INDEX COPERNICUS INTERNATIONAL

A randomized, double-blind, placebo--controlled study to investigate the use of bacteriophages in patients with chronic rhinosinusitis with nasal polyps

Konstantin Grigorievich Dobretsov[1], Oxana Kolenchukova[2], Alexander Sipkin[3], Luisa Maria Bellussi[4], Giorgio Ciprandi[5], Desiderio Passali[4]

[1]'Sokolov' Hospital N 122 of the Federal Medical-Biological Agency of Russia, Krasnoyarsk, Russia
[2]Federal Siberian Scientific and Clinical Center of Federal Medical-Biological Agency of Russia, Krasnoyarsk, Russia
[3]Federal Research Center "Krasnoyarskii Scientific Center" of the Siberian branch of the Russian Academy of Sciences, Scientific Research Institute of Medical Problems of the North, Russia
[4]IFOS, International Federation of Otorhinolaryngological Societies, Rome, Italy
[5]Outpatients Clinics Dept., Casa di Cura Villa Montallegro, Genoa, Italy

Article history: Received: 17.06.2021 Accepted: 29.06.2021 Published: 30.06.2021

ABSTRACT:

Introduction: A randomized, double-blind, placebo-controlled study investigates the use of bacteriophages in the treatment of chronic rhinosinusitis with nasal polyps.

Material and methods: 40 adult patients with chronic rhinosinusitis with nasal polyps were examined. All patients underwent functional endoscopic sinus surgery. After the surgery, 20 patients were administered an intranasal gel with bacteriophage mixture (Otofag, Micromir, Russia) twice a day for ten weeks, while 20 other patients received a placebo solution.

Results: On the 10th day, IL-1β secretion diminished (63 mg/ml versus 440 mg/ml in control). There was a decrease in the total number of microorganisms and *Enterobacteriaceae* (5.7 x 10^{6} CFU/ml *versus* 1.2 x 10^{9} CFU/ml in the control group) and the absence of *Streptococci* (*versus* 2.1 x 10^{9} CFU/ml in control) was noted on the 30th day of treatment in the group receiving bacteriophage mixture. On the 10th day, a decrease in the activity of secretory IL-1β and IL-8 strongly and very strongly correlated with a total number of microorganisms (r = 0.7; r = 0.9, respectively), as well as a decrease in the activity of secretory IL-8 correlated with the number of *Enterobacteriaceae* (r = 0.72) and *Staphylococci* (r = 0.65) in the experimental group treated with bacteriophages. On the 30th day, the decrease in serum IL-1β significantly correlated with the total number of microorganisms (r = 0.80) and *Enterobacteriaceae* (r = 0.90) in the experimental group.

Conclusions: The administration of bacteriophages restored the balance of microorganisms in the nasal cavity and decreased inflammatory response in chronic rhinosinusitis with nasal polyps. Changes such as an inflammation dampening could potentially contribute to reducing recurrent growth of polyp tissue in the future.

KEYWORDS: bacteria, bacteriophages, chronic rhinosinusitis with nasal polyps, cytokines, local treatment

ABBREVIATIONS

CFU – Colony Forming Unit
CRS – chronic rhinosinusitis
CRSwNP – chronic rhinosinusitis with nasal polyps
ENT – Ear, Nose and Throat

INTRODUCTION

The upper respiratory tract handles a particularly high microbial burden, as it is anatomically adapted for colonization of bacteria from the inhaled air. Although normal microbiota plays an important role in protecting the body from pathogenic microbes, it can also promote progress of various infectious diseases [1–6]. In physiological conditions, the human body contains hundreds of different microorganism types, among which bacteria constitute a dominant group.

The term "normal microbiota" includes microorganisms living in a specific environment, for example in the upper airways. The vast majority of them are saprophytes-commensals. As a rule, they do not cause any apparent harm to the human body. The species composition of the microbiota of various parts of the body, including the upper respiratory tract, changes periodically. However, it is often impossible to draw a clear line between saprophytes and pathogens that are part of the normal microbiota [7].

Cytokines are involved in the mechanisms following the penetration of pathogens into the nasal mucosa and the associated inflammation. Cytokines have pleiotropic biological effects on various

 DOI: 10.5604/01.3001.0015.0084

types of cells, mainly participating in the formation and regulation of the body's protective reactions to antigens. Local protection is developed by activating a typical inflammatory response after the interaction between pathogens and pattern-recognition receptors (membrane Toll-like receptors) and the following synthesis of the so-called pro-inflammatory cytokines. Synthesized in the area of inflammation, cytokines affect almost all cells involved in the development of inflammation, including granulocytes, macrophages, fibroblasts, endothelial and epithelial cells, as well as T-and B-lymphocytes in consequence. Cytokines are responsible for both sides of the relationship between non-specific protective reactions and specific immunity within the immune system. Hence, the level of cytokines may correlate with the concentration of bacteria in the inflammatory process, thus serving as a potential indicator of the effectiveness of therapy [8].

There are numerous chronic inflammatory diseases of the respiratory tract whose pathogenesis is not fully understood and in relation to which some conflicting theories have been proposed. Chronic rhinosinusitis with nasal polyps (CRSwNP) belongs to this category of diseases. Moreover, CRSwNP is one of the most common disease of the nasal cavity and paranasal sinuses [2–4]. Despite the overall prevalence of CRSwNP, there is currently no agreement regarding the recommendation whether antibacterial drugs should be used to treat this disease. Nevertheless, it has been recognized that CRSwNP is an inflammatory disease. Over the last years, some researchers have been adhering to the so-called "staphylococcal superantigen" theory which suggests that the colonization of *Staphylococcus aureus* leads to the formation of a superantigenic toxin that increases local eosinophilic inflammation and the formation of polyps [5, 9].

The effectiveness of CRSwNP treatment is probably not depending on the suppression of pathogenic microflora but concerns the restoration of eubiosis in the paranasal sinuses instead.

Bacteriophages can be suitable tool for achieving such restoration. The "Otophag" (Micromir, Russia) intranasal gel with bacteriophages contains a complex of 32 types of bacteriophages that inhibit the growth of a large number of pathogenic bacteria (*Bacteroides spp.*, *Escherichia coli spp.*, *Haemophilus influenzae spp.*, *Klebsiella spp.*, *Moraxella catarrhalis*, *Morganella morganii*, *Neisseria spp.*, *Proteus vulgaris spp.*, *Providencia rettgeri spp.*, *Pseudomonas aeruginosa spp.*, *Staphylococcus aureus spp.*, *Streptococcus pyogenes spp.).* The unique properties of the "Otophag" include inhibiting pathogenic bacteria's activity without disturbing normal microbiota, as well as lack of interaction with the body's physiological functions. As a result, the balance of microbiota in the nasal cavity and paranasal sinuses is restored, which could favorably affect CRSwNP recurrence.

To summarize, this study aims to investigate the use of the "Otophag" intranasal gel with bacteriophages in patients with CRSwNP.

MATERIALS AND METHODS

From 2018 to 2019, 40 patients with CRSwNP aged 18 to 64 years old were examined in the Center of Otorhinolaryngology of The Federal Siberian Research Clinical Center under FMBA of Russia (Krasnoyarsk, Russia). All patients were admitted to the hospital for planned surgical treatment (endoscopic removal of polyps). The inclusion criteria for this study accepted patients who had a primary form of CRSwNP and whose condition did not last for more than five years. Exclusion criteria were diagnosis of secondary CRSwNP (CRS associated with autoimmune diseases, selective immunodeficiency and primary ciliary dyskinesia) and previous sinus surgeries. The patients were randomly assigned to two groups:

- Group 1 (control) – 20 patients with CRSwNP received placebo treatment;
- Group 2 (experimental) – 20 patients with CRSwNP were treated by the "Otofag" intranasal gel with bacteriophages.

This study received approval from the local Ethics Committee of the Federal Siberian research clinical centre under the Federal Medical-Biological Agency of Russia (Krasnoyarsk, Russia). All patients signed an informed consent for participation in the study.

"Otophag" was applied on the nasal mucous membrane, mainly under the middle turbinate in the area of the middle nasal passage, twice a day for ten weeks after surgery. A placebo drug was also applied on the nasal mucous membrane with the same frequency and duration in the control group. The labeling of drugs was carried out according to a code available to neither doctors nor the patients.

We have therefore carried out a randomized, double-blind placebo-controlled study. Only objective research methods, such as bacteriological examination (10^{th} and 30^{th} days after treatment) and immunological analysis (10^{th} and 30^{th} days after treatment), were performed.

Microorganisms were cultured on differential diagnostic media (blood agar, yolk-salt agar, Endo agar, enterococcus agar). Sterile swabs with a commercial Ames transport medium were used to take samples of the pathological material from the nasal mucosa (mainly from the middle nasal passage) and transport them for further measurements. The obtained samples were cultured in sectors and incubated in a thermostat at 37°C for 24 hours.

The concentration of cytokines in blood serum and nasal lavage fluid (IL-1β, IL-8) was determined by the enzyme immunoassay method (JSC "Vector-Best", Russia).

The statistical processing of the results was carried out using the Statistics 7.0 application software package (StatSoft Inc., 2004). Sample descriptions were obtained by calculating the median (Me) and the interquartile range, defined as the difference between the 25^{th} and 75^{th} percentiles (C25 and C75). The reliability of differences between independent samples' indicators was evaluated by means of the nonparametric Mann-Whitney U-test, while the dependent samples were analyzed using the Wilcoxon test. Correlations were calculated with the Spearman's correlation. The critical significance level for statistical hypothesis testing in this study was established to be 0.05.

Tab. I. Microbial composition of nasal mucosa. Mean numbers of Colony Forming Units (CFUs) at different times in Placebo and Bacteriophages groups.

CFU/ML	PLACEBO		BACTERIOPHAGES	
	After 10 days	After 30 days	After 10 days	After 30 days
Streptococci	1.155×10^9	2.105×10^9	0 P = 0.0001	0 P = 0.0001
Enterobacteriaceae	1.105 x 109	1.18 x 109	1.775×10^7	5.755×10^6 P = 0.012
Staphylococci	1×10^6	1.3×10^6	5.1×10^4	1×10^4
Total number of bacteria	1.68×10^9	2.11×10^9	1.78×10^7	5.8×10^6 P = 0.019

Tab. II. Concentration cytokines in blood serum at different times in Placebo and Bacteriophages groups. Data is presented by the median and interquartile range values.

PG/ML	PLACEBO		BACTERIOPHAGES	
	After 10 days	After 30 days	After 10 days	After 30 days
IL-1β	30 (20–42)	5 (4–6) P = 0.024	32 (6–40) P = 0.019	6 (5–16) P = 0.021 (intragroup) P = 0.031(intergroup)
IL-8	18 (14–71)	10 (5–55)	55 (4–58)	31 (25–62)
TNF-β	15 (15–20)	5 (2–16)	18 (5–22)	4.5 (2–41)

RESULTS

Microbiological data

After 10 days of observation, a dramatic decrease in the number of *Streptococci* in the experimental group was recorded when compared to the placebo group (Tab. I.). A significant intergroup difference was detected. After 30 days, *Streptococci* remained absent in the experimental group, whereas the number of CFU increased in the placebo group. An intergroup difference was observed. The amount of *Enterobacteriaceae* decreased significantly in the experimental group both after 10 and 30 days of observation, with significant intergroup differences. *Staphylococi* colonies remained statistically unchanged. The total number of bacteria significantly decreased in the active group, in terms of both intragroup and intergroup differences.

Serum cytokines

The activity of interleukin-1β decreased significantly in the active group after 30 days of observation with significant intragroup and intergroup differences (Tab. II.). Moreover, a significant decrease in the placebo group was observed after 30 days. Other cytokines remained unchanged.

Nasal cytokines

Interleukin-1β secretion decreased in the active group after 30 days of observation, demonstrating a significant difference when compared to the placebo group (Tab. III.). Interleukin-1β secretion dropped significantly in the placebo group after 30 days of observation.

Furthermore, a significant decrease in the IL-8 levels was recorded in both groups after 30 days but no intergroup difference was noted. TNF-α levels remained unchanged.

In the control group, a single positive correlation was found between the concentration of IL-8 on the 30th day in the nasal lavage fluid and the concentration of *Staphylococci* ($r = 0.70$; $P = 0.023$). Several positive interactions were found in the experimental group (receiving bacteriophages). The concentration of IL-1β in the blood serum on the 30th day correlated with both the total number of microorganisms and the concentration of *Enterobacteriaceae* ($r = 0.8$; $P < 0.001$; $r = 0.9$; $P = 0.01$), while on the 10th day it correlated only with the total number of microorganisms ($r = 0.9$; $P = 0.018$). The level of IL-8 found in the nasal lavage fluid on the 10th day correlated positively with the total number of bacteria in the nasal cavity, as well as with the concentration of *Enterobacteriaceae* and *Staphylococci* ($r = 0.7$; $P = 0.036$; $r = 0.72$; $P < 0.001$; $r = 0.65$; $P = 0.016$, respectively).

DISCUSSION

Since the observed increase in the number of microorganisms referred to the opportunistic bacteria, it documenting the state of dysbiosis in the nasal cavity. The increase of the *Enterobacteriaceae* family indicated a dysbiotic change in the nasal mucosa and emphasized their undoubted role in the development of the inflammatory process. These microorganisms act quite aggressively when the immune system is suppressed. They cause various concomitant diseases of the ENT region and predispose to the development of polypoid tissue. The obtained results are therefore regarded as a microbiota disorder resulting from a decrease in the activity of both local and systemic immunity mechanisms in the nasal passages. As microflora of the nasal mucosa is smaller than the oral and pharyngeal ones, any minimal alterations in its composition may indicate the presence of dysbiotic disorders.

Measurement of cytokine levels in the blood serum showed an increased level of IL-1β on the 10th day in both the placebo and

Tab. III. Concentration of cytokines in the nasal lavage fluid at different times in Placebo and Bacteriophages groups. Data is presented by the median and interquartile range values.

PG/ML	PLACEBO		BACTERIOPHAGES	
	After 10 days	After 30 days	After 10 days	After 30 days
IL-1β	440 (50–600)	23 (5–45) P = 0.027	63 (50–430) P = 0.005	60 (50–100)
Il-8	386 (235–752)	41 (10–80) P = 0.009 (intragroup)	220 (93–517)	98 (16–546) P = 0.01 (intragroup)
TNF-α	5 (2–20)	9 (2–25)	7 (2–41)	2 (2–2)

bacteriophage group when compared to the control values, as well as a decrease in the amount of IL-1β on the 30th day to the level of specified parameters. IL-1β, a proinflammatory cytokine, induces the expression of adhesion molecules in vascular endothelial cells, thereby ensuring the arrest of neutrophils in the area of inflammation. Its increase indicates the activation of the phagocytic component of innate immunity. The concentration of IL-8 in the blood serum did not significantly differ neither in the control group (placebo) nor in the bacteriophage treatment group. The IL-8 cytokine activates neutrophil granulocytes and attracts them from blood vessels. The level of IL-1β in blood serum was significantly higher on the 10th and 30th days in the group of patients treated with bacteriophage mixture.

Evaluation of nasal cytokines showed a reduced level of IL-1β on the 10th day of treatment with bacteriophages when compared to the control group (placebo), as well as a decrease in the indicator on the 30th day in the control group (placebo). The level of IL-8 in the nasal lavage fluid in both groups decreased significantly on the 30th day of treatment. Notably, on the 30th day, the concentration of IL-8 in the nasal lavage fluid was higher in the experimental group than in the control group (placebo).

The concentration of TNF-α did not differ significantly in terms of the dynamic of changes or in comparison to the levels observed in the experimental and control groups.

Interestingly, relevant correlations were observed between the number of bacteria and pro-inflammatory cytokine levels. In other words, bacterial count correlated with inflammatory events.

Having obtained these results, we have reliably proved that the use of the "Otophag" bacteriophage solution in rhinosinusitis with polyps regulates the microbiocoenosis of the nasal cavity. In addition to a decrease in the total number of microorganisms on the 10th and 30th days in the experimental group, the *Streptococci* count was not determined, while the total number of *Enterobacteriaceae* decreased. On the contrary, an increase in the level of bacteria was recorded both on the 10th and 30th days in the control (placebo) group, with general and specific differentiation? This proved true mainly for the *Staphylococcus* bacteria. Restoration of normal microflora and suppression of pathogenic species led to a decrease in the activity of proinflammatory cytokines IL-1β and IL-8 in the nasal secretions on the 10th day of observation. Decrease in the activity of the proinflammatory cytokines in the nasal lavage fluid (IL-8) on the 10th day and in blood serum (IL-1β) on the 30th day was proved both by the total number of microorganisms and by the concentration of certain bacterial species (E*nterobacteriaceae*, *Staphylococci*).

Therefore, bacteriophages can represent a new strategy in the management of patients with CRSwNP, as they are capable of affecting the composition of microbiota and dampening inflammation. This study is the first demonstration that bacteriophages could be a valuable tool in CRSwNP therapy. In fact, their clinical implementation has only been hypothesised until now [10, 11].

On the other hand, this study had some limitations which include the lack of clinical and morphological assessment. Consequently, further studies should be performed to answer the needs which have not been met by this scientific work.

CONCLUSION

The restoration of balance in the nasal cavity microbiota could have a positive effect on the nasal mucosa in chronic rhinosinusitis with nasal polyps. It could potentially contribute to inhibiting inflammatory response reactions, leading to the normalization of all immune protection mechanisms, improved mucociliary clearance and, consequently, possible reduction of polyp tissue growth.

REFERENCES

1. de Boeck I., Wittouck S., Martens K.: Anterior Nares Diversity and Pathobionts Represent Sinus Microbiome in Chronic Rhinosinusitis. mSphere, 2019; 4(6): e00532–19.
2. Hastan D., Fokkens W.J., Bachert C. et al.: Chronic rhinosinusitis in Europe – an underestimated disease. A GA2LEN study. Allergy, 2011; 66(9): 1216–1223.
3. Klossek J.M., Neukirch F., Pribil C. et al.: Prevalence of nasal polyposis in France: A cross-sectional, case-control study. Allergy, 2005; 60(2): 233–237.
4. Tomassen P., Newson R.B., Hoffmans R. et al.: Reliability of EP3OS symptom criteria and nasal endoscopy in the assessment of chronic rhinosinusitis – a GA2LEN study. Allergy, 2011; 66(4): 556–561.
5. Bachert C, Zhang N, van Zele T. Staphylococcus aureus enterotoxins as immune stimulants in chronic rhinosinusitis. Clin Allergy Immunol, 2007; 20: 163–175.
6. Vickery T.W., Ramakrishnan V.R., Suh J.D.: The Role of Staphylococcus aureus in Patients with Chronic Sinusitis and Nasal Polyposis. Curr Allergy Asthma Rep., 2019; 19(4): 19–21.

7. Fekete S., Szabó D., Tamás L., Polony G.: The role of the microbiome in otorhinolaryngology. Orv. Hetil., 2019; 160(39): 1533–1541.
8. Dobretsov K., Negm K., Ralli M., Passali D.: The theory of a "staphylococcus superantigen" in chronic rhinosinusitis with nasal polyps: myth or reality? Eur Rev Med Pharmacol Sci, 2019; 23(1 Suppl.): 48–54.
9. van Cauwenberge P.B., Vermeiren J.S., van Kempen M.J.: Viral rhinitis and asthma. Curr. Opin Allergy Clin Immunol, 2001; 1(1): 21–25.
10. Miyake M.M., Bleier B.S.: Future topical medications in chronic rhinosinusitis. Int Forum Allergy Rhinol., 2019; 9(S1): S32–S46.
11. Tan N.C.W., Psaltis A.J.: Latest developments on topical therapies in chronic rhinosinusitis. Curr Opin Otolaryngol Head Neck Surg., 2020; 28(1): 25–30.

Word count: 3077 Tables: 3 Figures: – References: 11

DOI: 10.5604/01.3001.0015.0084 Table of content: https://otolaryngologypl.com/issue/14180

Competing interests: The authors declare that they have no competing interests.

Corresponding author: Giorgio Ciprandi; Outpatients Clinics Dept. Casa di Cura Villa Montallegro, Via Montezovetto 27, 16132, Genoa, Italy; Phone: +39 0103531284; E-mail: gio.cip@libero.it

Cite this article as: Dobretsov K.G., Kolenchukova O., Sipkin A., Bellussi L.M., Ciprandi G., Passali D.: A randomized, double-blind, placebo-controlled study to investigate the use of bacteriophages in patients with chronic rhinosinusitis with nasal polyps; Otolaryngol Pol 2021: 75 (6): 32-37