

IMMUNOMODULATORY AND CLINICAL EFFECTS OF LONG-TERM LOW-DOSE MACROLIDE TREATMENT OF CHRONIC RHINOSINUSITIS WITH NASAL POLYPOSIS

IMUNOMODULACIJSKI I KLINIČKI EFEKTI DUGOTRAJNE
NISKODOZIRANE PRIMENE MAKROLIDNOG ANTIBIOTIKA U TERAPIJI
HRONIČNOG RINOSINUZITISA SA NOSNOM POLIPOZOM

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Summary: Immunomodulatory treatment of chronic rhinosinusitis with nasal polyposis (CRSwNP) by macrolide antibiotics represents a challenging alternative to conventional therapy and surgery, still being at the very beginning. Immune and inflammatory processes in nasal and paranasal sinus mucosa, crucial in the etiopathogenesis of nasal polyps (NPs) are reflected in levels of various local mediators, found both in mucosa and nasal fluid. In this prospective study, we assessed the immunomodulatory and clinical effects of long-term low-dose oral macrolide treatment in the management of CRSwNP. Twenty-two ($n = 22$) nonasthmatic, nonallergic patients with CRSwNP were administered clarithromycin (CAM) 500 mg/day single oral dose for eight weeks. We measured the levels of proinflammatory cytokines TNF- α , TNF- β , and IL-1 β , Th1 cytokines IL-2, IL-12, and IFN- γ , Th2 cytokines IL-4, IL-5, IL-6, and IL-10, and chemokine IL-8 in the nasal fluid samples, before and after treatment, using a flow cytometric method. We also scored each of the 22 patients before and after therapy according to Tsicopoulos' global nasal symptom score and Malm's endoscopic score. Following treatment, we found significantly reduced levels of IL-8 ($p < 0.01$) and TNF- α ($p < 0.01$) in nasal secretions. Macrolide therapy decreased the size of polyps in 45.45% of the patients. We concluded that long-term low-dose treatment with CAM was effective in the management of

Kratak sadržaj: Mada je u samim začetima, imunomodulacijska terapija hroničnog rinosinuzitisa sa nosnom polipozom (HRSsNP) primenom makrolidnih antibiotika bi mogla predstavljati alternativu uobičajenoj konzervativnoj terapiji, kao i hirurškom lečenju. Imunski i zapaljenski procesi u sluzokoži nosa i paranasalnih sinusa, najznačajniji u etiopatogenezi nosnih polipa, reflektuju se na različite lokalne medijatore, detektovane u sluzokoži kao i u nosnom sekretu. U ovoj prospektivnoj studiji procenili smo imunomodulacijske i kliničke efekte dugotrajne niskodozirane oralne primene makrolidnog antibiotika u lečenju HRSsNP. Dvadeset dvoje ($n = 22$) neastmatičnih, nealergičnih pacijenata sa HRSsNP dobijalo je klaritromicin (CAM) u pojedinačnoj dnevnoj dozi od 500 mg tokom osam nedelja. Merene su koncentracije proinflammatoryh citokina TNF- α , TNF- β i IL-1 β , Th1-citokina IL-2, IL-12 i IFN- γ , Th2-citokina IL-4, IL-5, IL-6 i IL-10, kao i hemokina IL-8 u uzorcima nosnog sekreta, pre i nakon terapije, primenom protočne citometrije. Svaki od pacijenata klinički je klasifikovan pre i posle lečenja prema Tsicopoulosovom ukupnom nosnom simptom skoruu i Malm-ovom endoskopskom skoruu. Nakon lečenja, detektovane su značajno niže koncentracije IL-8 ($p < 0,01$) i TNF- α ($p < 0,01$) u nosnom sekretu. Terapija makrolidnim antibiotikom je smanjila veličinu polipa kod 45,45% od ukupnog broja pacijenata. Zaključeno je da je dugotrajna niskodozirana primena

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CRSwNP. We suggest that macrolides can be an alternative to topical and systemic corticosteroids in the management of CRSwNP.

Keywords: chronic rhinosinusitis, nasal polyposis, clarithromycin, nasal fluid, cytokines, IL-8, TNF- α

Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose and paranasal sinus mucosa that is present for at least 12 weeks without complete resolution. Chronic rhinosinusitis with nasal polyposis (CRSwNP), which is considered to be a subgroup of CRS, is defined as a chronic inflammatory disease of nasal and sinus mucosa leading to diffuse formation of benign polyps protruding from sinuses into the nasal cavity (1). Polyps are covered by pseudostratified columnar epithelium with some areas of squamous metaplasia, basement membrane thickening and an increased number of mucous glands. Lamina propria of nasal polyps (NPs) contains significantly more eosinophils, neutrophils, and plasma cells than does the nasal mucosa (1). Several mechanisms have been proposed for the formation of NPs. Clinical as well as experimental studies indicate that nasal polyp formation and growth are activated and perpetuated by an integrated process of mucosal epithelium, lamina propria and inflammatory cells, which, in turn, may be initiated by both infectious and noninfectious inflammation (1). The mechanisms responsible for selective accumulation of eosinophils in polyps are unknown. Nasal polyp fibroblasts could play a role in the recruitment of eosinophils through the release of RANTES (regulated on activation, normal T cell expressed and secreted) and GM-CSF (granulocyte-macrophage colony-stimulating factor) (2). Several cytokines (interleukin (IL)-4, IL-5, IL-6, IL-8, tumor necrosis factor- α (TNF- α), RANTES, GM-CSF have been shown to be upregulated in NPs, suggesting that resident structural cells can produce a number of molecules to attract inflammatory cells and prolong their survival. These inflammatory cells themselves can also produce cytokines which recruit more inflammatory cells in an autocrine fashion (1, 2).

Although surgery has been the preferred treatment for CRSwNP for a long time, a change in the treatment strategy in recent years has led to greater use of medications, especially nasal corticosteroids and antibiotics (1, 3). Macrolide antibiotics, such as erythromycin (EM), clarithromycin (CAM), and roxithromycin (RXM), have been used for decades as important chemotherapeutic agents in the treatment of infectious diseases. Their activities against Gram positive cocci but also against intracellular pathogens such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in particular have been of clinical significance (4). Recently, there has been considerable

CAM korisna u lečenju HRSsNP. Makrolidi mogu biti prihvaćeni kao alternativa topikalnim i sistemskim kortikosteroidima u terapiji HRSsNP.

Ključne reči: hronični rinosinuzitis, nosna polipoza, klari-tromicin, nosni sekret, citokini, IL-8, TNF- α

interest of Japanese and other investigators in the immunomodulatory and antiinflammatory action of 14-membered and 15-membered macrolides in the long-term low-dose treatment of CRS and NPs.

Nasal secretions contain minute amounts of cytokines, potent biologic factors involved in the regulation of inflammation and immune defense, and other inflammatory mediators expressed by various epithelial and nonepithelial cells (5). Because cytokines play a dominant role in the pathophysiology of airway disease, the cytokine profile in nasal fluid may help to recognize mechanisms underlying NPs and the immunomodulatory effects of treatment by antibiotics.

In this study, we compared cytokine levels in nasal fluid samples from patients with NPs before and after long-term low-dose macrolide treatment. We, also investigated the relationship between the changes in cytokine levels and in the clinical characteristics of NPs.

Materials and Methods

Study population

Twenty-two ($n = 22$) nonatopic, nonasthmatic patients with NPs were included in this prospective study which has been performed according to the Declaration of Helsinki. Written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia. The diagnosis of CRSwNP was based on a documented medical history and on the results of clinical examination, nasal endoscopy and computed tomography of the paranasal sinuses. The exclusion criteria were the presence of allergic rhinitis, lower airway obstruction symptoms, antrochoanal and sphenchoanal polyps, cystic fibrosis, and primary ciliary dyskinesia. All subjects included in this investigation did not have any acute respiratory tract infection and none of them were treated with oral and topical corticosteroids, antibiotics and antihistamines for at least three weeks before the enrollment. Skin prick tests were performed on all patients for sensitivity to 18 common allergens. A test result was considered positive when at least one of the induration diameters was 3 mm higher than that in the negative control. Subjects were considered allergic and excluded from the study if they had a serum IgE level > 160 IU/mL. Patients received 500 mg/day oral single dose of 14-membered rings macrolide antibiotic clarithromycin (CAM) for 8 weeks. There were no concomitant medications used during

the macrolide therapy. The exclusion criteria for long-term low-dose macrolide treatment were: pregnancy, macrolide hypersensitivity, younger than 18 years, liver and gastrointestinal dysfunction.

Clinical score

To investigate the effect of CAM, the presence of nasal symptoms associated with CRSwNP (obstruction, anosmia, sneezing, rhinorrhea, and itching) on the day of the enrollment in the study and after macrolide treatment was scored according to Tsicopoulos et al. (6) from 0 to 3: 0 for no symptoms, 1 for mild symptoms, 2 for moderate symptoms, and 3 for severe symptoms, so that the maximal global nasal symptom score is 15.

Endoscopic physical findings before and after macrolide administration were scored according to Malm (7). The degree of nasal polyps is classified in relation to fixed anatomical landmarks in four steps: 0 = »no polyposis«, 1 = »mild polyposis (small polyps not reaching the upper edge of the inferior turbinate)«, 2 = »moderate polyposis (medium sized polyps reaching between the upper and lower edges of the inferior turbinate)«, 3 = »severe polyposis (large polyps reaching below the lower edge of the inferior turbinate)«. The maximal endoscopic score is 6, bilaterally. Treatment results were divided into the following two categories: *improvement* and *no improvement*. We have defined *improvement* as observation of shrinkage of NPs by more than one grade (two grades bilaterally) after the macrolide treatment.

Sampling of nasal fluid and cytokine determination

Nasal fluid samples were collected from nasal cavities of all 22 patients before and after therapy with CAM using absorption technique with cotton wool sticks, which were inserted into the nasal cavity posterior to the mucocutaneous junction for 60 seconds, as previously described (8–10). All of the samples were put in a 2 mL eppendorf tube containing 1 mL of transfer media (phosphate-buffered saline with gentamycin 50 µg/mL, penicillin G 340 U/mL, fungizone 500 µg/mL) for 30 minutes because of diffusion of cytokines into the medium and then stored at 4 °C for a maximum of 2 h until processed. Nasal fluid samples were centrifuged at 1000 g for 10 minutes to separate the cellular components. After centrifugation, supernatants were portioned and stored at –70 °C until cytokine determination. The levels of eleven cytokines (TNF-α, TNF-β, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, and IFN-γ) were measured in each of the 44 samples using a commercial flow cytometric kit (Flow Cytomix, Bender MedSystems, USA) on the flow cytofluorimeter (Beckman Coulter XL-MCL, USA), which was connected with BMS Flow

Cytomix Pro 2.2 Software, according to the manufacturer's instruction. The sensitivities (lower limits) of detection were as follows: 11.2 pg/mL for TNF-α; 11 pg/mL for TNF-β; 12 pg/mL for IL-1β; 11.5 pg/mL for IL-2; 1 pg/mL for IL-4; 12 pg/mL for IL-5; 11 pg/mL for IL-6; 13 pg/mL for IL-8; 3.5 pg/mL for IL-10; 4 pg/mL for IL-12; 2 pg/mL for IFN-γ.

Statistical analysis

Data were expressed as mean ± standard deviation (± SD). Statistical analysis of the results was performed by using the nonparametric Wilcoxon signed rank test on SPSS software. Correlations between different parameters were made by the Pearson's correlation test. A *p* value less than 0.05 was considered to be statistically significant.

Results

We included in our study 6 female and 16 male patients with CRSwNP (mean age 44.36 ± 14.15 years, aged from 25 to 72 years). The average global nasal symptom (GNS) score was improved from 12.32 ± 2.34 before treatment with CAM to 9.13 ± 4.09 after treatment, but this difference was not statistically significant. We also did not find significant difference in the Malm endoscopic score before and after treatment (5.09 ± 1.02 vs. 4.32 ± 1.78). CAM decreased the size of NPs in 45.45% (10 of 22 cases) of the patients. We found that 54.55% of the patients (12 cases) showed no significant change in the size of polyps. We did not find significant differences in the levels of TNF-β, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, and IFN-γ in the nasal secretions before and after macrolide treatment. Only the concentrations of chemokine IL-8 and proinflammatory cytokine TNF-α in nasal fluid were highly statistically different, exactly lower after CAM treatment (from 293.59 ± 375.35 pg/mL to 75.30 ± 80.28 pg/mL, *p* < 0.01 for IL-8; from 153.05 ± 123.29 pg/mL to 44.22 ± 47.74 pg/mL, *p* < 0.01 for TNF-α) (Table I). Interestingly, the levels of IL-8 and TNF-α were decreased after therapy in almost all nasal fluid samples.

A very high positive correlation between reduction of nasal symptom score and reduction of endoscopic score (polyp size) was found (*r* = 0.760, *p* < 0.0001). We found positive correlation between reduction of endoscopic score and reduction of IL-8 and TNF-α levels in nasal secretions (*r* = 0.542, *p* = 0.041 for IL-8; *r* = 0.535, *p* = 0.035 for TNF-α) only in patients with polyp shrinkage after macrolide treatment.

Discussion

Recent years have seen numerous reports by Japanese and other authors regarding the efficacy of long-term, low-dose therapy using 14-membered ring

Table I Cytokine levels in nasal fluid before and after macrolide treatment.

| Parameter | Before treatment | After treatment | p value |
|---------------|------------------|-----------------|---------|
| | Mean ± SD | Mean ± SD | |
| IL-12 | 15.28±14.08 | 19.04±18.33 | p>0.05 |
| IFN- γ | 19.81±18.56 | 22.56±24.06 | p>0.05 |
| IL-2 | 148.76±142.87 | 132.65±115.05 | p>0.05 |
| IL-10 | 16.62±15.87 | 23.23±15.09 | p>0.05 |
| IL-8 | 293.59±375.35 | 75.30±80.28 | p<0.01 |
| IL-6 | 116.63±123.21 | 113.76±131.19 | p>0.05 |
| IL-4 | 227.82±295.70 | 248.74±287.44 | p>0.05 |
| IL-5 | 437.21±412.67 | 424.49±341.94 | p>0.05 |
| IL-1 β | 50.39±48.46 | 42.85±43.93 | p>0.05 |
| TNF- α | 153.05±123.29 | 44.22±47.74 | p<0.01 |
| TNF- β | 89.39±132.25 | 84.88±102.54 | p>0.05 |

All results of cytokine levels were expressed as pg/mL.

macrolides in the treatment of CRS and NPs. Ichimura et al. (11) found that roxithromycin (RXM) administered at 150 mg/day for at least 8 weeks shrank nasal polyp size in 52% of the twenty investigated patients. Yamada et al. (12) showed that CAM administered at 400 mg/day for 8–12 weeks resulted in marked shrinkage of polyps in 40% of twenty patients. Aydin et al. (13) demonstrated a significant improvement of the mucociliary transport and shrinkage of the polyps after treatment with RXM. Macrolides reduce mucus hypersecretion. An animal study showed that goblet cell hypersecretion in the guinea-pig trachea was reduced by CAM (14). Experience from diffuse pan-bronchiolitis suggests that it takes 6 weeks for the effect of macrolide treatment to set in. In CRS, the rate of improvement is related to the number of weeks the patient is treated. One study showed that response rate varied from 5% at 2 weeks to 71% at 12 weeks (15). A study performed by Cervin et al. (4) showed that further improvement in responders was seen at 12 months compared to 3 months regarding mucociliary transport, postnasal drip and headache. The results of the bacterial cultures suggest that the risk of selecting resistant bacteria is low (4). In a small number of patients the cultures were positive, but this was not always linked with an increase in symptoms, which could be due to the fact that in addition to the direct bacteriostatic effect of macrolides, they may in some cases reduce the virulence of bacteria without eradicating them (4).

However, the mechanisms of polyp shrinkage during macrolide treatment are not well known. CRSwNP is a chronic disease of the nasal and paranasal sinus mucosa characterized by inflammation and

structural abnormalities including thickening of the basement membrane and stromal fibrosis. Stromal fibrosis shows evidence of inflammatory cell accumulation and fibroblast hyperplasia. Nonaka et al. (16) demonstrated that *in vivo* RXM treatment directly suppressed nasal polyp fibroblasts (NPFs) proliferation, and that this effect of RXM on fibroblast growth was persistent, indicating that RXM may prevent the progression of NPs by inhibiting the development of fibrosis. Macrolides decrease proinflammatory mediators, neutrophil and eosinophil hemotaxis, leukocyte adhesion and oxidative burst and increase apoptosis (17). Interleukin-8 (IL-8), a potent neutrophil and also eosinophil chemoattractant and activating factor, is known to be released by monocytes, macrophages and airway epithelial cells (18, 19). Recent data have shown that airway fibroblasts are also important sources of this chemokine (18). Results published by Nonaka et al. (18) showed that although RXM did not directly suppress IL-8 production from NPFs, the reduction in the proliferation of fibroblasts suggests that RXM can indirectly reduce the total levels of IL-8 in the nasal polyps and thereby play a role in regulating inflammatory cell recruitment (18). Results of our investigation showed that the IL-8 and tumor necrosis factor-alpha (TNF- α) concentrations in nasal fluid from patients with NP were significantly decreased after treatment with CAM. The decreases in IL-8 and TNF- α levels in nasal secretions were associated with reduction in polyp size on endoscopic examination. TNF- α is a cytokine initially identified as a cause of hemorrhagic necrosis in certain tumors (20). It is secreted by macrophages, monocytes and NK cells and many other cell types (20). TNF- α mostly triggers apoptosis and necrosis in sensitive tissues and has been linked to osteomyelitis and apical periodontitis, but it can also participate in skeletal homeostasis, including osteoclastic formation, and bone resorption in the maxillofacial region (20). TNF- α , among other cytokines, can regulate fibroblast activity and collagen formation through modulation of collagenase activity (20–22). Relationship between decreased levels of TNF- α in nasal fluid and shrinkage of polyps can be explained by several recently published findings. Eosinophil infiltration is regulated by numerous chemokines and adhesion molecules such as eotaxin, regulated on activation normal T cell expressed and secreted (RANTES), and vascular cell adhesion molecule (VCAM)-1 (23). To infiltrate sites of inflammation, eosinophils leave the bloodstream and pass through the endothelium in four steps, namely rolling, adhesion, transendothelial migration, and chemotaxis (23). Adhesion molecules, such as VCAM-1 play an important role during adhesion to endothelial cells (23). Experiments performed by Otori et al. (23) demonstrated that TNF- α stimulation induces VCAM-1 protein production and mRNA expression in human nasal polyp fibroblasts. Epithelial and immunocompetent cells such as macrophages, mast cells and, especially, eosinophils produce TNF- α . These findings

suggest that TNF- α increases VCAM-1 production in nasal fibroblasts and activates the transmigration of eosinophils, which induce further production of TNF- α and accelerate the accumulation of eosinophils in NPs. Saji et al. (24) demonstrated that nasal polyp fibroblasts produced RANTES by stimulation with TNF- α and IL-1 β . Therefore, results published by Yoshifuku et al. (25) showed that eotaxin secretion from fibroblasts was induced by stimulation with IL-4 and synergistically enhanced by simultaneous stimulation with TNF- α and IL-4. Our results showed that treatment with CAM could suppress TNF- α production and the progression of CRSwNP due to lower stimulation or, maybe, inhibition of fibroblasts and eosinophil accumulation in NP tissue.

In conclusion, in our study CAM was used in the long-term low-dose treatment of CRSwNP. After eight

weeks of therapy, polyp size decreased in 45.45% of patients. Therefore, the levels of IL-8 and TNF- α in nasal fluid significantly decreased after macrolide administration. It was seen that a low-dose macrolide therapy was effective in the treatment of CRSwNP. Therefore, macrolide treatment may help to minimize surgical treatment. We suggest that macrolides can be an alternative to topical and systemic corticosteroids in the management of CRSwNP, especially in patients in whom steroid use is contraindicated.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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