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Interferons alpha and gamma, pidotimod, and tilorone in the treatment of acute respiratory infections in patients with allergic rhinitis: a prospective, cohort clinical and immunological study

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ABSTRACT

Aim. To compare the clinical efficacy and influence on interferon (IFN) production / sensing of drugs with immune-mediated antiviral effects, which potentiate type 1 (T1) immune responses, in the treatment of acute respiratory infections (ARI) in patients with allergic rhinitis.

Materials and methods. 146 ARI patients with remission of seasonal allergic rhinitis were divided into 4 cohorts. In addition to symptomatic therapy, patients received either 2,000 IU of IFNγ in each nasal passage 5 times a day; or rectal suppositories containing 10^6 IU of IFN-α2b and antioxidants (AO) twice a day; or gel with IFN-α2b and AO intranasally 3 times a day; or 400 mg of pidotimod *per os* twice a day; or 125 mg of tilorone *per os* on days 1, 2, 4, and 6. The severity of ARI was determined daily as the sum of 10-point scores for 15 symptoms. Serum concentrations of IFNα and IFNγ and the ability of blood cells to produce these cytokines *ex vivo* spontaneously and upon stimulation with Newcastle disease or phytohemagglutinin were studied using enzyme-linked immunosorbent assay (ELISA). The proportions of circulating lymphocytes expressing type I IFN receptor subunit 2 (CD118) or IFNγ receptor α-chain (CD119) were determined by flow cytometry.

Results. ARI symptoms in all cohorts generally regressed in a similar way. However, from day five of the treatment, pidotimod relieved symptoms more effectively than other drugs. In patients treated with tilorone, the regression of ARI manifestations was delayed in the first two to three days, followed by rapid symptom reduction. An initial decrease in the induced production of IFNγ was found in patients treated with pidotimod, and a tendency to a decrease in this parameter was noted in other cohorts. The induced production of IFNγ after the treatment in all groups did not differ from that in healthy donors. No significant changes and differences in the proportions of $CD118^+$ and $CD119^+$ lymphocytes were found between the cohorts, except for a decrease in the number of $CD118^+$ cells after the treatment with tilorone. In patients treated with IFN-α2b + AO, the proportions of $CD119^+$ and $CD118^+$ lymphocytes tended to increase slightly.

Conclusion. Drugs polarizing immune responses toward the Th1 type are a useful option for treating ARI in patients with allergic rhinitis.

Keywords: acute respiratory infections, interferon gamma, interferon alpha-2b, antioxidants, pidotimod, tilorone, interferon receptors, type 1 immune responses

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Интерфероны альфа и гамма, пидотимод и тилорон в лечении острых респираторных инфекций у пациентов с аллергическим ринитом: проспективное когортное клинико-иммунологическое исследование

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РЕЗЮМЕ

Цель – сравнить клиническую эффективность и влияние на выработку и рецепцию интерферонов (ИФН) препаратов с иммуноопосредованным противовирусным действием, потенцирующих иммунный ответ 1-го типа (Т1), в лечении острых респираторных инфекций (ОРИ) у пациентов с аллергическим ринитом.

Материалы и методы. Больные ОРИ (n = 146) с сезонным аллергическим ринитом в стадии ремиссии распределены на четыре когорты. Помимо симптоматической терапии пациенты получали либо 2 000 МЕ ИФН-γ в каждый носовой ход 5 раз/сут; либо ректальные свечи, содержащие 10^6 МЕ ИФН-α2b и антиоксиданты (АО), 2 раза/сут гель с ИФН-α2b и АО интраназально 3 раза/сут; либо 400 мг пидотимода *per os* 2 раза/сут; либо 125 мг тилорона *per os* в 1, 2, 4 и 6-е сут. Выраженность клинических проявлений ОРИ определяли ежедневно по сумме 10-балльных оценок 15 симптомов. Концентрации ИФН-α и ИФН-γ в сыворотке крови и способность клеток крови вырабатывать эти цитокины *ex vivo* спонтанно и при стимуляции вирусом болезни Ньюкасла или фитогемагглютинином изучали с помощью иммуноферментного анализа. Доли циркулирующих лимфоцитов, экспрессирующих субъединицу-2 рецептора ИФН I типа (CD118) или α-цепь рецептора ИФН-γ (CD119), определяли методом проточной цитофлуориметрии.

Результаты. Симптомы ОРИ во всех когортах регрессировали в целом сходным образом. Однако пидотимод с 5-х сут лечения купировал симптомы эффективнее других препаратов, а на фоне приема тилорона регрессия проявлений ОРИ задерживалась в первые 2–3 сут, после чего симптомы быстро угасали. Обнаружено исходное снижение индуцированной продукции ИФН-γ у пациентов, подлежащих лечению пидотимодом, и тенденция к уменьшению этого показателя в других когортах. После лечения индуцированная выработка ИФН-γ во всех группах не отличалась от таковой у здоровых доноров. Не установлено существенной динамики и отличий между группами по долям $CD118^+$- и $CD119^+$-лимфоцитов, за исключением снижения количества $CD118^+$-клеток на фоне приема тилорона. Лечение ИФН-α2b с АО вызывало незначительную тенденцию к увеличению доли $CD119^+$- и $CD118^+$-лимфоцитов.

Заключение. Препараты, поляризующие иммунный ответ в направлении Т2→Т1, являются полезной опцией в лечении ОРИ у больных с аллергическим ринитом.

Ключевые слова: острые респираторные инфекции, интерферон гамма, интерферон альфа-2b, антиоксиданты, пидотимод, тилорон, рецепторы интерферонов, иммунный ответ 1-го типа

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Исследование одобрено локальным этическим комитетом ЦНИИ эпидемиологии (протокол № 79 от 15.12.2017).

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INTRODUCTION

Treatment of acute respiratory viral infections (ARVI) remains largely the focus of discussions among scientists and doctors. The vast majority of used and studied etiotropic and pathogenetic drugs are far from the gold standard in this regard, especially in case of ARVI therapy in patients with concomitant allergic respiratory diseases. Allergic diseases and respiratory infections have a number of common and / or mutually potentiating links in their pathogenesis. Histamine, leukotrienes, prostaglandins, and many cytokines and chemokines are mediators of both allergic and infectious inflammation [1–5].

The development of allergic rhinitis and the most common phenotypes of other allergic respiratory diseases is associated with the predominance of type 2 (T2) immune response [6, 7]. At the same time, human rhinovirus, respiratory syncytial virus (RSV), and some other pathogens of ARVI polarize immune responses toward the Th2 type [8, 9]. Human rhinoviruses species A and B use the intercellular adhesion molecule 1 (ICAM-1) to enter the cell [10], and an increase in the expression of this molecule is an important link in the pathogenesis of respiratory allergic diseases [11, 12]. In addition, rhinoviruses themselves enhance the expression of ICAM-1 [13]. This does not include all the mechanisms by which respiratory viruses can provoke or aggravate allergic inflammation, and allergic respiratory disease can facilitate infection and create conditions for a more severe or atypical course of ARVI [14]. Patients with remission of allergic rhinitis retain minimal signs of persistent T2 inflammation, which makes them more sensitive to non-specific irritants [15] and *a priori* should affect the course of ARVI.

The aim of this study was to compare the clinical efficacy and effect on the production and sensing of type I and type II IFNs of several immunostimulants and drugs with immune-mediated antiviral effects, which are approved for clinical use in the Russian Federation for the treatment of acute respiratory infections (ARI) in patients with remission of concomitant allergic rhinitis.

Drugs were chosen as objects of this study due to their proven or implied ability to switch the balance of the prevailing immune response: T2→T1. Interferon-γ (IFN-γ) in nasal dosage forms was chosen as a key mediator of T1 immune responses and a stimulator of cellular antiviral defense [16]. IFN-α2b in topical and rectal dosage forms with added antioxidants was considered not only as the most important factor of antiviral innate immunity, but also as a molecule that potentiates the production of IFN-γ and suppresses the production of T2 cytokines [17, 18].

Pidotimod has proven to be effective in the prevention of respiratory infections [19], including patients with allergic respiratory diseases [20]. This synthetic dipeptide stimulated T cell immune responses, suppressed T2 inflammation, and increased the IFN-γ / interleukin (IL)-4 ratio in the serum of patients with respiratory allergies [21, 22].

The ability of tilorone to induce the production of type I IFNs has been known for more than 50 years [23]. Recently, it has been shown that this drug enhances (modulates) the production of IFN-γ, IFN-λ, and some other cytokines in intact animals and under experimental conditions of influenza *in vivo* [24, 25] and induces polarization of the immune response toward the T1 type [26].

MATERIALS AND METHODS

147 patients with ARI and remission of concomitant seasonal allergic rhinitis were followed up in City Polyclinic No. 180 of Moscow Healthcare Department, Scientific Advisory Clinical Diagnostic Center, and Clinical Department of Infectious Pathology of Central Research Institute of Epidemiology of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing

during the autumn – winter epidemic seasons of 2016–2019. The study was carried out in accordance with the requirements of the World Medical Association (WMA) Declaration of Helsinki "Ethical principles for medical research involving human subjects" as amended by the 52nd WMA General Assembly (2000) and "Rules of Clinical Practice in the Russian Federation" approved by the order of the Ministry of Health of the Russian Federation No. 266 dated June 19, 2003.

Inclusion criteria: clinical presentation of ARI within 48 hours after the onset of the first symptoms; history of seasonal allergic rhinitis verified at least 2 years ago; remission of allergic rhinitis; the level of immunoglobulins E (IgE) in the blood serum ≥ 100 IU / ml; age from 18 to 65 years; a voluntary informed consent to participate in scientific research.

Exclusion criteria: treatment with antiviral and immunomodulatory drugs within 1 month before therapy; the presence of ARI complications at the time of the initial visit; autoimmune diseases; chronic diseases of the cardiovascular system, gastrointestinal tract, and endocrine system, requiring medication intake during the study period; chronic obstructive pulmonary disease; tuberculosis; HIV infection; drug addiction; hypersensitivity to the components of drugs studied.

Withdrawal criteria: an allergic or other adverse reaction to any drugs manifested during the study; non-compliance with the frequency and dosage regimen; refusal of the patient to continue participating in the study.

Patients included in the study were divided into 4 cohorts (Table 1).

Table 1

Characteristics of the patients included in the study

Parameter		Patient groups				Statistically significant differences
Group number		1	2	3	4	
Treatment		IFN-γ	IFN-α + AO	Pidotimod	Tilorone	–
Number of patients		60	27	28	31	
Age, years, *Me* (Q_1–Q_3; *Min–Max*)		29 (26–38; 19–58)	46 (40–50; 27–62)	44 (40–50; 27–62)	29 (27–39; 18–57)	*
Sex	Men, *n* (%)	36 (60)	15 (56)	14 (50)	12 (39)	–
	Women, *n* (%)	24 (40)	12 (44)	14 (50)	19 (61)	
Erythrocyte sedimentation rate, mm / h, *Iu* (Q_1–Q_3; *Min–Max*)		6 (5–12; 2–25)	6 (4–9; 2–40)	10 (4–14; 1–24)	7 (2–5; 1–29)	–
Number of leukocytes in blood, ×10^3/mcl, Me (Q_1–Q_3; *Min–Max*)		6.7 (4.9–9.1; 3.1–13.1)	6.4 (5–7.7; 3.4–13.9)	6.5 (5.8–8.1; 3.1–12.8)	7.1 (5.7–9.8; 4.3–16.7)	–
The proportion of lymphocytes among blood leukocytes, %, *Me* (Q_1–Q_3; *Min–Max*)		36 (30–40; 11–48)	37 (30–44; 17–64)	36 (29–38; 14–64)	36 (30–40; 11–48)	–
Serum IgE concentration, IU / ml, *Me* (Q_1–Q_3; *Min–Max*)		297 (173–450; 102–822)	231 (159–417; 101–621)	218 (180–304; 100–598)	230 (185–394; 105–725)	–
Number of patients with identified ARI viruses, *n* (%)	Rhinoviruses	15 (25)	7 (26)	7 (25)	6 (19)	–
	Influenza A virus	19 (32)	10 (37)	9 (32)	10 (32)	–
	Influenza B virus	0	0	0	1 (3)	–
	Human parainfluenza viruses 1–4	4 (7)	1 (4)	1 (4)	1 (3)	–
	RSV	4 (7)	2 (7)	2 (7)	2 (6)	–
	Adenoviruses	5 (8)	2 (7)	1 (4)	4 (13)	–
	Coronaviruses	0	0	0	1 (1)	–
	Not identified	13 (22)	6 (22)	8 (29)	6 (19)	–

Note: RSV – respiratory syncytial virus.

* $p_{1,2,3,4} < 0.001$ (the Kruskal – Wallis test); $p_{1\text{-}2} < 0.001$, $p_{1\text{-}3} < 0.001$, $p_{2\text{-}4} < 0.001$, $p_{3\text{-}8} < 0.001$ (the Dunn's test).

In cohort 1 (IFN-γ group), the patients were treated with 2,000 IU of human recombinant IFN-γ (Pharmaclon, Russian Federation) in 2 drops of aqueous solution into each nasal passage 5 times a day for 7 days, which was a part of a complex therapy. In cohort 2 (IFN-α + antioxidants (AO) group), the patients received rectal suppositories containing 1 million IU of IFN-α2b, 0.055 g of alpha-tocopherol acetate, and 0.0081 g of ascorbic acid (Feron, Russian Federation) twice a day and a strip of gel 0.4–0.5 cm long, 1 gram of which contained 36,000 IU of IFN-α2b, 0.055 g of α-tocopherol acetate, 0.00128 g of benzoic acid, and 0.001 g of citric acid monohydrate (Feron, Russian Federation), 3 times a day in each nasal passage for 7 days. In cohort 3 (pidotimod group), the patients received 400 mg of pidotimod (Doppel Farmaceutici Srl, Italy) *per os* 2 times a day for 10 days. In cohort 4 (tilorone group), the patients received 125 mg of tilorone (Nizhpharm, Russian Federation) *per os* on days 1, 2, 4, and 6. In addition to these drugs, the patients received symptomatic treatment (irrigation procedures, decongestants, paracetamol at temperatures above 38.5°C).

All patients underwent a comprehensive examination, including history taking, physical examination, complete blood count, determination of the IgE level in the blood serum, verification of pathogens of respiratory infection, analysis of the interferon system and their receptors, and if necessary, clinical investigations (computed tomography of the sinuses, chest X-ray, electrocardiogram). Blood and nasopharyngeal samples for laboratory studies were taken in the first 48 hours from the onset of the disease before treatment and on day 7 of the treatment. All patients were followed up until complete recovery.

The main criterion (primary endpoint) for comparing the effectiveness of different treatment options was the influence of ARI clinical manifestations on regression. The overall severity of ARI was determined as the sum of 10-point scores for each of the symptoms listed below. Hyperthermia was assessed as follows: 1 point for body temperature ≥ 37°C; another 1 point for each additional temperature rise by 0.2°C; 10 points for any temperature ≥ 38.8°C. Other 14 symptoms and signs were assessed as follows: weakness, decreased appetite, nasal congestion, nasal discharge, itchy nose, itchy throat, sore throat, hoarseness, cough, sneezing, myalgia, headache, chest pain, and pain in the eyes. The results of the assessment of ARI clinical manifestations were recorded daily for 7 days in a specially developed individual diary, in which patients also had to register possible adverse events.

The pathogens of ARI in a nasopharyngeal swab were identified by the polymerase chain reaction using the AmpliSens ARVI-screen-FL and AmpliSens Influenza virus A/B-FL diagnostic test systems (Central Research Institute of Epidemiology, Russian Federation). Complete blood count was performed using a hematology analyzer (Beckman Coulter, USA) with determination of a standard set of parameters.

The ability of blood cells to produce IFN-α and IFN-γ *ex vivo* upon stimulation by the Kansas strain of the Newcastle disease virus (NDV) or phytohemagglutinin (PHA) (PanEco, Russian Federation), respectively, and without these stimuli was evaluated using the method proposed by S.S. Grigoryan et al. [27], as described earlier [28]. The levels of IFN-α and IFN-γ in the cell culture supernatant and blood serum were determined by ELISA using eBioscience kits (USA) and a fully-automated microplate reader Anthos 2020 (Anthos Labtec Instruments GmbH, Austria) at a wavelength of 450 nm with a correction at 620 nm.

The proportion of circulating lymphocytes expressing IFN-α / β receptor subunit 2 (CD118) and IFN-γ receptor α-chain (CD119) in peripheral blood was determined by flow cytometry on the EPICS XL cytometer (Beckman Coulter, USA) using PE-conjugated antibody to CD118 (Beckman Coulter, USA), PE-conjugated antibody to CD119 (eBioscience, USA), and BD FACS lysis solution (Becton Dickinson, USA) as described earlier [29]. Data from the analysis of biological samples collected from 30 healthy individuals comparable in gender and age with the studied population were used as a conditional norm of laboratory parameters.

A statistical analysis was carried out using the Statistica 18 software (StatSoft Inc., USA). Paired comparisons of independent and dependent samples in terms of quantitative characteristics were performed using the Mann – Whitney and Wilcoxon tests, respectively. The Kruskal – Wallis and Dunn's tests were used for multiple comparison of independent samples in terms of quantitative indicators. Independent groups were compared by nominal characteristics using the χ^2 test. All quantitative data

in the tables and in the figure are presented as *Me* (Q_1–Q_3; *Min–Max*), where *Me* is the median, Q_1 is the lower quartile, Q_3 is the upper quartile; *Min* is the minimum, *Max* is the maximum. The differences were considered statistically significant at $p < 0.05$. At $0.05 \leq p < 0.1$, a trend was claimed.

RESULTS

The cohorts of patients did not differ in the etiology of ARI, duration of infection during treatment, serum IgE level, erythrocyte sedimentation rate, and number of leukocytes and lymphocytes in peripheral blood. The groups were generally comparable in gender: a slight predominance of women in the tilorone cohort did not lead to statistically significant gender differences between the groups (Table 1). Sensitization to birch pollen allergens as a cause of seasonal allergic rhinitis, which was in remission during the study period, dominated in all cohorts. At the same time, patients in the IFN-α + AO and pidotimod groups were older than those in the IFN-γ and tilorone cohorts. Intergroup heterogeneity was also noted in terms of the initial severity of clinical manifestations of ARI. This was associated with a higher score for key symptoms of the disease in the tilorone cohort compared with patients who were prescribed IFN-γ and pidotimod (Fig. 1). The higher score in the tilorone group was mainly due to a high degree of myalgia, headache, decreased appetite, weakness, and eye pain. The groups did not differ significantly with respect to other ARI symptoms.

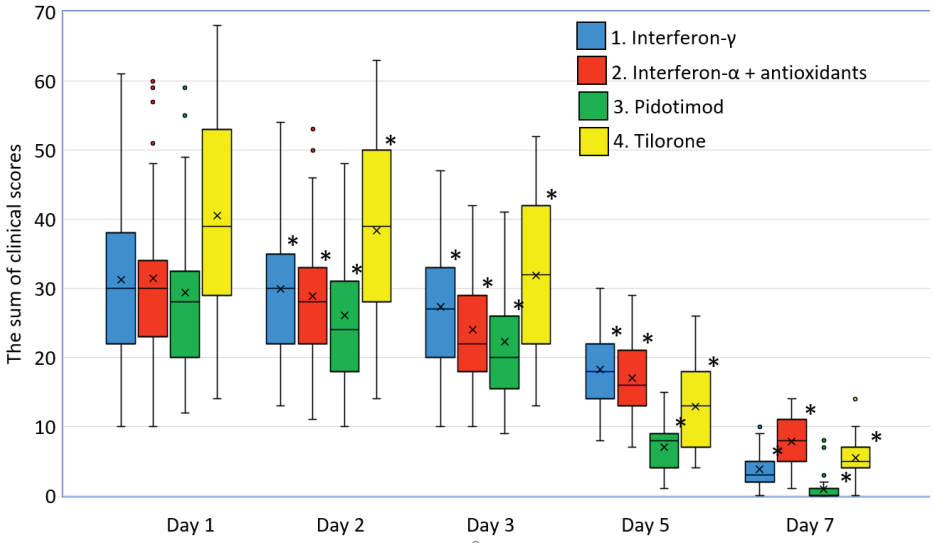

Figure. The dynamics of regression of ARI clinical manifestations in patients with concomitant allergic rhinitis who received different variants of antiviral therapy: × – mean values, points – outliers, *Me* (Q_1–Q_3; *Min–Max*)

* $p < 0.01$ compared with the parameters on the first day of the study (Wilcoxon test).

All the patients included in the study tolerated the treatment well. No cases of adverse events were noted. No patients were excluded or withdrawn from the study.

A statistically significant decrease in the total score for ARI clinical manifestations was observed in all the groups on day 2 of the treatment. At the same time, the severity of the disease in the cohort of patients treated with tilorone remained at a higher level compared with other groups (Figure, Table 2).

Subsequently, the main symptoms of the disease steadily regressed at approximately the same rate in patients with all the treatment options.

Table 2

Statistically significant intergroup differences		
Duration of the study, day	$p_{1,2,3,4}$ (the Kruskal – Wallis test)	The Dunn's test
1	0.008	$p_{1\text{-}4} = 0.039$, $p_{3\text{-}4} = 0.008$
2	0.001	$p_{1\text{-}4} = 0.047$, $p_{2\text{-}4} = 0.045$, $p_{3\text{-}4} = 0{,}001$
3	<0.001	$p_{2\text{-}4} = 0.032$, $p_{3\text{-}4} = 0.002$
5	<0.001	$p_{1\text{-}3} < 0.001$, $p_{1\text{-}4} = 0.002$, $p_{2\text{-}3} < 0{,}001$
7	<0.001	$p_{1\text{-}2} < 0.001$, $p_{1\text{-}3} < 0.001$, $p_{2\text{-}3} < 0.001$, $p_{3\text{-}4} < 0.001$

On day 5, the total score for patients in the tilorone group was lower than that in the IFN-γ cohort, and the pidotimod group had the highest regression rate of the main ARI symptoms. By day 7, the total score in the pidotimod group tended to zero and was significantly lower than in each of the other three groups, in which, despite a rapid decrease in the severity of symptoms, the residual ARI manifestations still persisted more clearly (Figure).

Since the pharmacological activity of all the studied drugs is largely associated with type I and type II IFN signals, we studied how the concentrations of IFN-α and IFN-γ in the blood serum changed as a result of different treatment options, the ability of blood cells to produce these cytokines *ex vivo*, and the proportion of peripheral blood lymphocytes expressing type I and type II IFN receptors.

A tendency toward an increase in the concentration of IFN-α in the blood serum of most ARI patients before the treatment was noted, and in the IFN-α + AO group, there was a significant increase in this parameter compared with healthy donors. The cohorts of patients were initially heterogeneous according to this criterion, as the level of IFN-α in the IFN-γ and pidotimod groups was lower than in the IFN-α + AO group. After the treatment, the concentration of IFN-α in the blood serum significantly decreased in patients of all groups and was in the range similar to the conditional norm (Table 3).

The content of IFN-α and IFN-γ in the supernatant of unstimulated peripheral blood cell cultures in the vast majority of cases (more than 75%) was below the detection limit in both healthy donors and patients with ARI. In addition, the sensitivity of the used test system was not enough to detect IFN-γ in the blood serum of more than 80% of patients and healthy people (data not provided).

NDV-induced production of IFN-α by blood cells *in vitro* in the patients from the IFN-γ, IFN-α + AO, and tilorone groups was initially higher than in healthy donors. In the pidotimod cohort, we only observed an upward trend for this parameter. No significant intergroup differences were detected before the treatment. After the treatment, the induced production of IFN-α in all the groups decreased; in the meantime, it was higher in the IFN-γ group than in the tilorone cohort and was at the level of a mathematically confirmed trend (Table 3).

Prior to the treatment, we revealed a decrease in the production of IFN-γ induced by PHA in the pidotimod group and a tendency toward a decrease in this parameter in other cohorts of patients. After the treatment, the induced production of IFN-γ increased to a level similar to the conditional norm, but this increase was statistically significant only in the IFN-α + AO and pidotimod groups. There were no mathematically confirmed intergroup differences both before and after the treatment (Table 3).

The proportion of lymphocytes expressing type I IFN receptor subunit 2 (CD118) in the peripheral blood in all groups of patients was initially higher than the conditional norm. After the treatment, a decrease in this parameter was observed in the tilorone group, which nevertheless remained at a higher level than in healthy donors. In other cohorts, the relative number of $CD118^+$ lymphocytes did not change.

When determining the proportion of lymphocytes expressing the IFN-γ receptor α-chain (CD119), no differences were found either between the groups of patients or between each of them and the conditional norm. Additionally, no significant changes were revealed in all the cohorts. At the same time, a slight upward trend in the proportion of $CD119^+$ and $CD118^+$ lymphocytes in the IFN-α + AO group was noted, that did not reach the level of the statistically confirmed trend (Table 3).

DISCUSSION

Higher baseline severity of ARI symptoms in the group of patients treated with tilorone makes it difficult to compare the clinical efficacy of this drug with that of other treatment options. However, a delay in the regression of symptoms in the first two to three days of the treatment was observed in the tilorone group, after which the clinical manifestations of ARI quickly faded away. This was probably due to the fact that tilorone at the first dose (on day 1) and second dose (on day 2) could act as an inducer of the production of not only IFNs of all types, but also other, mainly proinflammatory, cytokines. On the contrary, subsequent doses of this drug, taken on days 4 and 6 of the treatment could cause temporary hyporeactivity of IFN-producing cells and proinflammatory cytokines, which contributed to rapid relief of symptoms reflecting a local and systemic inflammatory response. In general, the features of the changes in the ARI clinical manifestations during the tilorone

Table 3

Changes in the parameters of production and sensing of types I and II IFNs in patients with ARI with concomitant allergic rhinitis who received different treatment options, *Me* (Q_1–Q_3; *Min–Max*)

Parameter	Healthy donors (conditional norm)	The duration of the study and the therapy option								Statistical significance of differences (multiple comparisons)#		Statistical significance of differences (paired comparisons)#
		Before the treatment				After the treatment						
		IFN-γ	IFN-α + AO	Pidotimod	Tilorone	IFN-γ	IFN-α + AO	Pidotimod	Tilorone			
		1	2	3	4	5	6	7	8	The Kruskal – Wallis test	The Dunn's test	The Wilcoxon test
Concentration of IFN-α in the blood serum, pg /ml	0 (0– 3.5; 0– 19.2)	0 (0–6.6; 0–37.8)	6.8 (2.2–9.9; 0–18.1)*	1.5 (0–5.6; 0–32.5)	0 (0–6.5; 0–37.9)	0 (0–1; 0-16)	0 (0–2; 0–6)	0 (0–1.8; 0–12.6)	0 (0–1; 0–8)	$p_{1,2,3,4} = 0.019$	$p_{1-2} = 0.044$; $p_{2-3} = 0.066$	$p_{1-5} = 0.002$; $p_{2-6} < 0.001$; $p_{3-7} = 0.016$; $p_{4-8} = 0.023$
IFN-α production *in vitro* induced by NDV, pg / ml	171 (75– 259; 20– 310)	252 (129–446; 0–670)*	213 (111–398; 8–600)*	183 (124–335; 4–647)	199 (108–328; 0–600)*	159 (104–252; 20–475)	151 (74–242; 20–271)	114 (46–182; 0–287)	143 (79–190; 29–407)	$p_{5,6,7,8} = 0.099$	$p_{5-8} = 0.081$	$p_{1-5} < 0.001$; $p_{2-6} = 0.006$; $p_{3-7} = 0.004$; $p_{4-8} = 0.012$
IFN-γ production *in vitro* induced by PHA, pg / ml	150 (56– 227; 6– 416)	72 (21–172; 0–745)	48 (12–123; 0–879)	29 (16–82; 0–879)*	79 (27–173; 0–1,369)	108 (22–188; 0–891)	174 (22–453; 0–1,364)	158 (19–444; 0–1,365)	129 (22–429; 0–1,385)	–	–	$p_{2-6} = 0.03$; $p_{3-7} = 0.003$
The proportion of $CD118^+$ lymphocytes in the blood, %	76 (68– 85; 60– 92)	90 (83–92; 63–97)*	91 (88–93; 78–94)*	94 (84–95; 74–98)*	92 (88–93; 77–94)*	89 (83–93; 68–97)*	92 (89–93; 81–97)*	89 (86–92; 76–96)*	89 (82–92; 66–95)*	$p_{1,2,3,4} = 0.024$	$p_{1-3} = 0.015$	$p_{4-8} = 0.036$
The proportion of $CD119^+$ lymphocytes in the blood, %	85 (80– 90; 61– 96)	85 (80–90; 6–96)	83 (79–89; 43–96)	88 (85–94; 60–96)	85 (80–87; 72–94)	85 (80–91; 75–97)	86 (84–90; 80–93)	87 (80–90; 58–93)	84 (81–89; 69–95)	–	–	–

Note: AO – antioxidants, NDV – Newcastle disease virus, PHA – phytohemagglutinin, # – the value of p is indicated only in cases when $p < 0.1$. * $p < 0.05$ compared with healthy donors (Mann – Whitney test).

treatment confirm the hypothesis about the mechanisms of clinical efficacy of tilorone, formulated earlier [30], and are consistent with the results of a recent study on the antiviral and cytokine-modulating activity of this drug in the model of influenza *in vivo* [25].

Conditional leadership of pidotimod in the relief of ARI symptoms, which manifested from day 5 of the study, can be partly associated with slightly lower initial severity of the disease in the group of patients who received this immunostimulant. Nevertheless, high effectiveness of pidotimod is of great interest because it is significantly further from classical antiviral agents than other comparison medicines used in this study in terms of the main mechanisms of the pharmacological action. Unlike pidotimod, tilorone, which has an immune-mediated antiviral effect, as well as IFN-γ and IFN-α2b with antioxidants, which are systematized as immunostimulants according to the anatomical, therapeutic, and chemical classification of drugs, are usually considered for etiotropic therapy of viral infections along with direct-acting antivirals. But it was pidotimod, whose effectiveness in the complex treatment of allergic rhinitis [31], asthma [22], as well as in the prevention of ARI [19] was previously proven, and which had a pronounced therapeutic effect in the acute phase of respiratory infection in patients with allergy in this study.

Probably, the use of a systemically acting immunostimulant that shifts the balance of the prevailing immune response in the T2→T1 direction was more important in terms of accelerating the relief of ARI symptoms for patients with concomitant allergic rhinitis than the use of drugs with a more pronounced antiviral effect. The synthetic dipeptide pidotimod like some immunomodulators of bacterial origin has immunoregulatory (anti-inflammatory or immune dampening) effects [32]. In this regard, it seems promising to continue studies of this drug not only as a stimulant of anti-infective defense, but also as a method of complex treatment of allergic rhinitis and other diseases accompanied by persistent inflammation in the respiratory tract.

Similar dynamics of regression of ARI clinical manifestations was observed in the groups of patients who received intranasally IFN-γ or a combination of topical and rectal dosage forms of IFN-α2b + AO. This result is interesting, since IFN-γ locally used a key mediator of the T1 response with proinflammatory activity[33] was comparable in clinical efficacy with a combination of systemic and topical dosage forms of IFN-α2b, a cytokine with a more pronounced antiviral effect and anti-inflammatory potential [34]. This is another indirect confirmation of a great importance of the T2→T1 immune response polarization for the regression of ARI clinical manifestations in patients with concomitant allergic rhinitis.

After IFN-α2 or another type I IFN binds two subunits of the corresponding receptor on the surface of the target cell and initiates biochemical cascades aimed at protecting against viruses, the ligand – receptor complex is internalized by endocytosis. This complex, when in the endosome, continues to exert biological (antiproliferative, immunomodulatory) effects for some time and only then undergoes lysosomal degradation [35]. However, the signals leading to the production of IFN-stimulated virostatic proteins are transduced through type I IFN receptor mainly when it is located on the cell surface. The ability of different type I IFNs to carry the receptor inside the cell correlates with the degree of affinity of the ligand – receptor interaction [36]. IFN-α2 is characterized by high affinity for type I IFN receptor [37], second only to IFN-β in this respect [38].

The interaction of IFN-γ with the receptor also finally leads to internalization and intracellular degradation of the ligand – receptor complex [39]. IFN-γ decreases the expression of its receptor in target cells by mechanisms independent of endocytosis [40]. Type I IFNs are also capable of suppressing the expression of IFN-γ receptors both as a result of blocking the transcription of the α-chain gene in this receptor [41] and secondarily due to stimulation of IFN-γ production [17], leading to the above-mentioned ligand-induced mechanisms for reducing sensitivity to IFN-γ.

In theory, these features of type I and type II IFN signal transduction could lead to a temporary decrease in the number of both types of IFN receptors on the plasmalemma of different cells (including circulating lymphocytes) in patients treated with systemically acting IFN-α2b. This could reduce the effectiveness of the natural antiviral mechanisms dependent on type I and type II IFN. The results of this study disavow this assumption. An upward trend in the proportion of lymphocytes expressing

type I IFN receptor subunit 2 and IFN-γ receptor α-chain was revealed on the last day of the 7-day treatment in the IFN-α + AO cohort and was not observed in other groups. This can be explained by the recirculation of internalized receptors [39] and / or the presence of previously unidentified positive feedback mechanisms leading to the restoration of the number of receptors after a ligand-induced decrease in their density on the surface of target cells. The results obtained are consistent with the data that it is IFN-α2b, but not IFN-β, that stimulates the recirculation of the internalized type I IFN receptor subunit 2 to the cell surface [42].

CONCLUSION

Generally similar clinical efficacy of IFN-γ in the nasal dosage form, the combination of rectal and nasal dosage forms of IFN-α + AO, pidotimod, and tilorone in the treatment of ARI in patients with allergic rhinitis was established. The results of this study allow to make a conclusion that drugs capable of polarizing the immune response in the T2→T1 direction are a useful option for the treatment of ARI in patients with concomitant allergic respiratory diseases. In this context, the choice of all the drugs investigated in this work should be recognized as justified. From the data obtained, a rational vector for the development of new effective agents for the pathogen-specific (etiotropic) treatment of ARVI in patients with respiratory allergy emerges: the search for natural and synthetic pharmacological substances that have both antiviral and T2→T1 properties.

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