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Study of the effect of a drug from the macromycetes Ganoderma lucidum on the phagocytic activity of peripheral blood neutrophils in patients with year-round allergic rhinitis

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Abstract

Modern complex therapy of patients with persistent (year-round, chronic) allergic rhinitis (AR), based on the use of antihistamines and intranasal glucocorticosteroids, in many cases is not effective enough, since drug therapy acts only on individual links in the pathogenesis, without preventing the progression of the allergic disease. At the same time, the quantitative and reserve capabilities of neutrophil granulocytes are depleted, which increases the level of antigenic load in patients with AR. The aim of this study is to investigate the phagocytic activity of

neutrophils in patients with AR who received basic therapy and patients who received basic therapy and a course of the drug from the macromycetes Ganoderma lucidum "Astmagan". We examined 40 patients of both sexes with AR, the duration of the disease ranged from 6 months to 2 years. Patients were divided into 2 equal groups: The 1st group consisted of patients who received basic therapy (oral Loratadine and intranasal Nasonex). The 2nd group consisted of patients who received basic therapy and a course of "Astmagan". The treatment course lasted 90 days. The control group consisted of 25 healthy individuals. After the treatment, patients in both groups showed a statistically significant positive dynamics of phagocytic system activity: in group 1, the phagocytic index (FI) increased by 30 % (p=0.001), in the second group this indicator reached the normal level. Also, in patients of group 1, the phagocytic number (PN) increased by 35 % (p=0.0001), but did not reach the normal level.

According to the results of comparative evaluation, a greater efficacy of the treatment complex with the additional use of "Astmagan" was established, which is manifested in the restoration of phagocytic activity of peripheral blood neutrophils, the effect persists for 6 months after treatment.

Key words: allergic rhinitis; phagocytic activity of neutrophils; macromycetes Ganoderma lucidum.

Introduction

Allergic rhinitis (AR) is a multidisciplinary problem because of its wide prevalence and various clinical and pathogenetic manifestations [1]. Every year more and more new cases are registered, and further growth of the disease is predicted. This pathology significantly reduces the quality of life and affects the ability to work in the socially active population [2].

These data indicate the need for further study of the nature of allergic inflammation to develop a sound approach to the use of existing treatments and to search for new pharmacological agents. Since ARs are immune-dependent diseases and rank among the first among ENT diseases in terms of social significance, the possibility of using new drugs in their treatment is very important.

Modern complex therapy of patients with persistent (year-round, chronic) allergic rhinitis, based on the use of antihistamines and intranasal glucocorticosteroids, in many cases is not effective enough, since drug therapy acts only on individual links in the pathogenesis, without preventing the progression of allergic disease [3]. Indicators of the phagocytic component of immune defense have a significant impact on the control of the disease in patients with AR. A decrease in the quantitative and reserve capacity of neutrophil granulocytes indicates insufficient neutrophil absorption activity and increases the level of antigenic load in patients with AR.

An effective antiallergic drug should be able to inhibit the development of the disease and not adversely affect homeostasis. Medicinal mushrooms are a rich source of pharmacologically active compounds. One of the mushrooms commonly used in traditional Chinese medicine is Ganoderma lucidum (G. lucidum). In Asian countries, it is treated as a nutraceutical, the regular use of which provides vigor and improves health. G. lucidum is a rich source of biologically active compounds and is widely used in traditional Chinese medicine [4]. The pharmacologically active fraction of G. lucidum polysaccharides has antioxidant, immunomodulatory, antitumor, antineurodegenerative, and antidiabetic activity [5-8]. Fungi of the genus Ganoderma have anti-inflammatory [9] and anti-allergic activity, and can affect inflammatory cytokines [6]. G. lucidum polysaccharides modulate immune function both in vivo and in vitro. The immunomodulatory effect of G. lucidum polysaccharides includes enhancing the function of antigen-presenting cells, mononuclear phagocyte system, humoral immunity and cellular immunity [10]. In connection with the above, it is of interest to include in the treatment of patients with AR a drug of plant origin "Astmagan", which is a lyophilized powder of the biomass of macromycetes G. lucidum.

Objective: to compare the parameters of neutrophil phagocytic activity (phagocytic index and phagocytic count) in patients with AR who received basic therapy and patients who received basic therapy and a course of "Astmagan".

Materials and methods of research

Forty patients with year-round allergic rhinitis (AR) (main group) were examined, of whom 15 (37.5 %) were men and 25 (62.5 %) were women. The average age of the patients was 37.58 ± 14.3. The duration of the disease ranged from 6 months to 2 years. Work with patients was carried out in accordance with their informed consent. These patients of the main group were divided into 2 subgroups: Subgroup 2 (20 people) - patients who received basic therapy (orally 1 tablet (10 mg of Loratadine) once a day and Nasonex 2 injections (50 mcg each) in each nostril once a day (total daily dose - 200 mcg). The 3rd subgroup (20 patients) consisted of patients who received basic therapy and a course with a drug from the macromycetes G. lucidum "Astmagan", 1 caps. 2 times a day. The course of treatment was 90 days. The 1st control group consisted of 25 healthy individuals.

Neutrophils were isolated from whole venous blood by gradient centrifugation. Venous blood in a ratio of 2:1 was layered on a density gradient of ficoll (solution density 1.1192 g/cm^3, manufactured by Granum Laboratory, Ukraine). Centrifuged at 200 g for 20 min, the cell ring formed at the interface was transferred to a clean centrifuge tube, washed twice with saline, resuspended and centrifuged each time for 10 min at 200 g. The resulting cell suspension of neutrophilic granulocytes (0.1 ml) was placed in a plate for immunological studies and mixed with the same amount of 10% polystyrene latex suspension for phagocytosis (1.5 μm) (manufacturer DIA-M, Ukraine), then incubated at 37^0 C for 30 minutes. They were centrifuged for 10 minutes at 100 g, fixed, smeared, and stained with hematoxylin. By light microscopy (Euromax BioBlue LabSeries Lab Binocular microscope, objective x100, eyepiece x10), 100 neutrophils were counted and the percentage of cells with absorbed latex particles was determined. A neutrophil that captured 1 or more latex cells was considered a phagocytic cell. The following indicators of phagocytic activity were calculated: 1) phagocytic index (PI) - the percentage of cells that entered phagocytosis out of the total number of cells; 2) phagocytic number (PN) - the average number of latex particles that are intracellularly located (the quotient of dividing the total number of absorbed latex particles by the number of cells that entered phagocytosis).

The results were statistically processed using parametric and nonparametric statistical methods. Statistical analysis of the data was performed using the computer program Statistica 8.0. The research results are a database, which is a spreadsheet in the format of the above program. To evaluate the numerical indicators, the arithmetic mean (M) and the error of the mean (m) were calculated using Student's t-test for independent and dependent samples. The results of special studies performed in the groups of patients with AR were compared with each other and the results of these studies in practically healthy individuals. The Shapiro-Wilks test was used to check the distribution of quantitative data.

The study was conducted in accordance with the basic bioethical standards of the Helsinki Declaration, the Council of Europe Convention on Human Rights and Biomedicine (1977), as well as the relevant provisions of the WHO and the Ministry of Health № 281 of 01.11.2000. The work is an integral part of the research work (RW) of the Department of Otorhinolaryngology of Odesa National Medical University: "Development of differential diagnostic criteria and etiopathogenetic methods of treatment of allergic, inflammatory and tumor diseases of the upper respiratory tract and ear", state registration number 0160U1.

A decrease in phagocytic number (PN) by 39 % ($p=0.0001$) and phagocytic index (PI) by 42 % ($p=0.0001$) in patients with AR before treatment compared with the control group of healthy individuals may indicate significant allergization of the body, which can lead to depletion of the reserve capacity of peripheral blood neutrophils.

After the treatment in patients of the 2nd (baseline therapy) and 3rd groups, a statistically significant positive dynamics of the studied indicators of phagocytic system activity was noted. In group 2, the PI index increased by 30 % ($p=0.001$) compared to the level before treatment. In group 3, this indicator not only increased by 38 % ($p=0.0001$), but also reached the normal level of 60.1 ± 2.2 %. It should be noted that in this group of patients, 6 months after the course of treatment with, the PI remained at the normal level (58.3 ± 2.8 %). However, in patients of group 2, PI after baseline treatment did not reach normal values.

Results and discussion

The study of neutrophil phagocytic activity (NP) before treatment showed that there was a statistically significant suppression of phagocytic reactions in the examined patients with AR compared with the control group (Table 1). A decrease in phagocytic number (PN) by 39 % (p=0.0001) and phagocytic index (FI) by 42 % (p=0.0001) in patients with AR before treatment compared with the control group of healthy individuals may indicate significant allergization of the body, which can lead to depletion of the reserve capacity of peripheral blood neutrophils.

Table 1. Dynamics of changes in phagocytic activity in the blood of patients with allergic rhinitis, before and after treatment, (M±m)

Indicators	1st group	2nd group			3r dgroup		
		Before treatment 2a	After 3 months of treatment 2b	After 6 months of treatment 2c	Before treatment 3a	After 3 months of treatment 3b	After 6 months of treatment 3c
PI, %	57,45±4,2	35,2±2,2	49,2±5,8	45,9±3,3	37,3±3,6	60,1±3,6	58,3±2,8
	P1-2a=0,0001 P1-2b=0,0001 P1-2c=0,0001 P1-3a=0,0001		P1-3b=0,038 P2a-2b=0,001 P2b-2c=0,006 P2a-2c=0,0001		P1-3c=0,345 P3a-3b=0,001 P3b-3c=0,026 P3a-3c=0,0001		
PN, ум. од.	3,8±0,16	2,2±0,14	3,4±0,13	2,4±0,19	2,1±0,14	3,8±0,09	3,7±0,14
	P1-2a=0,0001 P1-2b=0,0001 P1-2c=0,0001 P1-3a=0,0001		P1-3b=0,0001 P2a-2b=0,0001 P2b-2c=0,0001 P2a-2c=0,003		P1-3c=0,046 P3a-3b=0,0001 P3b-3c=0,009 P3a-3c=0,0001		

Notes: n - number of patients; P - level of significance of the difference between the indicators; AR - allergic rhinitis; FI - phagocytic index; FN - phagocytic number.

After the treatment in patients of the 2nd (baseline therapy) and 3rd groups, a statistically significant positive dynamics of the studied indicators of phagocytic system activity was noted. In group 2, the FI index increased by 30% (p=0.001) compared to the level before treatment. In group 3, this indicator not only increased by 38 % (p=0.0001), but also reached the normal level of 60.1 ± 2.2 %. It should be noted that in this group of patients, 6 months after the course of treatment with, the PI remained at the normal level (58.3 ± 2.8 %). However, in patients of group 2, PI after baseline treatment did not reach normal values.

A similar trend was observed in the study of PF in the 2nd and 3rd groups of patients. Prior to treatment, PF in both groups of patients was statistically significantly reduced by 43 % compared to group 1, control (p=0.0001). After the basic treatment in group 2, the FF increased by 35 % (p=0.0001). In the 3rd group of patients who took baseline therapy and "Astmagan", the PEF increased by 45% compared to this indicator before treatment and reached the level of normal, and after 6 months this indicator did not change. After 6 months of treatment, the PEF in the 2nd group of patients returned to the level of the pretreatment value.

Our studies have shown that after discontinuation of Astmagan, the patients' general condition improved significantly: increased efficiency, significantly improved sleep, improved nasal breathing, and rhinoconjunctival syndrome disappeared. In addition to the clinical improvement, the phagocytic system was normalized. It should be noted that in the group of patients receiving basic therapy, despite a statistically significant increase in phagocytosis, their normalization did not occur. We attribute such a positive effect of "Astmagan" (the active ingredient of which is the lyophilized biomass of macromycetes G. lucidum) on the body of patients with AR to its antioxidant, detoxification, hepatoprotective and immunomodulatory properties, which have been established in other studies [4, 10 - 14]. Thus, our studies have shown that the addition of "Astmagan" to the basic course of treatment has a positive effect on the phagocytic activity of peripheral blood neutrophils, and this effect persists for 6 months.

Based on the above data, we can consider the inclusion of "Astmagan" in the treatment regimen for patients with allergic rhinitis to be pathogenetically sound and clinically promising.

Conclusions

1. A statistically significant decrease in the phagocytic number by 39 % and the phagocytic index by 42 % was found in the examined patients with year-round allergic rhinitis compared with the control group.

2. It was shown that after the treatment in patients of group 2 who received basic therapy, the phagocytic index significantly increased by 30 % compared with the level before treatment. In group 3, patients who received "Astmagan" as part of the basic therapy, the phagocytic index reached the normal level (60.1 ± 2.2%). 6 months after the course of treatment in patients of group 3, the phagocytic index remained at the level of group 1 (58.3 ± 2.8 %). However, in patients of group 2, such a positive effect was not observed, and the phagocytic index remained at the level of the pretreatment index after 6 months, i.e. significantly reduced compared to the level of group 1.

3. It was determined that in patients of group 2 after the basic course of treatment, the phagocytic number statistically significantly increased by 35 %, and almost reached the level of group 1 of the control group. After 6 months, this figure decreased significantly and did not differ significantly from the level of the pretreatment figure. In patients of group 3, the phagocytic count recovered, reached the normal level, and remained stable for 6 months.

Thus, our studies have shown that the use of Astmagan in the complex treatment of patients with year-round allergic rhinitis is reasonable and appropriate, as it has a corrective effect on the phagocytic activity of peripheral blood neutrophils, has a prolonged effect, and this effect persists for 6 months after treatment.

Thus, our studies have shown that the use of "Astmagan" in the complex treatment of patients with year-round allergic rhinitis is reasonable and appropriate, as it has a corrective effect on the phagocytic activity of peripheral blood neutrophils, has a prolonged effect, and this effect persists for 6 months after treatment.

Author Contributions

Conceptualization, Bohdanov V.K.; methodology, Bohdanov V.K.; formal analysis, Bohdanov V.K.; data curation, Bohdanov V.K.; writing—original draft preparation, Bohdanov V.K.; writing—review and editing, Bohdanov V.K. & Gushcha S.G.; supervision, Gushcha S.G.

All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Bioethics of Odesa National Medical University (31/2021, 31 May 2021).

Informed Consent Statement

All participants were informed about the details of the investigation.

DataAvailabilityStatement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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