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POLYOXIDONIUM EFFECT ON IMMUNOREACTIVITY OF PATIENTS WITH CHRONIC STAPHYLOCOCCOSIS PHARYNGITIS

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In this paper we have studied the effectiveness of Polioksidony in the treatment of patients with chronic staphylococcus's pharyngitis on background. It was found that the inclusion in the complex treatment of patients Polioksidony normalizes biotsinoz oropharyngeal secretions, increases the phagocytic activity of neutrophils and biocide, opsonizing properties serum normalizes the major classes of immunoglobulins in oropharyngeal secretions. Polioksidony has a positive effect on the clinical course of chronic pharyngitis and warns its recurrence, reduces the number of acute respiratory diseases and their complications.

KEY WORDS: polioksidony, chronic pharyngitis, antimicrobial immunity

ВПЛИВ ПОЛІОКСІДОНІУ НА ІМУНОРЕКТИВНІСТЬ ХВОРИХ НА ХРОНІЧНИЙ СТАФІЛОКОККОВИЙ ФАРИНГІТ

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В роботі вивчалась ефективність застосування Поліоксідонію у комплексній терапії хворих на хронічний стафілококовий фарингіт. Встановлено, що включення в комплексне лікування хворих Поліоксідонію дозволяє нормалізувати біоценоз ротоглоткового секрету, підвищити фагоцитарну та біоцидну активність нейтрофілів, властивості сироватки, що опсонуються, нормалізувати вміст основних класів імуноглобулінів в ротоглотковому секреті. Поліоксідоній зумовлює позитивний вплив на клінічний плин хронічного фарингіту та попереджує його рецидування, зменшує кількість гострих респіраторних захворювань та їх ускладнень.

КЛЮЧОВІ СЛОВА: поліоксідоній, хронічний фарингіт, антимікробний імунітет

ВЛИЯНИЕ ПОЛИОКСИДОНИЯ НА ИММУНОРЕАКТИВНОСТЬ БОЛЬНЫХ ХРОНИЧЕСКИМ ФАРИНГИТОМ НА ФОНЕ СТАФИЛОКОККОНОСИТЕЛЬСТВА

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В работе изучена эффективность применения Полиоксидония в комплексной терапии больных хроническим фарингитом на фоне стафилококконосительства. Установлено, что включение в комплексное лечение больных Полиоксидония позволяет нормализовать биоциноз ротоглоточного секрета, повысить фагоцитарную и биоцидную активность нейтрофилов, опсонизирующие свойства сыворотки, нормализовать содержание основных классов иммуноглобулинов в сыворотке крови и ротоглоточном секрете. Полиоксидоний оказывает положительное влияние на клиническое течение хронического фарингита и предупреждает его рецидивирование, снижает количество острых респираторных заболеваний и их осложнений.

КЛЮЧЕВЫЕ СЛОВА: полиоксидоний, хронический фарингит, антимикробный иммунитет

INTRODUCTION

Long-term inflammatory processes occurring in the airway mucosa lead to inhibition of local defense mechanisms and reduce the overall immunoreactivity body [1, 2].

With the development of inflammation of the upper airway disorder observed in the immune system, including insufficient mucosal immunity. Thus, the greatest interest in the treatment of these patients and immunorehabilitation are immunomodulatory drugs or immunostimulatory activities [3, 4, 5].

Among immune preparations is difficult to identify drugs with distinct only immunocorrective or just immunostimulatory properties. The observed effect (or immunostimulatory immunocorrective) of using this group of drugs is determined by the initial status of the immune system of the patient and treatment scheme.

The effectiveness of the therapy of infectious and inflammatory diseases including increases in the range of therapeutic interventions drugs immunocorrective orientation. Today, a wide clinical application has Polioksidony (Petrovax Pharm, Russia), with a wide range of influence on the cells and organs of the immune system [6, 7].

In its chemical structure Polioksidony substances are similar to of natural origin. N-oxide groups are the base of the drug. They are commonly found in the human body, as through the formation of NO- oxide occurs the metabolism of nitrogenous compounds. The drug has a wide range of pharmacological effects on the body - an immunomodulatory, detoxifying, antioxidant and membrano-protektiv. Polioksidony factors are capable of activating innate immunity (monocytes/macrophages, neutrophils, NK), stimulate the production of cytokines that enhance antibody and cell mediated immunity and improve the quality of life of the patient. All these properties define it as an immune stimulant drug of first choice for all kinds of secondary immunodeficiency, complex treatment and prevention of diseases, including infectious origin, holding immunorehabilitation. It is important that the contraindications of Polioksidony were not detected [6]. Antioxidant and detoxifying membrano-protektiv properties of Polioksidony allow the use of this medication with antibiotics. There are studies proving the effectiveness of Polioksidony treatment of inflammatory diseases of the nasal cavity and paranasal sinuses, chronic inflammatory process in pharyngeal plexus [7].

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OBJECTIVE

The aim of this work was to study the effectiveness of Polioksidony in treating patients with chronic staphylococcus's pharyngitis (ChPh), its impact on the clinical course of the disease and the body immunoreactivity parameters.

MATERIALS AND METHODS

We observed 62 patients on chronic staphylococcus's pharyngitis in age from 21 to 45 years, who were treated in the Communal health institution «Kharkiv city hospital № 6». The first group (basic group) amounted to 32 patients, together with anti-inflammatory therapy (topical Lizak 1 tablet 3 times daily for 7 days, physiotherapy for 5days, treatment of the posterior pharyngeal wall Lugol solution 3 times a day for 5 days) was obtained Polioksidony (6 mg every 24 hours to 5 injections, then two times a week, 10 injections for the course). The second group (control group) consisted of 30 patients who received similar therapy without Polioksidony.

Clinical, microbiological, immunological studies were performed before the treatment, at 7 and 30 days after the end of the therapy.

As indicators of standards the results of 30 healthy individuals were used.

Immunological studies included the determination of the phagocytic activity of neutrophils, their biocidity, opsonizing properties serum titers of antibodies to the causative infectious agents, concentrations of the major classes of immunoglobulins in saliva and serum.

Phagocytic activity of blood neutrophils was determined by thick smear [8]. As the object of phagocytosis using an inactivated strain of staphylococcus culture daily 209. Phagocytic number (PhN - number of phagocytic cells) and phagocytic index (PhI - the number of bacteria absorbed by one cell) were determined. The efficiency of phagocytosis of bacteria opsonized was studied in a similar manner. The opsonization of bacteria were performed in Hanks solution containing 20 % of sera from patients with thermally activated (autoserum) or serum from healthy donors (pool of 5 donors) for 30 min at 37°C.

Biocidal phagocytes were estimated by using the S. Nielsen method [9]. The number of absorbed, but live bacteria was determined after seeding cell lysate Golda Petri method dishes

with plain agar. Lysis of leukocytes was performed by adding 3-fold amount of water.

Antibody titer (T.Ab) to opportunistic microorganisms and antigenic determinant common (CAD) bacteria were determined by ELISA [10].

Ultrasonic disintegrants were used as the bacterial antigens prepared from one-day culture of microbial cells killed by heating for 2 hours on a boiling water bath [11].

Serum levels of immunoglobulins M, A, G were determined spectrophotometrically [12].

Mathematical processing of the data was performed using Microsoft Excel 2007 and programs MedStat (Serial number MS000055) SPDE «Alpha», Donetsk, according to the recommendations of statistical processing of biomedical materials. To identify significant differences compared indicators Student's t-test was used. The differences were considered significant at a significance level of $p < 0.05$. Data was given in the text as the arithmetic mean M and the standard deviation sd .

RESULTS AND DISCUSSION

Patients before treatment complained of pain and sore throat (84 %), foreign body sensation in the throat (32 %), dry cough (40 %) and low-grade fever (52 %).

Microbiological examination of oropharyngeal secretions ChFh patients revealed a 34 % *S. pneumonia* ($3,6 \pm 0,3$) $\times 10^5$ CFU/ml, at 24 % - *S.aureus* ($3,5 \pm 0,4$) $\times 10^6$ CFU/ml.

Microbial associations were sown in 45 % of cases, which consisted of *S. pneumonia*, *S.aureus* and *Candida Albicans*.

Immunological studies have shown that patients with chronic staphylococcus's pharyngitis of increasing concentrations of IgG and mIgA and reducing values sIgA and lysozyme in oropharyngeal secretions (tab. 1).

It has also been found that digesting and absorbing capability bacterial particles neutrophils peripheral blood of patients with ChPh lower than that of healthy individuals (tab. 2). Low phagocytic ability of cells to patients with HF was observed in respect of opsonized autoserum bacteria (tab. 3).

In all patients of the main group and the comparison group revealed a significant increase in antibody titer to bacterial etiological factors and common antigenic determinative (CAD) bacteria. High antibody titers were detected for almost all microbes studied (tab. 4).

The clinical observations have shown that under the influence of Polioksidony patients with chronic staphylococcus's pharyngitis, on the 7th day after the treatment has been a marked decrease or complete disappearance of the major clinical symptoms: sore throat (25 %), foreign body sensation in the throat (at 13,8 %). all patients receiving Polioksidony markedly improved overall health (normalization of body temperature in 70 %, fatigue 18 %).

Table 1

Content of lysozyme, sIgA, mIgA, IgG in the oropharyngeal secretions of patients 1 and 2 groups before and after the treatment, (M \pm sd)

Indicators	Healthy persons	ChFh Patients		
		Before treatment	After treatment	
			7 days	1 month
sIgA g/l	0,30 \pm 0,03	<u>0,22 \pm 0,02*</u> 0,21 \pm 0,02*	<u>0,26 \pm 0,02*</u> 0,22 \pm 0,02*	<u>0,28 \pm 0,02†‡</u> 0,23 \pm 0,02*
IgA g/l	0,15 \pm 0,02	<u>0,23 \pm 0,02*</u> 0,23 \pm 0,02*	<u>0,19 \pm 0,02*</u> 0,21 \pm 0,02*	<u>0,16 \pm 0,02†‡</u> 0,21 \pm 0,02*
IgG g/l	0,063 \pm 0,03	<u>0,076 \pm 0,08*</u> 0,075 \pm 0,08*	<u>0,068 \pm 0,007*</u> 0,071 \pm 0,007*	<u>0,064 \pm 0,008†‡</u> 0,071 \pm 0,008*
Lysozyme mg/l	458,6 \pm 25,9	<u>338,3 \pm 30,6*</u> 337,8 \pm 30,5*	<u>452,5 \pm 30,2†‡</u> 351,2 \pm 30,4*	<u>460,4 \pm 30,6†‡</u> 395,1 \pm 30,4*

Note: Above the line - parameters of patients of group 1, under the dash - 2 groups

* $p < 0.05$ - significant differences parameters of patients from healthy individuals,

† $p < 0.05$ - significant differences parameters of patients after treatment on the performance of patients before treatment,

‡ $p < 0.05$ - significance of differences between the indices of patients 1 and 2 groups.

Table 2

Phagocytic and biocidal activity of blood neutrophils of patients 1 and 2 groups before and after the treatment, (M ± sd)

Indicators	Healthy persons	ChFh Patients		
		Before treatment	After treatment	
			7 days	1 month
Phagocytic number (PhN),%	75,7 ± 7,1	<u>55,6 ± 6,3*</u>	<u>70,2 ± 6,4†‡</u>	<u>74,8 ± 6,8†‡</u>
		55,4 ± 6,3*	55,5 ± 6,3*	57,1 ± 6,1*
Phagocytic index (PhI)	5,6 ± 0,5	<u>3,1 ± 0,4*</u>	<u>4,7 ± 0,4†‡</u>	<u>5,3 ± 0,6†‡</u>
		3,1 ± 0,4*	3,2 ± 0,4*	3,7 ± 0,4*
Bactericidal activity,%	4,8 ± 0,6	<u>17,7 ± 1,6*</u>	<u>6,4 ± 0,6*†‡</u>	<u>5,4 ± 0,6†‡</u>
		17,8 ± 1,6*	17,2 ± 1,8*	15,2 ± 1,4*†

Note: Above the line - parameters of patients of group 1, under the dash - 2 groups
 * p < 0.05 - significant differences parameters of patients from healthy individuals,
 † p < 0.05 - significant differences parameters of patients after treatment on the performance of patients before treatment,
 ‡ p < 0.05 - significance of differences between the indices of patients 1 and 2 groups.

Table 3

Phagocytic activity of neutrophils of patients 1 and 2 groups regarding auto serum opsonized bacteria before and after the treatment, (M ± sd)

Indicators	Healthy persons	ChFh Patients		
		Before treatment	After treatment	
			7 days	1 month
Phagocytic number (PhN),%	81,0 ± 7,1	<u>58,4 ± 6,6*</u>	<u>78,2 ± 6,4†‡</u>	<u>80,2 ± 7,6†‡</u>
		58,5 ± 6,6*	58,7 ± 6,5*	63,7 ± 6,1*
Phagocytic index (PhI)	5,8 ± 0,5	<u>3,1 ± 0,3*</u>	<u>4,7 ± 0,4†‡</u>	<u>5,9 ± 0,6†‡</u>
		3,1 ± 0,3*	3,2 ± 0,3*	4,1 ± 0,4*
Bactericidal activity,%	3,1 ± 0,4	<u>17,3 ± 1,8*</u>	<u>4,9 ± 0,5†‡</u>	<u>3,7 ± 0,4†‡</u>
		17,2 ± 1,8*	17,0 ± 1,6*	11,9 ± 1,3*†

Note: Above the line - parameters of patients of group 1, under the dash - 2 groups
 * p < 0.05 - significant differences parameters of patients from healthy individuals,
 † p < 0.05 - significant differences parameters of patients after treatment on the performance of patients before treatment,
 ‡ p < 0.05 - significance of differences between the indices of patients 1 and 2 groups.

Table 4

The Antibody titer (relative unit) to infectious agents in patients 1 and 2 groups before and after the treatment, (M ± sd)

Microbes	ChFh Patients		
	Before treatment	After treatment	
		7 days	1 month
S. pneumoniae	<u>1,5 ± 0,1*</u>	<u>1,8 ± 0,1*†</u>	<u>2,0 ± 0,2*†‡</u>
	1,5 ± 0,1*	1,7 ± 0,1*	1,7 ± 0,1*
S. aureus	<u>1,4 ± 0,1*</u>	<u>1,7 ± 0,1*†</u>	<u>1,8 ± 0,1*†‡</u>
	1,4 ± 0,1*	1,6 ± 0,1*	1,6 ± 0,1*
C bacteria	<u>1,5 ± 0,1*</u>	<u>1,8 ± 0,1*†</u>	<u>1,9 ± 0,1*†‡</u>
	1,5 ± 0,1*	1,6 ± 0,1*	1,6 ± 0,1*

Note: Above the line - parameters of patients of group 1, under the dash - 2 groups
 * p < 0,05 - significant differences parameters of patients from healthy individuals,
 † p < 0,05 - significant differences parameters of patients after treatment on the performance of patients before treatment,
 ‡ p < 0,05 - significance of differences between the indices of patients 1 and 2 groups.

When instrumental examination of all patients of the main group and the comparison group on the 7th day after the end of treatment was observed swelling and hypertrophy of the mucous membrane of the posterior pharyngeal wall.

On the 30th day after the end of treatment, 71.8 % of the study group patients without complaints (in the comparison group - 30 % of patients), and their clinical status was characterized by the norm. Pharyngoscope at 84,3 % of the patients of the main group and in all patients of the comparison group experienced a slight hypertrophy of the mucous membrane of the posterior pharyngeal wall.

Microbiological examination of the main group found in 84,3 % of cases the absence of pathogens (from 9,3 % - *S. pneumonia* $(3,1 \pm 0,3) \times 10^3$ CFU/ml, at 6,4 % - *S.aureus* $(2,3 \pm 0,3) \times 10^3$ CFU/ml), marked decrease in microbial associations (in 15,6 % of patients) without the appearance of fungal flora and 68 % of the pathogenic microflora (*S. epidermidis* $(2,1 \pm 0,3) \times 10^3$ CFU/ml, *S. saprophyticus* $(3,3 \pm 0,2) \times 10^2$ CFU/ml).

In the control group half of the patients (52 %) was sown pathogenic flora (*S. pneumonia* $(3,1 \pm 0,3) \times 10^4$ CFU/ml, *S.aureus* $(2,3 \pm 0,3) \times 10^5$ CFU/ml) a slight decrease in the amount of microbial associations (36.6 % cases).

Immunological studies have shown that under the influence of the dynamic changes occurring Polioksidony and immunoreactivity in patients. However, they are somewhat delayed in time as compared with the clinical improvement.

Under the effect of Polioksidony the increase of the secretory IgA and serum immunoglobulins (IgA and IgG), and lysozyme occurred in the content of oropharyngeal secretions (tab. 1).

On the 7th day after the treatment the patients receiving Polioksidony, had a significant increase in the phagocytic activity of neutrophils and biocide effect, and increase serum opsonizing properties (tab. 2, 3).

The autoserum increased both in the absorption capacity of neutrophils and their biocidal effect. Full restoration of the functional activity of phagocytic cells in these patients occurred by 30 days after treatment. To compare the group of patients we have studied that the properties of cells and serum opsonizing properties recovering very slowly and by 30 days were significantly different from the norm.

On the 7th and 30th days after the end of the treatment the patients who received

Polioksidony, had an increase in antibody titer to infectious etiologic pathogens and IgG antibodies to CAD bacteria (tab. 4).

It appears that the long circulation in patients with high titers of antimicrobial antibodies with high affinity is an important factor in the suppression and elimination of infectious agents, as well as a factor preventing the recurrence of the disease. The patients who did not receive Polioksidony such dynamic improvements in humoral immunity were observed. By 30th days after the treatment the patients in a comparison group the antibody titer to infectious etiologic pathogens and their affinity were not significantly changed compared to their values before treatment (tab. 4).

The monitoring of patients during the year showed that the main group was not observed recurrence ChFh. They are much less likely than the comparison group was ill patients with acute respiratory infections, which are mild and not accompanied by complications. In the study group acute respiratory infections 2-4 times a year have been reported in 17 % of patients.

Patients with ChFh comparison group relapses occurred in 40 % of cases, of which 25 % of patients - 2-3 times a year, 70 % of patients 2-4 times a year suffered acute respiratory illness, which in 23,8 % of cases complicated by acute bronchitis. In 3 patients (10 %) were diagnosed with pneumonia.

CONCLUSIONS

1. Polioksidony has a positive effect both on the clinical course of chronic pharyngitis and prevention of its recurrence, and the immunoreactivity of the body.

2. Polioksidony stimulates the increase of phagocytic and biocidal activity of white blood cells, the production of high affinity antibodies antimicrobial, increased serum opsonin properties.

The findings showed promising applications and high therapeutic efficacy of Polioksidony in the treatment of chronic staphylococcus's pharyngitis.

PROSPECTS FOR FUTURE STUDIES

Prospects for future studies is a comparative analysis of the treatment of chronic and acute pharyngitis by immunomodulatory drugs.

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