MUCOUS

The production of mucous in the respiratory tract, and its clearance through the mucociliary transportation system, constitute a very important defense mechanism in chronic pulmonary bronchitis and inflammation provokes mucous hypersecretion and reduction of its clearance. *In vitro* erythromycin had demonstrated capacity for reducing glycoconjugate secretions of the respiratory tract at baseline and those stimulated by histamine or metacholine.[42]

In a randomized, double blind, placebo controlled study, clarithromycin reduced the volume of expectorant sputum in individuals with chronic bronchitis, bronchiectasis or panbronchiolitis (median of 51 ± 6 to 24 ± 3 g/d); increased the percentage of solid composition from 2.44 ± 0.29 to 3.01 ± 0.20%, and increased the elastic capacity from 66 ± 7 to 87 ± 8 dynas/cm$^2$, without changing its dynamic viscosity. Also it was demonstrated that clarithromycin and erythromycin inhibit secretion of mucous and expression of MUC5AC mRNA in the NCI-H292 cells and in human nasal epithelial cells.[6,43]

Macrolides reduce hypersecretion of mucous provoked by bacterial infection and allergic inflammation. Short-term administration of clarithromycin reduced chronic hypersecretion of mucous, and possibly inhibited chloride secretion and the consequent secretion of water through the respiratory tract.[44]

In another study, clarithromycin was administered (5 to 8mg/kg/day) for more than two months in 55 children with otitis media. Curation percentage was significantly greater (66%) for those receiving clarithromycin in comparison with the control group (16%). Of the children receiving clarithromycin, those with otitis media related to chronic sinusitis had a higher cure percentage.[45]

## EFFECTS ON HYPERREACTIVITY OF THE RESPIRATORY TRACT

Erythromycin and roxithromycin increased the necessary concentration of metacholine for provoking a decrease of 20% in FEV1 in patients with bronchial asthma.[46,47] Erythromycin, roxithromycin and clarithromycin demonstrated reduction, depending on the concentration used, in the contractile response of bronchial tissue isolated *in vitro*.[48] Erythromycin and clarithromycin suppress secretion of endothelin-1 protein and mRNA of bronchial epithelial cells and attenuate the bronchoconstrictive response.[49]

## BIOACTIVE PHOSPHOLIPIDS AND EPITHELIAL DAMAGE

Bioactive phospholipids, the platelet activation factor, the activation/lysis factor packaging and lysophosphatidylcholine cause damage to the human ciliated epithelium with or without phagocyte-derived reactive oxidants, roxithromycin, clarithromycin and azithromycin, which protect the ciliated epithelium of these phagocyte-derived reactive oxidants.[50]

Ketolides attenuate direct and indirect forms of epithelial damage induced by phospholipids.[51]

## MODULATION OF BACTERIAL VIRULENCE

### Bacterial adherence

Adherence of bacteria to mucosa is a decisive phenomenon for the initiation of the pathogeny

of the majority of bacterial infections. With exposure to erythromycin, *Pseudomona aeruginosa* pili significantly reduced its adherence to the tracheal epithelium, which is damaged by acid (animal-rat model), compared with bacteria exposed to other antibiotics.[52] *P. aeruginosa* has greater adherence to oral epithelial cells of individuals with cystic fibrosis, but is reduced at normal concentrations with administration at low doses.[53] Erythromycin reduces adherence of *P. aeruginosa* to type 4 collagen of the basal membrane (*in vitro*).[54]

#### Effects on biofilm formation

Gram-negative bacteria such as *Pseudomonas* form biofilms through various sequential stages. After the bacteria adhere to the surface (such as the epithelium) lose their pili and flagela; are transformed from flagellar mobility to mobility through spasmodic contractions and produce exopolysaccharides or alginates through dehydrogenase of guanosine-diphosphate-d-mannose. Bacteria in the interior of a biofilm have a reduced metabolism and are relatively protected from the effect of antibiotics. Therefore, antibiotics at concentracions that were frequently efficacious, are not efficacious against biofilm.

The majority of gram-negative organisms are frequently considered macrolide resistant. However, in the animal model of the biofilm related to chronic respiratory infection by *P. aeruginosa*, clarithromycin was capable of reducing the number of viable bacteria.[55] Macrolides reduce the formation of biofilm and inhibit the production cycle of dehydrogenase of guanosine-diphosphate-d-mannose.[56] Also, they prevent mobility through fimbria-dependent spasmodic contractions.[57]

Flagellin is the largest component of bacterial flagellar filament, which allows mobility for a grand diversity of bacterial species. Expression of flagellin and mobility of *P. aeruginosa* are reduced by use of macrolides.[58,59]

#### Effect of virulent factors of *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* infection begins with adherence to host cells through diverse adhesins, including lectins. Afterwards, *Pseudomonas aeruginosa* secretes toxins and enzymes and causes damage. Autoinduction signals (N-acyl-L-homoserine lactones) can control production of adhesins and compute these cytotoxins. Erythromycin, at subinhibitory concentrations, is capable of suppressing *Pseudomonas aeruginosa* lectins, hemagglutinate activity in the cellular surface, extracellular proteases, hemolytic activity and autoinduction activity.[60] Macrolides suppress synthesis of a greater stress protein "Gro-EL" in *P. aeruginosa* at concentrations less than the inhibitory minimum for 90% of the bacterial innoculate. This could be related with inhibition of *Pseudomonas aeruginosa* virulence and bacterial death.[61]

### CHRONIC RESPIRATORY TRACT ILLNESSES; MACROLIDES TREATMENT

#### Diffuse panbronchiolitis

Panbronchiolitis is a chronic inflammatory pulmonary disease of unknown origin. It is reported principally in east Asia and is characterized by progressive loss of pulmonary function. This illness begins during adolescence and the majority of patients have chronic sinusitis and have not been exposed to tobacco smoke. Typical symptoms are: chronic cough, production of sputum and exercise induced dyspnea. Pulmonary function tests generally show a mixed pattern (obstructive and restrictive), principally with advanced illness. Bacterial species that infect the respiratory tract include: *Staphylococcus* and *H. influenzae*; *P. aeruginosa* is the predominant organism with disease advancement. *Pseudomonas* infection is related with greater and faster disease progression.[62]

Long-term administration of erythromycin, clarithromycin or azithromycin significantly reduces the symptoms of patients with panbronchiolitis, even those infected with mucoid *P. aeruginosa* resistant to macrolides antibiotics.[63] Erythromycin, at low doses for a prolonged period of time (400 to 600 mg/day), achieved in the 1980s, greater survival of patients with panbronchiolitis (from 26 to 94%).[3,64] Kadota et al.[65] undertook a prospective open study

with oral clarithromycin (200 mg/day) over 4 years. Patients had significant improvement of FEV 1, from 1.74 ± 0.12 to 2.31 ± .22 L ($p < 0.01$); sputum cultures in the majority of patients were negative at six months after commencement of treatment. All patients continued improving or remained stable with continuous treatment. There were no reports of collateral effects of clarithromycin.

Ten percent of patients with panbronchiolitis do not respond to macrolides.[66] The committee on pulmonary illnesses of the Japanese Ministry of Health recommended the following guidelines for the use of macrolides:

*1. Medication of choice*

Initial: erythromycin 400 or 600 mg/day, orally.

Second choice: clarithromycin 200 or 400 mg/day or roxithromycin 150 or 300 mg/day, orally, if efficacy is poor or the results are adverse with erythromycin.

*2. Evaluation of reaction and duration of treatment*

Clinical reaction is evaluated in the first 2 to 3 months after initiating treatment; however, it should be continued for 6 months and evaluated at that point. If it turns out to be efficacious, treatment should be continued for two years until clinical, pulmonary and radiological stability is achieved, along with improved quality of life.[67] In advanced illness with extensive bronchiectasis, or respiratory failure, if the reaction is positive, treatment should be continued for more than two years.

*3. Notes*

Fourteen or 15 carbon atom macrolides treatment should be commenced early after establishment of diagnosis, given that the clinical reaction will be better in early stages of the disease. Treatment should be reinitiated if the symptoms reappear after suspension of treatment.

**Cystic fibrosis**

It is a recessive autonomic process, provoked by the mutation of a unique gene, in the long arm of chromosome 7, and which codifies for the CFTR (cystic fibrosis transmembrane regulator) protein.[68-70] It is the most common mortal genetic illness in Caucasians and affects approximately 30,000 individuals in the United States and 70,000 in the world.[71] At an early age, respiratory tract infections are very frequent and almost always are caused by *Staphylococcus aureus* and *H. influenzae*.

Afterwards, patients are infected with *Pseudomonas aeruginosa* and this converts into the predominant organism, which provokes progressive deterioration of pulmonary function. In the adult age, less than 80% of patients with cystic fibrosis are infected with this organism.[72] These infections, and the intense neutrophilic inflammatory response, provoke destruction of the respiratory tract, bronchiectasis and obstructive respiratory tract illness. Cystic fibrosis has high similarity with panbronchiolitis.

In a recent study of adults with cystic fibrosis, azithromycin was administered for 3 months (250 mg/day); it was a prospective, randomized, double blind, placebo controlled study.[73] FEV1 and FVC remained stable in the azithromycin group, while in the control group it fell by -3.62% (1.78%) FEV1 ($p = 0.047$) and to 5.73% (1.66%) FVC ($p = 0.001$). Patients treated with azithromycin required a lower quantity of IV antibiotics (0.-37 *vs* 1.13; $p = 0.016$). C-reactive protein fell in the group treated from 10 to 5.4 mg/mL and remained without changes in the placebo group ($p < 0.001$). Improvement was observed in quality of life ($p = 0.035$).

One group in the Royal Brompton hospital underwent a 15-month prospective, randomized, double blind, placebo controlled and crossed study using azithromycin in 41 children with cystic fibrosis between 8 and 18 years of age. For six months, the patients received 250 mg/day of azithromycin when their weight was less than 40 kg and 500 mg/day when greater than 40 kg. Changes observed in tests of pulmonary function were positive in patients with active treatment and the median difference between them was of 5.4% (95% of CI, 0.8 to 10.5).[8]

A multicenter, prospective, randomized, double blind, placebo controlled study was undertaken in

23 cystic fibrosis centers in the United States from December 15, 2000 to May 2, 2002.[74] The group treated with azithromycin had an increase of FEV1 of 0.097 L compared to 0.003 in the placebo group (median difference 0.094 L 95% CI).

With respect to the control, 17% more participants from the treatment group had nausea (p = 0.01), 15% more had diarrhea (p = 0.009) and 13% more had wheezing (p = 0.007). Patients in the group treated with azithromycin had fewer exacerbations (p = 0.03) and, at the end of the study, had higher weight (average of 0.7 kg) in comparison with patients from the control group (p = 0.02). Currently, azithromycin or clarithromycin are recommended treatments in patients with cystic fibrosis and pulmonary disease.

## CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease is characterized by chronic limitation of expiratory air flow in patients with chronic bronchitis or emphysema. Obstruction is generally progressive, but can be partially reversible and can be accompanied by hyperreactivity of the respiratory tract. Smoking is the greatest risk factor for this (more than 80% of patients).[75] Isolation of *H. influenzae*, *Moraxella*, *Catarrhalis* or *Streptococcus pneumoniae* is related with increased risk of pulmonary exacerbation.[76] Macrolides are recommended to reduce exacerbation of obstructive pulmonary disease.[77] In a prospective, randomized, double-blind, placebo controlled study of 109 individuals with obstructive pulmonary disease, erythromycin was administered and there was reduction of viral exacerbations. In 12 months, the number of common colds was significantly lower in the group treated with erythromycin compared to the control group (1.24 ± 0.07 *vs* 4.54 ± 0.02, p = 0.0002). Thirty individuals from the control group (56%) and six from the erythromycin group (11%) had one or more exacerbations. A greater number of patients in the control group required hospitalization for acute exacerbations in comparison with the group treated with erythromycin (p = 0.0007).[78] Patients with chronic infections of the lower respiratory tract, upon administration of roxithromycin (150 mg two times per day, for three months), had better pulmonary volume, reduction of IL 8, neutrophil elastase and B4 leukotriene in epithelial liquid.[79]

## ASTHMA

Macrolides have been used since 1970 as corticoid spacing agents for patients with severe asthma dependent on corticoids. Clinically, clarithromycin reduces hyperreactivity of the respiratory tract provoked by metacholine and diminishes the number of eosinophils in the sputum of patients with asthma.[80]

In asthma, there is an increase of cytokines derived from TH2 lymphocytes of the mononuclear cells of the peripheral blood. Macrolides help revert imbalances between TH1 and TH2 lymphocytes, and reduce TH2 cytokine expression.[21,81] In asthma, lymphocyte apoptosis is altered, while spontaneous lymphocyte apoptosis increased during remission. Roxithromycin induces apoptosis of *Dermatophagoides farinae* activated lymphocytes in patients with asthma sensitive to this mite, and induces the Fas/Fas ligand system and reduces expression of Bcl -2.[82]

Some asthma exacerbations are related to infections from organisms such as *Chlamydia pneumoniae* or *M. pneumoniae.* Also, persistent infections from these organisms contribute to the severity of asthma.[83] High concentrations of IgG and IgA *vs C. pneumoniae* are related to the severity of asthma.[84] In another study, individuals with severe asthma, with positive C reactive protein testing in upper or lower respiratory tract secretions *vs M. pneumoniae* or *C. pneumoniae*, demonstrated significant improvement in FEV1 (2.50 ± 0.16 to 2.69 ± 0.19 L) after treatment with 500 mg of clarithromycin, two times daily, for six weeks.[85]

## CHRONIC SINUSITIS

Chronic sinusitis is an illness of diverse origins. Frequently, it is not known where the infectious,

inflammatory factors and other poorly signaled causes intervene, and is accompanied by diverse symptoms, such as: sinusal pain, headache, nasal congestion, posterior or anterior mucopurulent rhinorrhea, altered quality of life and fatigue for at least 12 weeks. Diagnosis is confirmed radiographically through computed tomography. Chronic sinusitis is characterized by obstruction of the ostium of the paranasal sinuses that promotes stagnation and accumulation of secretions and highly viscous mucous and mucociliary dysfunction. Stagnated liquid is easily colonized and infected, which promotes inflammation. The pathogenesis becomes a vicious circle between inflammation and bacterial reinfection (in case it provokes infection). These bacterial infections initiate a series of phenomena that promote the chronic state of the illness. The infection produces chemotaxis and infiltration toward the paranasal sinuses by neutrophils, mononuclear cells and T cells. Excessive quantities of proteases are released by the neutrophils that damage the mucosa and facilitate hypersecretion of mucous. These immune cells and the epithelium produce IL 8, TGF b and TNF to proinflammatory cytokines.[38] Inflammation of the sinusal mucosa and poor sinus ventilation are provoked by bacterial products such as: lipopolysaccharides, elastases produced by inflammatory cells and other inflammatory mediators such as vasoactive amines, immune complexes and arachidonic acid metabolites and leukotrienes. Sinusal inflammation produces inflammation of the contiguous nasal mucosa and obstructs the sinus ostium. The poor ventilation of the paranasal sinuses reduces ciliary activity, increases bacterial growth and proliferation of small capillaries[86] and provokes the vascular escape of blood components. Platelets and fibroblasts are activated, as well as hyperplasia of the glandular mucosa. Glandular hyperplasia induced by growth factors, such as the epidermic growth factor and the keratinocyte growth factor,[87] produce hypersecretion of mucous,[88] and fibroblast activation provokes widening of the sinus mucosa. Hypersecretion of abnormal viscoelastic mucous provokes mucociliary dysfunction,[89] which accelerates accumulation of sinus secretions and produces chronic inflammation.

The majority of studies in patients with chronic sinusitis, which show efficacy of long-term macrolides treatment, are small and open, and in general show clear advantages in patients with this recalcitrant illness. For example, in an open and prospective study, administration of clarithromycin (400 mg/day) for 8 to 12 weeks reduced symptoms and rhinoscopic findings in 45 adults with chronic sinusitis, of whom 20 were resistant to surgical intervention. The ranges of improvement were related with the duration of treatment and varied by 5% at two weeks and by 71% in week 12. After this time, half of the patients had reduced quantity and viscosity of nasal secretions, retronasal secretion and nasal obstruction. No significant collateral effects were reported.[90] Some similar findings were observed in patients with infection from *H. influenzae* resistant to macrolides treated with roxithromycin.[91]

In Japan, Korea and China, macrolides are administered very frequently, and for a prolonged period of time, to patients with persistent symptoms after surgical sinus intervention, who have chronic sinusitis, nasal polyposis and sinusitis. In the United States, in a two week study of individuals with nonbacterial rhinitis treated with clarithromycin, the volume of nasal mucous (500.1 *vs* 28.3 mg; p = 0.01) was reduced, with an increase in mucociliary mobility in 30% (0.76 *vs* 0.99; p = 0.005); mucous viscoelasticity did not change.[92] In another study, clarithromycin was administered to 18 individuals with chronic sinusitis for four weeks.[93] The result was that cohesion and solid composition of the nasal mucous increased, with decreases in all samples of the relation between viscosity and elasticity (G"/G'). These studies suggest that clarithromycin modulates the properties of nasal mucous in chronic sinusitis and promotes mucociliary transportation.

In a retrospective study, there was an analysis of long-term effects of treatment with erythromycin at low dose, after endoscopic sinus surgery, in

individuals with panchronic sinusitis and nasal polyposis.[94] Upon examination of the ethmoid sinuses, the ostium of the frontal sinus and the maxillary sinus had less inflammation and edema, and symptoms, after the intervention, reduced significantly in the erythromycin group compared to the untreated group. In one prospective study, 12 to 17 individuals with persistent chronic sinusitis, after sinusal surgical intervention, and treatment with erythromycin or clarithromycin for one year, there was improved mucociliary function ($p < 0.05$), less congestion and nasal obstruction, thinner secretions and reduction of rhinorrhea ($p < 0.01$ with visual scale clinical evaluations).[95]

## NASAL POLYPS

Many patients with chronic sinusitis have nasal polyps that increase nasal obstruction. Although the origin of nasal polyps is not certain, there is a relationship between inflammation and nasal polyposis. Some inflammatory factors affect nasal polyps, such as: IL-1b, IL-5, IL-6, IL-8 and RANTES.[96,97]

There are two studies that show the efficacy of macrolides treatment for reducing the size of nasal polyps in patients with chronic sinusitis. In the first, 20 patients with chronic rhinosinusitis received clarithromycin, 400 mg/day, for 8 to 12 weeks and significantly reduced the size of the polyps.[98] This reduction was related to the reduction in the concentration of IL-8 in the nasal lavage. Patients who did not have reduction of IL-8 did not react favorably to treatment.

In an open study in Japan, roxithromycin (150 mg/day for eight weeks) was administered to 20 patients with nasal polyposis and chronic sinusitis. In half of the patients, nasal polyps were reduced following treatment.[5]

Macrolides reduced the size of polyps in spite of allergy or intense eosinophilic infiltration. These results suggest that IL-8, an important influential factor in the accumulation of neutrophils in the respiratory tract, is related to reduction of the size of nasal polyps with suppression of production of cytokines in the inflammatory cells of the sinusal epithelium.[5]

One *in vitro* study showed that macrolides reduce proliferation of fibroblasts of nasal polyps.[98]

## OTITIS MEDIA WITH EFFUSION

Otitis media with effusion is frequently related to chronic sinusitis and is characterized by chronic inflammation with calciform cell hypersecretion.[38] Improvement and curation of otitis was observed with prolonged treatment with erythromycin. In a model of rats with otitis media, previous treatment with erythromycin inhibited adhesion of neutrophils to WK-5 endothelial cells and accumulation of neutrophils.[99] In horses with otitis media, with histamine-induced effusion, erythromycin also reduced neutrophil accumulation from $0.11 \pm 0.04$ to $0.04 \pm 0.03$ ($p < 0.001$).[100]

In another study in Japan, 16 individuals with sinobronchial syndrome and otitis media with effusion received erythromycin (600 mg/day) for more than four months. Thirteen of the 16 patients were cured and in the majority the symptoms were significantly reduced.[101]

## CONCLUSION

Long-term macrolides treatment at low doses is the treatment of choice for patients with panbronchiolitis. Currently, it is recommended for the treatment of pulmonary disease in patients with cystic fibrosis.[102,103] The data from this study suggest that they have efficacy in patients with chronic sinusitis, or without nasal polyposis, and in steroid-dependent patients with severe asthma. There are reports of a wide variety of effects of the inflammatory cascade; however, the underlying mechanisms are still to be determined. Research also should focus on and evaluate which group of patients would obtain advantages through the long-term use of macrolides. There is intense work underway to develop better immunomodulatory macrolides without antimicrobial properties that

could favor its efficacy, without questioning the bacterial resistance that undoubtedly could arise with prolonged use.

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