Thorax 1999;**54**(Suppl 2):S58–S62

The anti-inflammatory effects of macrolides

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Introductory article

Erythromycin and clarithromycin attenuate cytokine-induced endothelin-1 expression in human bronchial epithelial cells

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Erythromycin and its fourteen-member macrolide analogues have attracted attention for their efficacy in bronchial asthma. However, their mechanisms of action remain unclear. We evaluated the effects of the macrolide antibiotics on endothelin-1 (ET-1) expression in normal and transformed human bronchial epithelial cells, one of the sources of this potent bronchoconstrictor important in the pathogenesis of asthma. Human bronchial epithelial cells were obtained from the resected bronchi, and the effect of several antimicrobial and antiasthmatic drugs on the production and messenger ribonucleic acid (mRNA) levels of ET-1 was evaluated. Bronchoepithelial cells were also isolated from the mucosa of asthmatic patients under fibreoptic bronchoscopy, and the modulating effects of the drug were studied. Erythromycin and clarithromycin uniquely suppressed mRNA levels as well as the release of ET-1 at therapeutic and non-cytotoxic concentrations (percentage inhibition of ET-1 protein release: 26.4$\pm$5.22% and 31.2$\pm$7.45%, respectively, at 10^{-6} M). Furthermore, erythromycin and clarithromycin inhibited ET-1 expression in bronchoepithelial cells from patients with chronic, stable asthma. A glucocorticosteroid, dexamethasone, also inhibited ET-1 expression. In contrast, theophylline, salbutamol and FK506 had no effect on ET-1 production. Our findings demonstrated that these fourteen-member macrolide antibiotics had an inhibitory effect on endothelin-1 expression in human bronchial epithelial cells. Moreover, this new mode of action may have some relevance to their clinical efficacy in bronchial asthma. (*Eur Respir J* 1998;**12**:57–63)

Macrolide antibiotics are widely used in the treatment of infection. They show broad spectrum antibacterial activity against Gram positive bacteria—for example, *Streptococcus pneumoniae*—and intracellular bacteria—for example, *Mycoplasma pneumoniae*, *Chlamydia* and *Legionella* species—and combine this with good tissue penetration. It has been known for many years that macrolide antibiotics have an effect on host cell function as well as an antimicrobial effect. Erythromycin and troleandomycin were shown to improve the clinical status of patients with steroid dependent asthma over 20 years ago[1] and, more recently, long term low dose erythromycin has been shown to reduce bronchial hyperreactivity.[2] However, the mechanisms by which these effects occur have remained obscure. In the Introductory Article by Takizawa *et al* erythromycin and clarithromycin have been shown to suppress endothelin-1 expression and release by human bronchoepithelial cells which may provide new insight into how this effect is achieved.[3]

Endothelin-1, a polypeptide, is the most potent vasoconstrictor known[4] and also has potent bronchoconstrictor effects.[5] It has been reported to stimulate mucus secretion[6] and to cause mucosal oedema.[7] In experimental work it has been shown to play a key role as a mediator of airway inflammation. A considerable increase in the concentration of endothelin-1 in bronchoalveolar lavage (BAL) fluid of the rat occurred during the early phase of experimental inflammation[8] and this was associated with a rise in the total cell, eosinophil, and neutrophil counts. Treatment with an endothelin-1 receptor antagonist inhibited the increase in BAL fluid eosinophils and reduced the inflammatory reaction in the lung tissue. Bronchial smooth muscle cells have been shown to possess specific binding sites for endothelin-1[9] and the bronchial epithelial cells of asthmatic patients express preproendothelin-1 mRNA and release large amounts of biologically active endothelin-1.[10] Corticosteroids have been shown to reduce the production of endothelin-1 and, for the first time, this effect has been shown by macrolides.

In this study a clinical role for endothelin-1 as a bronchoconstrictor was suggested by a negative correlation with peak expiratory flow rate; surprisingly, no relationship was found with forced expiratory volume in one second (FEV_1) but the number of subjects studied was small. A number of antimicrobial and anti-asthma drugs were also tested but inhibition of endothelin-1

Table 1 Macrolides

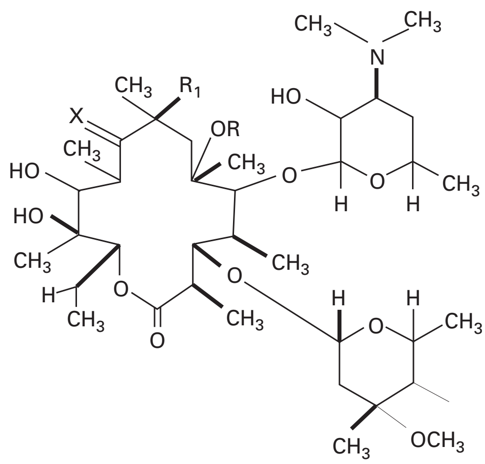
Ring structure	*Name*
14-membered	Erythromycin, clarithromycin, roxithromycin, dirithromycin
15-membered	Azithromycin
16-membered	Josamycin, spiramycin

release was found to occur only with the 14-membered ring macrolides erythromycin and clarithromycin. Interestingly, no such effect was seen with josamycin, a 16-membered ring macrolide, or with FK506 (table 1).

An effect on endothelin-1 is one of the most recently described of a number of anti-inflammatory properties shown by the 14-membered ring macrolides (fig 1). The following sections describe some of these mechanisms and lead on to a description of other studies of the use of macrolides in asthma and other conditions characterised by inflammation.

Macrolides and the inflammatory response

The inflammatory process is multifactorial and macrolides have been shown to act in a number of different ways, their effects being demonstrated in various animal models (table 2). Oedema produced by the injection of carrageenin into the paw of a rat can be suppressed by pretreatment of the animal with a macrolide.[11] Roxithromycin has been shown to reduce oedema formation with an effect almost equal to that of the non-steroidal anti-inflammatory drug nimesulide, while azithromycin and clarithromycin showed lesser anti-inflammatory effects. Roxithromycin has been further evaluated in a variety of rat models including poly–L–arginine induced paw oedema, croton oil inflamed ear assay, and intraperitoneal polyester sponge granuloma.[12] It produced a marked anti-oedema effect similar to that of indomethacin in poly–L–arginine assay, significant inhibition of croton oil induced inflammation in the ear, but failed to reduce the development of granuloma induced by implanted polyester sponges. Pretreatment with erythromycin decreased neutrophil counts in BAL fluid from the lungs of mice in which inflammation had been



	X	R	R_1
Erythromycin A	O	H	H
Clarithromycin	O	CH_3	O
Roxithromycin	$NOCH_2O(CH_2)_2OCH_3$	H	H

Figure 1 Fourteen-membered macrolide ring structure.

Table 2 Anti-inflammatory mechanisms shown for macrolides

Mechanism	*References*
Reduced endothelin-1	3
Inhibition of neutrophil oxidant burst	15, 16, 17, 18, 19, 31
Inhibition of neutrophil chemotaxis	13, 14, 15, 20, 29
Reduced production of IL-6, IL-8 and IL-1β	20, 21, 22, 32, 33, 34
Reduced soluble intercellular adhesion molecule 1 (sICAM-1)	20
Reduced production of TNF-α	22
Suppression of granulocyte-macrophage colony stimulating factor	21
Reducted defensin production	32

induced by intratracheal instillation of lipopolysaccharide[13] or aerosolised *Proteus mirabilis* and *Staphylococcus aureus*.[14] Thus, an anti-inflammatory effect has been repeatedly demonstrated in animal models with roxithromycin, seemingly more potent than azithromycin and clarithromycin, with erythromycin having the least effect. It has been postulated that this could be due to better cell penetration by the newer macrolides than by erythromycin.

Macrolides and the neutrophil oxidant burst

Reactive oxidant products of neutrophils are known to damage tissue and the intracellular accumulation of macrolides may limit their production, although data on this are conflicting. Lambro *et al*[15] have shown that only roxithromycin strongly decreased the polymorphonuclear neutrophil (PMN) oxidative burst as assessed by luminol amplified chemiluminescence, superoxide anion generation, and myeloperoxidase mediated iodination of proteins. This effect was noted to vary significantly between individuals and may relate to the high concentration of roxithromycin achieved within the neutrophil. Anderson *et al*[16] found that both erythromycin and roxithromycin selectively inhibited superoxide generation by activated neutrophils. Hand *et al*[17] observed that roxithromycin which readily enters the phagocyte was an efficient inhibitor of the PMN superoxide generation stimulated by formyl–methionyl–leucyl–phenylalanine (FMLP) and concanavalin A. Clearly, it would be of interest to minimise the oxidative response of human PMNs whilst preserving their bactericidal and phagocytic functions.

Macrolides and neutrophil chemotaxis

Stimulation of neutrophil migration has been described in two studies.[16 18] In one, adult volunteers were given a single oral dose of 500 mg erythromycin stearate and a significant increase in PMNL migration in response to a leucocyte attractant was observed at 90 minutes. Conflicting data were produced in a study by Torre *et al*[19] who observed decreased PMN chemotaxis following the ingestion of erythromycin, josamycin, miokamycin, roxithromycin, and rokitamycin for four days by adult volunteers. Reduction in chemotaxis was observed by other workers,[13 14] raising concerns that the bactericidal effects of these antibiotics might be reduced. However, the relevance of these findings in the clinical setting is not yet known.

Macrolides and cytokine production

Cytokines are small proteins involved in the orchestration of the inflammatory process. They can be either pro-inflammatory (for example, tumour necrosis factor (TNF) alpha, interleukin (IL)-6, IL-8 and IL-12, and gamma interferon) or anti-inflammatory (for example, IL-10). Macrolides, particularly those derived from erythromycin A, have been shown to impair the production of pro-inflammatory cytokines. *Haemophilus influenzae* induces the release of IL-6, IL-8, and soluble intercellular adhesion molecule 1 (sICAM-1) from airway epithelial cells and this effect can be reduced by erythromycin.[20] Similarly, roxithromycin has been shown to suppress the production of IL-6 and IL-8 and granulocyte–macrophage colony stimulating factor, in addition to inhibiting neutrophil adhesion to epithelial cells.[21] Erythromycin also caused a dose dependent decrease in heat killed *Streptococcus pneumoniae* (HKSP) induced production of TNF-α and IL-6 in human whole blood in vitro.[22] The production of IL-1, IL-12, and gamma interferon was only affected at the highest concentration of erythromycin. The production of TNF-α and IL-6 in whole blood obtained from healthy subjects after a 30 minute infusion of 1 g of erythromycin was lower after ex vivo stimulation with HKSP than blood drawn before infusion. Effects on the cytokine pathways are complex but reduction in the pro-inflammatory cytokines in experimental models provides some insight into how these effects might be achieved in vivo.

Macrolides and asthma

Macrolides have been shown to affect bronchial hyperresponsiveness by a non-antibiotic mechanism. Rosenberg *et al*[23] described a patient with corticosteroid dependent asthma in whom the addition of daily troleandomycin allowed corticosteroids to be weaned without clinical deterioration (table 3). Erythromycin has been shown to reduce the severity of bronchial hyperresponsiveness in adult asthmatics who were not corticosteroid dependent[24]; 200 mg of erythromycin three times a day given over a 10 week period resulted in a significant increase in the PC_{20} in both atopic and non-atopic patients, supporting the observation of an improvement in asthma control by macrolides by workers in the early 1970s.[1] The interaction of erythromycin with theophylline, reducing its clearance and increasing plasma theophylline levels, could partly explain the beneficial effects of erythromycin. However, in this study 600 mg erythromycin daily produced no change in serum theophylline levels. A further study was performed on children with asthma using roxithromycin, an antibiotic with little effect on the pharmacokinetics of theophylline.[25] Again, the PC_{20} significantly increased with 150 mg roxithromycin daily after four and eight weeks of treatment. This study also reported no change in the liver enzymes SGOT and SGPT or morning cortisol levels and concluded that roxithromycin did not affect corticosteroid metabolism.

Reduced production of reactive oxygen species by polymorphomuclear leucocytes or an effect on neutrophil chemotaxis or cytokine production may be involved. Decreased production of neutrophil chemotactic lymphokines has also been postulated.[14] Experimentally, erythromycin has been shown to reduce the electrical field stimulation induced contraction of isolated human bronchial strips in a dose dependent fashion, suggesting that macrolides may inhibit the cholinergic neuroeffector mechanism possibly by reducing acetylcholine release at nerve terminals.[26] However, the effect on endothelin-1 described in the Introductory Article is likely to be another important mechanism by which bronchoconstriction is reduced.

Table 3 Clinical conditions where a benefit from macrolides has been shown

Condition	*References*
Asthma	1, 2, 23, 24, 25, 26
Diffuse panbronchiolitis	13, 27, 28, 29, 30, 31, 32, 33, 34, 37
Bronchiectasis	35, 36
Cystic fibrosis	28, 39
Atheroma	40, 41, 42, 43, 44, 45
Cancer	46, 47
Arthritis	48

Macrolides and diffuse panbronchiolitis (DPB)

A clinical role for macrolides, which is not due to a direct antimicrobial effect, is perhaps best shown in DPB. This disease is characterised by chronic bronchial sepsis and airflow obstruction with chronic inflammation of the respiratory bronchioles, stenoses, and obstruction. Initial infections are with *H influenzae*, *Strep pneumoniae*, and *Staph aureus*, and eventually patients become colonised with *Pseudomonas aeruginosa*. Death due to respiratory failure occurs after repeated cycles of infection. The disease occurs commonly in Japan with occasional cases reported in Italy and North America. The prognosis of DPB has been dramatically improved in recent years by the use of erythromycin and other macrolide antibiotics in this condition. In 1984 the five year survival rate was only 26% in cases with *P aeruginosa* and 55% for all other types of DPB. Since erythromycin has become widely used, the 10 year survival for all types of DPB has increased to 94%.

The clinical efficacy of erythromycin in this condition was first noticed in 1982 and has since been confirmed by clinical trials[27 28] but the mechanism of action is still unknown. Studies indicate a crucial role for the polymorphonuclear leucocyte in the pathogenesis with influx of these cells into the airways and the production of oxidants and proteolytic enzymes producing inflammatory change. Bronchoalveolar lavage (BAL) fluid from sufferers has demonstrated increased numbers of neutrophils, neutrophil derived elastolytic-like activity, IL-8, IL-1β, and leukotriene B4 (LTB4). After treatment with erythromycin the number of neutrophils and amount of neutrophil derived elastolytic-like activity in BAL fluid has been shown to decrease significantly with a coincident improvement in lung function.[29] This was also described by Kadota *et al*[13] who found increased numbers of neutrophils and neutrophil chemotactic activity in the BAL fluid of patients compared with normal subjects which was reduced by erythromycin treatment; this was also found in an animal model. Benefit is not confined to erythromycin and has been demonstrated with other 14-membered macrolides. An efficacy of 79% for erythromycin (400 mg or 600 mg), 86% for roxithromycin (150 mg or 300 mg), and 67% for clarithromycin (200 mg or 400 mg) taken daily for at least two months has been reported.[30] Azithromycin, a 15-membered macrolide, has also been shown to be effective.[31] Defensins, antimicrobial and cytotoxic peptides which occur in high concentrations in the BAL fluid of patients with DPB are reduced with macrolide treatment[32] and several studies have shown a reduction in levels of IL-8 and IL-1β.[32–34] Reduced adhesion molecule macrophage activating complex 1 (MAC-1) on peripheral blood neutrophils has also been shown to occur after macrolide therapy.[33] Sixteen-membered macrolides including josamycin are not beneficial in DPB.

Macrolides and bronchiectasis/cystic fibrosis

The dramatic effect of macrolides on the prognosis of

DPB has led to trials in other conditions characterised by chronic bronchial sepsis including bronchiectasis and cystic fibrosis. Both conditions are associated with copious sputum production, rhinosinusitis, progressive airway destruction, and chronic *P aerurginosa* infection of the airways. A double blind, placebo controlled trial of low dose erythromycin in bronchiectasis has recently been published.[35] Erythromycin was given to 11 patients with bronchiectasis in a dose of 500 mg twice daily for an eight week period and FEV_1, forced vital capacity (FVC), and sputum volume over 24 hours were significantly improved compared with placebo. However, no parallel improvement in sputum pathogens, leucocytes, IL-1α and IL-8, TNF-α or LTB4 was found. Erythromycin is unlikely to be bactericidal in view of the low dosage and poor penetration into the bronchial tree, and the authors postulate that inhibition of glycoconjugate release and chloride secretion by airway epithelium and macrophage mucus secretagogue production might result in decreased sputum water content and volume. Airway responsiveness in bronchiectasis has also been shown to improve with long term, low dose macrolide administration. Roxithromycin was given to 13 children with increased airway responsiveness and bronchiectasis and a group of 12 controls received placebo.[36] Methacholine challenge tests were performed at baseline and after 12 weeks. The PC_{20} increased significantly in the roxithromycin group while no change was seen in the placebo group. In addition, improvement in sputum features was noted after six weeks in the treatment group.

That macrolides might be efficacious in these conditions by a non-bactericidal effect on *P aeruginosa* has been postulated by Howe and Spencer.[37] Diffuse panbronchiolitis, bronchiectasis, and cystic fibrosis are all associated with chronic *P aeruginosa* infection and inhibition of this organism could be a mode of action. Two mechanisms by which macrolides might be effective are proposed: an effect on the immune system to modify the inflammatory response to infection or a direct effect on *P aeruginosa* to decrease its virulence. In addition to the effects on the immune system described above, macrolides inhibit endotoxin A, total protease, elastase, phospholipase C, DNase, lecithinase, gelatinase, lipase, pyocyanin and motility, all virulence factors associated with *P aeruginosa* infection. Strains of *P aeruginosa* found in these conditions produce a mucoid alginate by which they closely adhere to the airway surface. In this "biofilm" the bacteria are resistant to attack by antibacterial agents and interaction with neutrophils. The alginate works as an antigen and serum titres of anti-alginate antibody IgG and IgA are significantly higher in *Pseudomonas* positive patients with DPB than in *Pseudomonas* negative patients. In experimental models the alginate induced antigen–antibody reaction resulted in lymphocyte infiltration around small airways which gradually developed into granuloma-like infiltration containing macrophages. The state of antigen excess resulting from persistent colonisation of mucoid alginate producing *P aeruginosa* may generate an immune complex in the host. The levels of serum immune complexes in patients with DPB have been positively correlated with clinical symptoms. These immune complexes deposit on lung tissue and stimulate neutrophil chemotaxis.[38] Long term treatment with clarithromycin has resulted in most cases in a decrease in serum immune complexes and an improvement in clinical symptoms.[28] Erythromycin, clarithromycin, and azithromycin have been shown to be effective inhibitors of antigen–antibody reaction in vitro but not 16-membered macrolides or cephalosporins. Long term treatment with azithromycin has been shown to improve lung function in patients with cystic fibrosis.[39]

Combining macrolides with antipseudomonal antibiotics may be more effective than administering antipseudomonal antibiotics alone.[28] In vitro incubation of biofilm *P aeruginosa* with twice the minimum bactericidal concentration of ciprofloxacin resulted in 85% of bacteria remaining viable. However, when clarithromycin or azithromycin was added, the bactericidal effect of ciprofloxacin was far greater. This offers new prospects for clinical trials of these antibiotics in combination in patients with cystic fibrosis and bronchiectasis.

Atheroma, arthritis, and cancer

An effect of macrolides has been described in each of these conditions. Whether the mechanism relates to its anti-inflammatory effect, antimicrobial effect, or some other effect remains to be discovered. The development of atheroma has been linked to chronic chlamydial infection.[40–43] Two clinical trials have been undertaken of macrolide therapy as secondary prevention in patients with known coronary artery disease. In the first by Gupta *et al*[44] 220 male survivors of acute myocardial infarction were screened for IgG antibody to *Chlamydia pneumoniae* by microimmunoflorescence. Those with titres of >1/64 on two consecutive occasions were randomised to receive azithromycin 500 mg daily for three days and repeated at three months or placebo. Patients were followed for a mean of 18 months. A five fold reduction in adverse cardiovascular events was found ($p = 0.03$) in the azithromycin treated group compared with placebo. A second study by Gurfinkel *et al*[45] enrolled 202 patients with unstable angina or non-Q wave myocardial infarction. Patients were randomised to receive roxithromycin 150 mg twice daily for 30 days or placebo, independent of *C pneumoniae* serostatus. They were followed for six months and the primary end point was a composite of three outcomes—cardiac death, acute

LEARNING POINTS

- **Multiple in vitro and in vivo anti-inflammatory effects of macrolides have been demonstrated.**
- **Anti-inflammatory effects appear to be limited to macrolides with 14-membered and 15-membered ring structure.**
- **A clear clinical role for macrolides has so far only been shown in DPB.**
- **Future work should focus on the molecular mechanisms of the macrolide–cell interaction leading to the development of new, more specific, anti-inflammatory macrolides.**
- **Inhibition of endothelin-1 may be partly responsible for an anti-asthma effect.**

myocardial infarction, and severe recurrent ischaemia; 2% of the roxithromycin group and 9% of the placebo group reached the triple end point (p = 0.032). The double end point of myocardial infarction or cardiac death was reached by 4% in the placebo group and by none of the roxithromycin treated group (p = 0.058). An association between *C pneumoniae* and coronary artery disease has been shown but whether the relationship is causal or not is unknown. These two small studies of macrolides in secondary prevention will stimulate further studies in this field.

Helicobacter pylori, another macrolide susceptible organism, may also play a role in coronary artery disease but is definitely responsible for gastric and duodenal ulcers and plays an important part in the development of gastric cancer. Eradication therapy is an appropriate way of treating gastric and duodenal ulcers and clarithromycin in combination with another antibiotic and a proton pump inhibitor is usually employed. Whether there will be a reduction in the incidence of gastric cancer remains to be shown.

An anti-cancer effect of clarithromycin has been demonstrated in various aminal models[46 47] but efficacy in man has yet to be demonstrated.

A possible role for macrolides in arthritis has been considered but further research is needed.[48]

Conclusions

Multiple in vitro and in vivo anti-inflammatory effects of macrolides have been demonstrated. As the complex molecular interactions which determine the inflammatory cascade become better understood, it is likely that more will be found. A clear clinical role for macrolides has so far only been shown in DPB. Future work should focus on the molecular mechanisms of macrolide–cell interaction leading to the development of new, more specific, anti-inflammatory macrolides. The door is open for further clinical trials of macrolides in a number of clinical areas.

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