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New life for macrolides

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This article is an attempt to analyze and discuss the role and the purported mechanisms of azithromycin (AZM) in non-eosinophilic severe asthma, including antineutrophil activity, an effect on gastroesophageal reflux or antibacterial activity against an underlying chronic infection, such as *Chlamydia pneumoniae*. Macrolides have an expanding role in the therapy of chronic inflammatory diseases based on their additional anti-inflammatory and immunosuppressive properties. Many studies have been performed in lung transplantation field and maintenance treatment has been proved to be effective in cystic fibrosis, bronchiectasis, diffuse panbronchiolitis, and in bronchiolitis obliterans syndrome and in the prevention of exacerbations in patients with chronic obstructive pulmonary disease. Pathobiological studies of people with severe, refractory asthma focused on its heterogeneity encouraging more targeted and personalized approaches to asthma therapy. In neutrophilic asthma corticosteroids are not very effective, while the immunomodulatory action of macrolides is particularly relevant on neutrophils. Recently, The AZIthromycin in Severe ASThma (AZISAST) study, published on the April number of Thorax, provided evidences on the efficacy and safety of long-term add-on treatment with AZM in severe non-eosinophilic asthma. Despite concerns about an increased proportion of macrolide-resistant organism and about the effects of macrolides on cardiovascular events, there was no evidence of an increased risk of pneumonia or other adverse events. Because the AZISAST study was not able to demonstrate significant improvement in lung function and use of rescue medication, there is still a need for new data confirming the efficacy of AZM in severe non-eosinophilic asthma.

KEY WORDS: Asthma - Azithromycin - Lung transplantation.

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This article takes its cue from an article by G. Brusselle on prevention of exacerbations with long-term add-on treatment with azithromycin in non-eosinophilic severe asthma, published on the April number of Thorax.[1] However, it addresses the current role of macrolides in many other chronic neutrophilic airway diseases.

Macrolides are widely used as antibiotics and they have an expanding role in the therapy of chronic inflammatory diseases based on their additional anti-inflammatory and immunosuppressive properties.[2] The immunomodulatory action of macrolides is particularly relevant on neutrophils.

Maintenance treatment with macrolides such as azithromycin (AZM) has been proved to be effective in chronic neutrophilic airway diseases including cystic fibrosis, bronchiectasis, diffuse panbronchiolitis, and post-transplant bronchiolitis obliterans syndrome (BOS) and in the prevention of exacerbations in patients with chronic obstructive pulmonary disease (COPD). Developments in this class include tacrolimus and its derivatives that target macrophyllin-12 (FK506 binding protein) and are potent immunosuppressive agents.[3-5] Macrolides are able to inhibit intracellular signaling in a least two important inflammatory pathways; blocking extracellular signal-regulated kinase (ERK)1/2 phosphorylation in the mitogen-activated protein kinase pathway and inhibiting inhibitor of kB phosphorylation, a key step in nuclear factor-kB signaling. The consequence is a reduced secretion of cytokines and chemokines, such as IL-1b, IL-8, TNF-α and GM-CSF, from epithelium and inflammatory cells in response to stimuli. Macrolides have been shown to reduce neutrophil adhesion, increase neutrophil apoptosis and increase the phagocytosis of apoptotic neutrophils by macrophages. Finally, it is worth noting that macrolides can reduce the clearance of corticosteroids, potentially enhancing their anti-inflammatory effect.

Recent studies have suggested a role for AZM in managing airway inflammation and remodeling of post-transplant obliterative bronchiolitis in which epithelium mediators play an important role. Lipopolysaccharide up regulates release of IL-8 and GM-CSF from primary bronchial epithelial cells derived from stable lung allografts. Sub-microbicidal concentrations of AZM attenuate this and may, therefore, alleviate infection-driven neutrophilic airway inflammation and remodeling in the allograft airway. AZM demonstrated an effect on lipopolysaccharide-mediated epithelial release of factors relevant to airway neutrophilia and remodeling in a unique population of primary bronchial epithelial cells derived from stable lung allografts.[6] Studies demonstrating a marked beneficial effect of the macrolide antibiotic, erythromycin, in improving lung function and survival in patients suffering from diffuse panbronchiolitis appeared in the mid-to-late 1990s. These reports were seized upon by the lung transplant community, as it was recognized that there are similarities between diffuse panbronchiolitis and Bronchiolitis Obliterans Syndrome (BOS), a form of chronic lung allograft rejection, in the nature of the airway inflammation present and the physiological defects that develop. This led to a number of small retrospective and prospective, open-label, non-placebo controlled studies in lung transplant (LTx) recipients with BOS using the newer 15-ringed macrolide, AZM.[7]

Chronic lung allograft rejection is the single most important cause of death in LTx recipients after the first postoperative year, resulting in a 5-year survival rate of approximately 50%, which is far behind that of other solid organ transplantations. However, the introduction of AZM in the field of LTx as of 2003 made it clear that some patients with established BOS might in fact benefit from such therapy due to its various anti-inflammatory and immunomodulatory properties. Particularly in patients with an increased bronchoalveolar lavage neutrophilia (i.e., 15%-20% or more), AZT treatment could result in an increase in FEV1 of at least 10%. More recently, it has become clear that prophylactic therapy with AZT actually may prevent BOS and improve FEV1 after LTx, most likely through its interactions with the innate immune system.[8] AZM shares this activity with other macrolides. Since 2000, clarithromycin has been used as long-term therapy in LTx recipients with BOS or potential BOS. Long-term clarithromycin therapy effectively improves lung function in more than one-third of recipients with BOS or potential BOS, comparable to results reported with long-term AZM.[9]

BOS is an important pulmonary complication also after hematopoietic stem cells transplant (HSCT) for treatment of both acute and chronic leukemia as well as aplastic anemia. BOS usually develops as a late complication after HSCT (after the first 100 days after transplantation) and is believed to be part of the chronic graft *versus* host disease (GVHD) phenomenon. The mainstay of therapy for BOS after HSCT is treatment of chronic GVHD with augmentation of immunosuppression. Previous experience with BOS after LTx has suggested a possible anti-inflammatory role for macrolides. A beneficial effect of AZM on lung function in BOS patients after HSCT has also been shown.[10, 11] At variance, there was no significant benefit of 3 months of oral AZM on the respiratory symptoms and lung function in patients with relatively late BOS after HSCT in a randomized placebo-controlled study.[12]

Anecdotal data suggest the AZM effects on cryptogenic organizing pneumonia (COP) and idiopathic pulmonary fibrosis (IPF).[13, 14]

The AZIthromycin in Severe ASThma (AZISAST) study of treatment effects in severe asthma, published on the April number of Thorax, provides evidences to support that long-term low dose AZM halved asthma attack frequency in patients with non-eosinophilic asthma.[1] This randomized double-blind placebo-controlled parallel-group multicenter study was aimed to assess whether long-term add-on treatment with AZM decreases the frequency of acute exacerbations and lower respiratory tract infections (LRTI) in adult patients with severe asthma and frequent exacerbations. The primary efficacy outcome was the rate of severe asthma exacerbations and/or LRTI requiring antibiotics (primary endpoints) during the 26-week treatment phase. Secondary efficacy outcomes included lung function (FEV1 pre- and post-bronchodilation), morning and evening peak expiratory flow (PEF), quality of life (AQLQ score) and asthma control (ACQ score).

Guy Brusselle and colleagues' AZISAST study is the first randomized controlled trial examining the efficacy and safety of add-on treatment with low-dose AZM in severe asthma. Although there was no effect on the primary outcome in the total population, AZM seems to be a new option for prevention of exacerbations in patients with non-eosinophilic severe asthma. Since severe asthma is a heterogeneous syndrome, the authors had predefined to analyze the efficacy of AZM according to the type of underlying inflammation (non-eosinophilic [mainly neutrophilic] or eosinophilic asthma) as defined by a FeNO lower than the upper limit of normal and a blood eosinophilia ≤200/mL.

Besides immunomodulatory and anti-inflammatory effects, macrolide antibiotics may affect gastroesophageal reflux (GER) by modifying esophageal and gastric motility. Acid and weakly acidic GER was measured with 24-h pH-impedance monitoring in 47 LTx patients (12 patients "on" AZT). Patients "on" AZT had a significant lower total number of reflux events, number of acid reflux events, esophageal acid exposure, bolus exposure, and proximal extent of reflux. AZT reduced the concentration of bile acids in BALF without affecting levels of pepsin. LTx patients "on" AZT have less GER and bile acids aspiration. This effect might be due to enhanced esophageal motility and accelerated gastric emptying.[15] Studies showed a high prevalence of GER and aspiration of gastric components (pepsin and bile acids) after LTx and both have been implicated as contributory non alloimmune factors in the pathogenesis of BOS. Aspiration of gastroesophageal refluxate, especially bile acids, may interact with the respiratory epithelium and induce neutrophil recruitment by promoting the expression of cytokines such as IL-8 by structural airway cells. AZM is able to reduce GER severity and aspiration of bile acids after LTx, probably due to a prokinetic effect on esophageal and gastric motility, but AZM does not seem to protect against the long-term allograft dysfunction caused by GER and aspiration and an additional treatment targeting aspiration may be indicated in those LTx patients.[16]

The purported mechanisms of AZM in non-eosinophilic severe asthma include also antibacterial activity against an underlying chronic infection, such as *Chlamydia pneumoniae*, antineutrophil activity, or an effect on airway mucus.

Severe asthma has been shown to be a risk factor for LRTI, including pneumonia.[17] Currently, the guideline for Community-acquired pneumonia (CAP) recommends dual β lactam and macrolide therapy for patients admitted to hospital not only because dual therapy may be more effective at rapid control of bacterial numbers during infection with non-'atypical' organisms but also because CAP is often associated with a strong inflammatory response that contributes towards the development of consolidation, septic shock and acute lung injury. A recent retrospective study by Rodrigo et al published on Thorax in May supports the addition of a macrolide to a β lactam because this approach reduce mortality of patients with moderately severe CAP.[18]

Unfortunately, chronic treatment with AZM for airways diseases may induce resistance to macrolides in the individual patients and extensive use of macrolides may reduce the efficacy for patients with pneumonia.[19, 20] In fact, there is evidence that marked resistance of *Staphlycoccus aureus* and *Haemophilus influenzae* to macrolides develops in cystic fibrosis patients receiving long-term macrolides, with resistance to *Staphlycoccus aureus* reaching 100% within 3 years.[21] In addition, long-term macrolide use might induce resistance in nontuberculous mycobacteria, which are emerging as an increasingly important class of lung pathogens in the cystic fibrosis population.[22]

Consistently with this concern in the COPD Clinical Research Network study, long-term treatment with AZM was associated with an increased proportion of macrolide-resistant nasopharyngeal streptococci.[23] However, in both the COPD Clinical Research Network study and the AZISAST study, there was no evidence suggesting that colonization with macrolide-resistant organisms increased the risk of LRTI or pneumonia.

Another concern is safety. Long-term treatment with AZM in the study by Brusselle appeared to be safe, since the frequency and severity of adverse events was not different from placebo. In particular, no subjects in the AZM-treated group mentioned hearing loss, which contrasts with the hearing decrements reported by Albert et al in patients with chronic obstructive pulmonary disease (COPD) and there were no serious cardiac adverse drug reactions.[23] However, Bruselle *et al.* excluded patients with significant cardiovascular disease, a prolonged corrected QT interval or use of drugs known to cause QT prolongation. Moreover, the use of a macrolide such as clarithromycin in the setting of acute exacerbations of chronic obstructive pulmonary disease or community acquired pneumonia has been recently associated with increased cardiovascular events and mortality.[24] Therefore, the long term effects of macrolides on cardiovascular events when used for respiratory diseases are still unclear.

Pathobiological studies of people with severe, refractory asthma reinforced the concept of asthma heterogeneity with the finding that some of these individuals had neutrophilic inflammation. Although asthma has been considered as a single disease for years, the study of Bruselle et al., as well as other studies, have increasingly focused on its heterogeneity. The characterization of this heterogeneity (that is indeed present in individuals with mild to moderate asthma), has promoted the concept that asthma consists of multiple phenotypes or consistent groupings of characteristics leading to more targeted and personalized approaches to asthma therapy. In neutrophilic asthma corticosteroids are less effective, perhaps because of the absence (or suppression) of the TH2 process.[25] In the year 2008, Simpson and colleagues have already demonstrated that add-on treatment with clarithromycin significantly reduced airway concentrations of IL-8 and neutrophil numbers in severe asthma.[26] However, the lack of neutrophil-targeted interventions limits the ability to determine whether neutrophilia is a biomarker or a target for therapy. More-

over, the lack of efficacy of anti–TNF-α in severe asthma treated with high doses of corticosteroids raises questions about neutrophilic asthma. Several studies, most on mild-to-moderate asthma, have examined whether macrolides are beneficial in adult patients with asthma. However, the duration of these single-centre studies was too short and the number of patients too small to examine the effect of macrolides on exacerbations.[27-30]

Much more studies have been performed to date in Lung transplantation field. Recent clinical observations, supported by research findings, have revealed a dichotomy in the clinical spectrum of BOS with the so-called neutrophilic (partially) reversible allograft/airways dysfunction (NRAD) that is responsive to AZM and the fibroproliferative BOS or classical obliterative bronchiolitis that is not responsive to AZM. These subsets of BOS patients were identified according to their response to AZM (with respect to FEV1, bronchoalveolar lavage neutrophilia/IL-8) and to the large collection of data that are available in BOS patients, consisting of histology specimens, physical and radiological examination, FEV1 and BAL examination.[31] On thin-section computed tomography NRAD was characterized by more centrilobular abnormalities with respect to non-responders to AZM and, at follow-up, showed improvement in all CT abnormalities including air trapping.[32]

The acknowledgment of this dichotomy could improve understanding of the heterogeneous pathological condition that constitutes BOS, thus encouraging a more accurate diagnosis and, ultimately, better tailored treatment.[33] Remarkably, in a retrospective cohort study of consecutive LTx recipients who developed BOS, AZM treatment initiated before BOS stage 2 was independently associated with a significant reduction in the risk of death.[34]

There are similarities between the AZISAST study and the Fungal Asthma Sensitization Trial (FAST) Study, a randomized controlled trial with the antifungal agent itraconazole.

In the AZISAST study there were no significant differences between the AZM and placebo groups in the change from baseline in AQLQ score, ACQ score, FEV1 (pre- and postbronchodilator), morning PEF, evening PEF, use of rescue medication and FeNO. The FAST study showed no benefit in FEV1, a modest improvement in rhinitis and morning peak flow, but a significant improvement in quality of life and a fall in IgE in patients with severe asthma sensitized to one of several common fungi after oral antifungal therapy.[35] As most of the fungi are common in air, the direct external exposure or low levels of colonization may be sufficient to induce an allergic response and pulmonary inflammation in many patients with severe asthma. Another possibility is that itraconazole has a direct and profound immunologic effect and interactions with inhaled steroids such as budesonide, and probably fluticasone.[36, 37] As well as fungal spore, *Staphylococcus aureus*-derived enterotoxins are a group of molecules with superantigenic activity and potent stimulatory effect on T-cell, eosinophils, neutrophils and other inflammatory cells involved in asthmatic inflammation. Sensitization to Staphylococcal enterotoxins and multiclonal IgE synthesis may play a role in the pathogenesis of severe refractory asthma.[38]

Both the AZISAST study and the FAST study strongly support the need to use accurate phenotyping when evaluating new therapies in severe asthma and show unexpected positive effects of drugs not previously designed for asthma.

However, in the AZISAST study the Authors considered non-eosinophilic asthma as defined by FeNO lower than the upper limit of normal and a blood eosinophilia ≤200/mL. All patients received high-dose ICS for at least 6 months prior to study entry and continued this treatment throughout the entire study. Remarkably, in the interpretation of FeNO the weight placed on results depends on whether the test is being used diagnostically in a symptomatic steroid-naive subject, or whether it is used to assess the response to ICS in a patient with a confirmed diagnosis of asthma on anti-inflammatory medications; low FeNO

may indicate a significant response to anti-inflammatory therapy in eosinophilic asthma and not necessary a non-eosinophilic disease.[39] Moreover, the Authors state that the peripheral blood eosinophilia is a sensitive and specific biomarker for airway eosinophilia citing an article in which the sensitivity and specificity of peripheral blood eosinophils as biomarkers of sputum eosinophilia were calculated using data from all subjects who had not been treated with ICS.[40] Thus, peripheral blood eosinophils and FeNO cannot be relied on to identify asthma phenotypes, because both of these tests are not sufficiently sensitive in context of the AZISAST study.

In the AZISAST study, the eosinophilic subgroup had more exacerbations when taking AZM. This finding is judged as unexpected and unexplainable by the authors. However, the proportion of subjects colonized by streptococci resistant to erythromycin was increased in the AZM group and it is well known that asthma exacerbations may be caused by respiratory infections. On the other hand, airway inflammation in this subgroup of patients with eosinophilic asthma was not expected to benefit from macrolides and they may have only the negative effects of the add-on treatment with AZM.

There is still a need for new data on the long-term add-on treatment with AZM in severe asthma because the AZISAST study was not able to demonstrate significant improvement in lung function and use of rescue medication. It is possible that with a more accurate and restrictive definition of non-eosinophilic asthma this outcomes could be obtained. From a speculative point of view, it would be interesting to investigate whether the prevention of exacerbations in patients with non-eosinophilic severe asthma is caused by the antibiotic or anti-inflammatory effects of macrolides; moreover, it would be interesting to contextually evaluate whether sensitization to Staphylococcal enterotoxins play any role. Finally, there is a need for a randomized controlled trial to confirm the impact of AZM on survival in lung transplant recipients, and we have to be aware that a more intensive anti-inflammatory use of AZM, not only in a little number of patient (lung transplantation), but also in obstructive lung diseases (asthma and COPD) much more represented in about 10-15% of population, could be detrimental on the antibiotic point of view, causing the selection of macrolides cross resistance and drug resistant bacteria. Anti-inflammatory activity could erase the antibiotic role and the new life could not survive with the old one.

Riassunto

Nuovi scenari nell'utilizzo dei macrolidi

Questo articolo costituisce il tentativo di analizzare e discutere il ruolo dei presunti meccanismi d'azione dell'azitromicina nell'asma grave non-eosinofilo; fra questi l'attività antineutrofili, un effetto sul reflusso gastroesofageo e l'attività antibatterica contro un'infezione cronica sottostante, ad esempio da *Chlamydia pneumoniae*. Grazie alle loro proprietà antiinfiammatorie e immunosoppressive, che si aggiungono a quelle antibatteriche, i macrolidi hanno un ruolo crescente nella terapia delle malattie infiammatorie croniche. Numerosi studi sono stati condotti nel campo del trapianto polmonare e il trattamento a lungo termine si è dimostrato efficace nella fibrosi cistica, nelle bronchiectasie, nella panbronchiolite diffusa, nella sindrome da bronchiolite obliterante e nella prevenzione delle esacerbazioni in pazienti con BPCO. Studi anatomopatologici effettuati in persone con asma grave, refrattario alla terapia, hanno evidenziato l'eterogeneità della malattia e la necessità di approcci più mirati e personalizzati. Nell'asma neutrofilico i corticosteroidi non sono molto efficaci mentre è particolarmente rilevante l'azione immunomodulante sui neutrofili dei macrolidi. Il recente studio "AZIthromycin in Severe ASThma (AZISAST)", pubblicato sul numero di aprile di Thorax, fornisce evidenze circa l'efficacia e la sicurezza del trattamento aggiuntivo a lungo termine con azitromicina nell'asma grave non eosinofilo. Nonostante le preoccupazioni riguardo un possibile aumento della percentuale di resistenza ai macrolidi e i possibili effetti cardiovascolari, non c'è stata alcuna evidenza di un aumento del rischio di polmonite o di altri eventi avversi. Rimane ancora la necessità di ulteriori dati che confermino l'efficacia dell'azitromicina nell'asma grave non eosinofilo perché lo studio AZISAST non è riuscito a dimostrare un significativo miglioramento della funzione polmonare e una riduzione dei farmaci al bisogno.

PAROLE CHIAVE: Asma - Azitromicina - Polmone, trapianto.

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