INFLUENCE OF ALPHA-GLUTAMIL-TRYPTOPHAN ON THE BACKGROUND AND INDUCED ACTIVITY OF FACTORS OF ADAPTIVE IMMUNITY FOR PREVENTION

Ruleva A.A. [1],
Krasnov A.A. [2],
Petlenko S.V. [3],
Zaplutanov V.A. [4],
Apryatina V.A. [5]

[1] Pediatric Research and Clinical Center for Infectious Diseases, Federal Medical Biological Agency (Professora Popova str. 9, Saint Petersburg 197022, Russian Federation)
[2] Herzen State Pedagogical University (Embankment of the Moika River 48, Saint Petersburg 191186, Russian Federation)
[3] Golikov Research Center of Toxicology (Bekhtereva str. 1, Saint-Petersburg 192019, Russian Federation)
[4] St. Petersburg Institute of Bioregulation and Gerontology (Dinamo Ave. 3, Saint Petersburg 197110, Russian Federation)
[5] Institute of Translational Biomedicine, St. Petersburg State University (Universitetskaya emb. 7–9, Saint Petersburg 199034, Russian Federation)

Corresponding author:
Anna A. Ruleva,
e-mail: Ruleanna@yandex.ru

ABSTRACT

Background. *During the ongoing COVID-19 pandemic and in the season of rising incidence of other respiratory infections, it is relevant to use preventive measures of non-specific prophylaxis. Synthetic peptides are widely considered as a tool. The representative of this group is the synthetic analogue of thymus regulatory peptides Thymogen, which has been used in Russia for more than 20 years in the treatment of acute and chronic infection diseases.*

The aim of the study. *To evaluate the effect of Thymogen, a dosed nasal spray, on induced parameters of the immune system during prophylactic use in healthy volunteers.*

Materials and methods. *Twenty healthy volunteers received Thymogen nasal dosed spray (OOO Cytomed, Russia) at a dose of 25 μg twice a day for 10 days. A comparative assessment of immunological parameters was carried out in dynamics: before the start of therapy, on days 6 and 11 of taking the drug and 14 days after the end of the course. Clinical observation was carried out from day 1 to day 11, registration of adverse events – the entire period of the study for 24 days.*

Results. *A statistically significant increase in virus-induced α-interferon production by blood cell culture on day 11 of Thymogen administration was revealed. This effect persisted for another 14 days after the end of the course. No statistically significant differences in the dynamics of bactericidal and phagocytic activity of neutrophils, serum α- and γ-interferon were observed.*

Conclusion. *The use of the Thymogen spray preparation at a dose of 25 μg for 10 days was considered safe, did not affect the morphofunctional state of the immune system, but promoted a statistically significant increase in the production of α-interferon in response to the inducing effect of the in vitro viral pathogen. As a result, the preparation can be recommended for prophylactic use during the period of high incidence in acute respiratory infections.*

Key words: *alfa-glutamyl-tryptophan, interferon-alpha, viral infections, healthy volunteers, non-specific prophylaxis, factors of innate immunity*

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ВЛИЯНИЕ АЛЬФА-ГЛУТАМИЛ-ТРИПТОФАНА НА ФОНОВУЮ И ИНДУЦИРОВАННУЮ АКТИВНОСТЬ ФАКТОРОВ ВРОЖДЁННОГО ИММУНИТЕТА ПРИ ПРОФИЛАКТИЧЕСКОМ ПРИМЕНЕНИИ

Рулева А.А. [1],
Краснов А.А. [2],
Петленко С.В. [3],
Заплутанов В.А. [4],
Апрятина В.А. [5]

[1] ФГБУ Детский научно-клинический центр инфекционных болезней ФМБА России (197022, г. Санкт-Петербург, ул. Профессора Попова, 9, Россия)
[2] ФГБОУ ВО «Российский государственный педагогический университет им. А.И. Герцена» (191186, г. Санкт-Петербург, набережная реки Мойки, 48, Россия)
[3] ФГБУ «Научно-клинический центр токсикологии имени академика С.Н. Голикова Федерального медико-биологического агентства» (192019, г. Санкт-Петербург, ул. Бехтерева, 1, Россия)
[4] АННО ВО НИЦ «Санкт-Петербургский институт биорегуляции и геронтологии» (197110, г. Санкт-Петербург, просп. Динамо, 3, Россия)
[5] Институт трансляционной биомедицины, ФГБОУ ВО «Санкт-Петербургский государственный университет» (199034, г. Санкт-Петербург, Университетская наб., 7–9, Россия)

Автор, ответственный за переписку:
Рулева Анна Александровна,
e-mail: Ruleanna@yandex.ru

РЕЗЮМЕ

В период продолжающейся пандемии COVID-19 и в сезон подъёма заболеваемости другими респираторными инфекциями остаётся актуальным использование неспецифической профилактики. В этой связи в качестве инструмента широко применяются синтетические пептиды. Представителем этой группы является синтетический аналог регуляторных пептидов вилочковой железы Тимоген, который применяется в России уже более 20 лет для лечения острых и хронических инфекционных заболеваний.

Цель исследования. *Оценить действие препарата Тимоген спрей на индуцированные показатели иммунной системы при профилактическом применении у здоровых добровольцев.*

Материалы и методы. *20 здоровых добровольцев получали препарат Тимоген спрей (АО «МБНПК «Цитомед», Россия) в дозе 25 мкг 2 раза в сутки в течение 10 дней. Иммунологические показатели оценивали в динамике: до начала терапии, на 6-й и 11-й дни приёма и через 14 дней после окончания курса. Клиническое наблюдение осуществляли с 1-го по 11-й дни, регистрацию нежелательных явлений – в течение всего периода исследования (24 дня).*

Результаты исследования. *Выявлено статистически значимое увеличение вирус-индуцированной продукции α-интерферона культурой клеток крови к 11-му дню приёма Тимогена. Этот эффект сохранялся ещё в течение 14 дней после окончания курса. Статистически значимых различий в динамике бактерицидной и фагоцитарной активности нейтрофилов, сывороточного α- и γ-интерферона не получено.*

Заключение. *Использование препарата Тимоген спрей в дозе 25 мкг в течение 10 дней было безопасным, не влияло на морфофункциональное состояние иммунной системы, но способствовало статистически значимому увеличению продукции α-интерферона в ответ на индуцирующее воздействие вирусного патогена in vitro. Это позволяет рекомендовать препарат для профилактического применения в период подъёма заболеваемости острыми респираторными инфекциями.*

Ключевые слова: *альфа-глутамил-триптофан, альфа-интерферон, вирусные инфекции, здоровые добровольцы, неспецифическая профилактика, факторы врожденного иммунитета*

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INTRODUCTION

Innate immunity is one of the most important factors determining the outcome of infection. Essentially all cells can participate in its realisation. The main structures responsible for innate immunity are monocytes, macrophages, dendritic cells (DCs), neutrophils and NK (natural killers) cells [1–4]. Innate immune cells carry receptors (PRR, pattern recognition receptors) such as Toll-like receptors (TLR), RIG-I-like receptors (RLR), NOD-like receptors (NLR), C-type lectin superfamily (CLSF), recognizing nonspecific structures of microorganisms (PAMP, pathogen-associated molecular patterns) [2, 3, 5]. After PRR binding to the ligand, intracellular biochemical cascades are triggered, leading to cellular activation. Activated cells initiate phagocytosis, secretion of reactive oxygen species and a number of cytokines (tumour necrosis factor α (TNF-α), interleukin (IL) 1β, IL-6, IFN (interferon) type 1), which in turn induce inflammation and other antiviral responses [2–4]. Phagocytosis is a multi-step process initiated by pathogen recognition that leads to pathogen uptake, phagosome maturation to eliminate the pathogen and then phagolysosome disintegration and pathogen inactivation [6].

The response of NK cells includes cytotoxicity and the release of cytokines (TNF-α and IFN-γ), which is closely related to the activation of receptors that activate NK cells and the blocking of inhibitory receptors on their surface [5]. The balance of these receptors' engagement protects normal cells from the harmful effects of NK cells while activating them to destroy virus-infected target cells [1, 5].

A key mechanism of innate immunity is the production of IFN type 1. It represents a highly optimised systemic response that provides a first line of defense against a wide range of viral infections. Failure to generate an effective IFN response against the virus leads to chronic infection, while excess IFN production leads to autoaggression [7]. Natural and recombinant interferons are among the most widely used biological therapeutic immunotropic agents in the world [8].

Currently, three major families of IFNs are known – types 1, 2 and 3. IFNs of type I (IFN-α/β) and 3 (IFN-λ) are primarily produced as the first line of defense and can be produced by most cell types, while IFNs of type 2 (IFN-γ) are mainly produced by secondary specialized immune subpopulations (e.g., NK cells or T cells) [2, 9]. Consequently, genetic determination of IFN type 1 and 3 synthesis is critical for controlling the susceptibility and course of viral infections. These IFNs are induced by a variety of systems including dozens of recognition receptors such as RIG-1, MDA-5 (melanoma differentiation-associated protein 5), TLR, RLR, STING (stimulator of interferon genes) [9]. It is also generally acknowledged that type 1 IFN production is induced by interaction with bacterial receptor ligands of innate immune cells. Furthermore, interferons activate higher-order processes (cellular activity, proliferation, differentiation and T cell function) that are critical for controlling viral infections. Deficiencies in the production or signalling of IFN-1 or IFN-3 nor in the antiviral proteins they induce are closely associated with the severe course of the disease [9].

Indeed, genetic deficiencies associated with the induction of IFN-α as well as IFN-neutralising autoantibodies have been identified in individuals with severe COVID-19 [10]. These correlations have epidemiologic significance. For example, 1.5 % of severe COVID-19 cases can be attributed to a specific TLR deficiency, while about 10 % of people with severe COVID-19 have autoantibodies against one or more type 1 or type 3 IFNs. In addition, ISG OAS1 allelic variants also predict the severity of COVID-19. These data definitively indicate that interferons are indispensable for the control of SARS-CoV-2 and prevention of severe COVID-19, as well as other respiratory viral infections (particularly influenza), where, due to the peculiarities of pathogens, disturbances in the system of immune response regulation are possible [11].

Thus, the innate immune response involves a coordinated chain of induced gene products, pre-formed immune effectors, biochemical signalling cascades and specialised cells [2, 12]. An important mechanism in the antiviral defense chain is the regulation of interferon production, and the degree of this induction allows predicting the course and outcome of infectious diseases, primarily of viral nature. Excessive activation of nocifensors can cause the development of pathological processes. A balance between pathogen defense and pathophysiological manifestations is important [13]. In fact, the presence of prolonged hyperinterferonemia during prophylactic use of interferon inducers (and some of this group of drugs can be used for up to 4 weeks or more) in the absence of a pathogen may contribute exclusively to the development of side effects, of which this group of cytokines (IFNs) has a huge number (from intestinal dysfunction and "flu-like state" to the development of "chronic fatigue and immune dysfunction syndrome" and dementia) [1, 14, 15]. Consequently, in our opinion, it is much more reasonable and effective, pathogenetically justified and safe to use drugs that do not induce but regulate the endogenous interferon synthesis in compliance with the needs and condition of the organism in order to prevent infectious diseases.

Synthetic peptides have been considered as a tool for regulation and activation of the interferon system by a number of studies. Key transcriptional peptide factors involved in the regulation of the immune response, such as NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells), JAK-STAT (Janus kinase – signal transducer and activator of transcription) and IRFs (interferon regulatory factors), were originally discovered in bony fishes. The critical regulators of type 1 IFNs are IRF3 and IRF7, while IRF1, IRF5 and IRF8 trigger IFN responses in a cell-specific manner [16]. In the work of R. Pandey et al. [17], devoted to the development of immunoprophylaxis methods for visceral leishmaniasis, it was revealed that individual synthetic peptides or their mixtures significantly activated IFN-γ secretion. This was confirmed by an increase in intracellular cytokines with a significant increase in IFN-γ produced by $CD4^+$ T cells.

Since 2003, a synthetic dipeptide identical to the natural compound isolated by chromatographic method from thymus extract, the drug Thymogen, has been registered and used in Russia [18]. One of the main points underlying the present study was to investigate the immunologic mechanisms underlying the prophylactic effect of the preparation against acute and chronic viral and bacterial diseases of the upper respiratory tract in healthy volunteers.

In this academic research work, the key objective was to evaluate the effect of Thymogen in relation to baseline and induced immune system parameters during prophylactic use in healthy volunteers. Bactericidal and phagocytic activity of neutrophil granulocytes, spontaneous and virus-induced production of IFN-α by peripheral blood cells, and the content of IFN-α and IFN-γ in serum were studied. We also analysed the safety and tolerability of the studied drug in healthy volunteers during its prophylactic administration for 10 days. The terms of the study – from June 04, 2021, to July 05, 2021.

MATERIALS AND METHODS

A total of 20 healthy male volunteers aged between 18 and 40 years participated in the study. The inclusion criteria were absence of acute and exacerbation of chronic diseases at least 1 month before inclusion in the study; any vaccination at least 6 months before the commencement of the study.

The study preparation was Thymogen, nasal dosed spray (manufacturer — Medical and Biological Research and Production Complex OOO Cytomed, Russia). The active substance is alpha-glutamyl-tryptophan (Thymogen sodium in terms of Thymogen) 25 μg/dose. Excipients: sodium chloride 900 μg; benzalkonium chloride 10 mcg; water, purified to 0.1 ml.

The study was approved by the independent ethical committee 'BioEthics' (Protocol No. 156 dated May 27, 2021), Saint Petersburg, Russia.

All subjects included in the study received Thymogen spray according to a single regimen at a dose of 25 μg (1 injection) into each nasal passage 2 times a day in the morning and evening for 10 days. After completion of the course of preparation administration, the volunteers were monitored for another 14 days. Complaints, life and medical history, data related to the use of medications, and physical examination were collected before commencement of drug administration as well as at each medical surveillance.

Physical examination included measurement of vital signs: systolic (SBP) and diastolic (DBP) blood pressure, heart rate (HR), respiratory rate (RR), body temperature (BT). To measure vital signs, we used certified instruments (ASG, Japan; Armed YX200, Russia) designed for use in clinical trials. The total drug administration and observation period for each volunteer was 24 days and included 4 visits (day 1 – before drug administration; day 6, day 11; day 24), during which a complete physical examination and blood collection for laboratory diagnosis were performed.

Immunological examination included determination of bactericidal activity of peripheral blood neutrophil granulocytes (according to the NBT-test (nitroblue tetrazolium reduction test) spontaneous and induced by zymosan) by spectrometry on a spectrophotometer (Infinite F50; Austria); assessment of phagocytic activity of peripheral blood neutrophil granulocytes (percent of neutrophils that absorbed yeast; phagocytic index; completion of phagocytosis) by light microscopy (Leica DM LS2; Leica, USA); assessment of spontaneous and induced IFN-α production and the content of IFN-α and IFN-γ in serum by immunoenzyme method (commercial kits of OOO Vector Best, Russia). Spontaneous and Newcastle disease virus-induced IFN-α production was determined in supernatants of daily whole blood culture. The bactericidal stimulation index was calculated as the ratio of induced bactericidality to spontaneous bactericidality; the phagocytosis index was calculated as the average number of yeasts absorbed by one phagocyte. Phagocytosis completion was calculated as the ratio of phagocytic indices in samples with and without the addition of fetal serum.

Safety and tolerability of Thymogen was assessed by the frequency of adverse events (AEs) and serious adverse events (SAEs) according to the tolerability grading: "good" – absence of AEs; "satisfactory" – presence of mild AEs not requiring medication correction of the condition; "unsatisfactory" – presence of several AEs requiring medication treatment. Also within the framework of safety assessment we studied the dynamics of vital signs: SBP and DBP, HR, RR, BT. Tolerability was assessed based on subjective assessment by volunteers and by maintaining adherence to the study drug regimen (compliance).

Statistical analysis

Statistical data processing was performed using specialized software Statgraphics Centurion 18, version 18.1.12 (Statgraphics Technologies, Inc., USA) and RStudio, version 1.1.442 (RStudio PBC, USA), Rmisc packages (version 1.5), psych (version 2.1.6), nortest (version 1.0-4). To test the normality of the compared samples, the Lilliefors test was applied with a critical value of $p = 0.2$. Comparisons between related groups were performed using one-way ANOVA with repeated measures. The sphericity of the data was analyzed using Mauchly's test with a statistical significance level of 95 %. If the distribution of the parameter in the group deviated from normal, the nonparametric Friedman test was applied. The obtained data are presented as mean (M), standard deviation (σ), standard error of the mean (SE) or median (Me), quartile deviations (Q25%; Q75%) or interquartile range (IQR) (Q25%–Q75%), minimum (min) and maximum (max) values. The critical p value was considered to be 0,05.

In case differences between groups (at different time points) were revealed, posterior (post-hoc) tests for pairwise comparison were used to determine them: Fisher's

method after analysis of variance, Bonferroni criterion after Friedman's test with adjustments for multiple comparisons (with a power of 95 %).

The object of research

The mean age of the volunteers included in the study was 28.25 ± 6.69 years, with a minimum age of 18 years and a maximum age of 40 years. The group was homogeneous in terms of demographics and anthropometrics. A full course of the drug was provided to 17 volunteers and an incomplete course to 3 of them. All 20 study subjects were included in the analyses.

RESULTS

Assessment of spontaneous and induced IFN-α production under the influence of the drug

When the spontaneous production of IFN-α by daily culture of cells isolated from the peripheral blood of volunteers was examined on day 1 (Me = 5 pg/ml; IQR = 2.75 pg/ml) before drug administration compared to the values on day 6 (Me = 5 pg/ml; IQR = 0 pg/ml) (p = 0.026), statistically significant differences were found; however, these values were similar and such differences are not clinically significant (Table 1).

When Newcastle disease virus-induced IFN-α production by whole blood cells was assessed, statistically significant differences in this parameter were observed on days 1 and 11, on days 1 and 24, and between days 6 and 24 (F = 31.7; p = 0.000000604317) (Fig. 1).

In the analysis of the study results, a statistically significant increase in induced IFN-α production by blood cells during prophylactic administration of Thymogen for a course of 10 days was observed by day 11 of follow-up (day 11: Me = 390.5 pg/ml, IQR = 290.75 pg/ml; day 1: Me = 194.5 pg/ml, IQR = 149.25 pg/ml; $p < 0.001$). Furthermore, a statistically significant ($p < 0.001$) increase in this index 14 days after the end of the course (day 24: Me = 550.5 pg/ml; IQR = 190.25 pg/ml) compared to day 1 (Me = 194.5 pg/ml; IQR = 149.25 pg/ml) and day 6 (Me = 223.5 pg/ml; IQR = 164.5 pg/ml) was also revealed (Fig. 1).

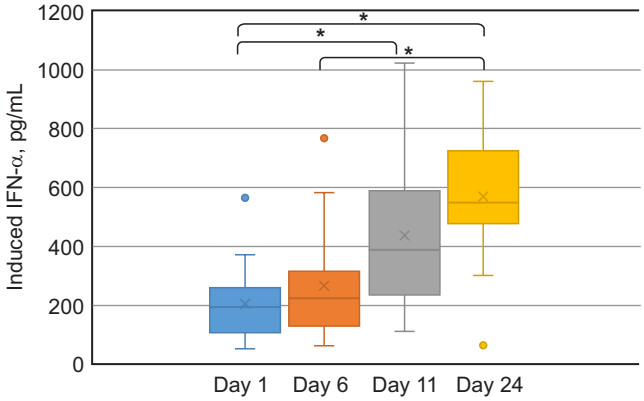

FIG. 1.

*Virus-induced production of IFN-α by daily culture of whole blood from volunteers in dynamics during 10-day administration of Thymogen spray (n = 20): * – statistically significant differences ($p < 0.001$), Friedman's nonparametric test; day 1, 6, 11 and 24 – control points of the study*

Accordingly, the present study reveals that virus-induced IFN-α production, reflecting one of the mechanisms of increasing the organism's immunity to viral pathogens, statistically significantly increases by the end of the drug administration (after 10 days) and continues to increase during 2 weeks of further follow-up. Consequently, prophylactic use of Thymogen in the spray form allows activating the ability of peripheral blood cells to produce IFN-α in response to viral pathogen exposure, and this effect persists for at least 14 days after the end of the course of the studied drug application.

Results of serum IFN-α and IFN-γ assessment

Comparison of IFN-α levels in the serum of healthy volunteers during prophylactic administration of Thymogen revealed statistically significant differences in this parameter on days 6, 11 and 24, despite the fact that the serum IFN-α levels were similar in absolute values (Me = 5 pg/ml; IQR = 0 pg/ml) compared to the values on day 1 (Me = 5 pg/ml; IQR = 2.25 pg/ml; p=0.0004), however, this change was not clinically significant (Table 1).

TABLE 1

SPONTANEOUS PRODUCTION OF IFN-α BY DAILY WHOLE BLOOD CULTURE

Indicators	Reference values	Control points	Me	Q25%	Q75%	min	max
IFN-α spontaneous, pg/ml	3–30	Day 1	5	5	7.75	5	19
		Day 6	5*	5	5	5	5
		Day 11	5	5	5	5	15
		Day 24	5	5	5	5	13

Note. * – statistically significant differences compared to the initial value ($p < 0.05$); Me – median; Q25%, Q75% – 25th and 75th quartiles, respectively; min – minimum; max – maximum.

Examination of IFN-γ in serum revealed no statistically significant differences in all controls ($p > 0.05$). The index did not change throughout the study (Me = 2 pg/ml; IQR = 0 pg/ml) (Table 2).

Results of the assessment of phagocytic and bactericidal activity

According to the results obtained, no statistically significant differences in the dynamics of such parameter as neutrophil bactericidal activity, including spontaneous and induced, were revealed in the present study. The indices had no statistically significant variations in the study controls (Table 3).

Similar results were obtained when studying the parameters of neutrophil phagocytic activity (Table 4), including neutrophil phagocytosis, phagocytosis completion and phagocytosis index.

The present study reveals that in healthy volunteers without immune system disorders and in the absence of acute diseases / conditions prophylactic use of Thymogen spray 2 times a day for 10 days does not affect the parameters of bactericidal activity of neutrophil granulocytes of peripheral blood, determined by NBT-test, and does not lead to changes in the phagocytic activity of neutrophils.

Analysis of the safety and tolerability

As part of the safety and tolerability analysis, vital signs were evaluated in dynamics: SBP, DBP, HR, RR and BT, which did not reveal statistically significant differences ($p > 0.05$) in healthy volunteers throughout the entire follow-up period (i.e. at the control points of the study – days 1, 6, 11 and 24) (Table 5).

In 11 (55 %) volunteers at different control points of the study, there were observed episodes of blood pressure rise, and in all cases the increase in SBP and / or DBP did not exceed 10 mmHg compared to the values taken as reference. The recorded abnormalities were classified by the authors as clinically insignificant, not requiring therapeutic measures and not being associated with the use of Thymogen.

No SAEs were observed during the study conducted. Among the recorded AEs, mucous nasal discharge ($n = 1$) and nasal congestion ($n = 1$) were observed on days 1–10 of the study, which resolved independently. Mucous discharge was observed in the mornings within 1 h after Thymogen administration and nasal congestion was observed 20 min after taking the drug within 3–4 h, which were considered by the authors to be drug-related conditions. The AEs were of mild severity. No clinical manifestations of allergic reactions, such as urticaria, rash, anaphylactic reactions, were observed during the use of the drug.

Thus, the obtained data about prophylactic use of Thymogen spray 2 times a day over a 10-day period indicate satisfactory tolerability of the drug, its safety and absence of adverse effects on the main vital signs.

DISCUSSION

According to the results obtained in the course of the study, a statistically significant increase in virus-induced IFN-α production by daily culture of peripheral blood cells was revealed after 10 days of Thymogen administration and continued growth of this indicator for 14 days after the end of the course. Consequently, prophylactic use of Thymogen spray increases the ability of peripheral blood cells to produce IFN-α in response to viral pathogen exposure during the period of drug administration and for at least 2 weeks afterwards. This mechanism of biologi-

TABLE 2
SERUM IFN-α AND IFN-γ LEVELS

Indicators	Reference values	Control points	Me	Q25%	Q75%	min	max
IFN-α serum, pg/ml	0–5	Day 1	5	2.75	5	1	5
		Day 6	5*	5	5	5	5
		Day 11	5*	5	5	5	5
		Day 24	5*	5	5	5	5
IFN-γ serum, pg/ml	0–5	Day 1	2	2	2	2	2
		Day 6	2	2	2	2	22
		Day 11	2	2	2	2	2
		Day 24	2	2	2	2	2

Note. * – statistically significant differences compared to the initial value ($p < 0.05$); Me – median; Q25%, Q75% – 25th and 75th quartiles, respectively; min – minimum; max – maximum.

TABLE 3

RESULTS OF ASSESSMENT OF NEUTROPHIL BACTERICIDAL ACTIVITY IN THE DYNAMICS OF THE STUDY

Indicators	Reference values	Control points	Me	Q25%	Q75%	min	max
Spontaneous neutrophil bactericidality, units/million cells	70–120	Day 1	98	74.25	112.5	68	116
		Day 6	93	85	104	62	124
		Day 11	92	87.25	102	71	124
		Day 24	88	82	98	68	116
Induced neutrophil bactericidality, units/million cells	150–200	Day 1	140	116	177.2	94	202
		Day 6	152	138	169.8	84	198
		Day 11	145	131.5	170.8	88	198
		Day 24	156.5	139.8	167.5	102	195
Stimulation index	1,2–2	Day 1	1.55	1.3	1.725	1.2	1.8
		Day 6	1.6	1.4	1.8	1.3	1.9
		Day 11	1.6	1.5	1.725	1.2	1.9
		Day 24	1.8	1.575	1.8	1.4	1.9

Note. Me – median; Q25%, Q75% – 25th and 75th quartiles, respectively; min – minimum; max – maximum.

TABLE 4

RESULTS OF ASSESSMENT OF NEUTROPHILS PHAGOCYTIC ACTIVITY IN THE DYNAMICS OF THE STUDY

Indicators	Reference values	Control points	Me	Q25%	Q75%	min	max
Neutrophil phagocytosis, %	65–88	Day 1	68	66	68	65	69
		Day 6	67.5	66	68	65	69
		Day 11	68	66	68	65	69
		Day 24	68	67	68	65	69
Completion of phagocytosis, coefficient	1–1,2	Day 1	1	1	1	0.9	1
		Day 6	1	1	1	0.9	1
		Day 11	1	1	1	0.9	1
		Day 24	1	1	1	1	1
Phagocytosis index	2,3–3	Day 1	2.3	2.3	2.4	2.2	2.8
		Day 6	2.3	2.3	2.4	2.2	2.7
		Day 11	2.3	2.3	2.4	2.2	2.6
		Day 24	2.3	2.3	2.4	2.2	2.5

Note. Me – median; Q25%, Q75% – 25th and 75th quartiles, respectively; min – minimum; max – maximum.

TABLE 5
DYNAMICS OF VITAL SIGNS DURING THE STUDY

Indicators	Reference values	Control points	M	σ	min	max
SBP, mmHg	110–135	Day 1	128.2	7.47	115	145
		Day 6	126.45	7.24	110	139
		Day 11	125.3	8.09	104	138
		Day 24	126.0	7.28	115	139
DBP, mmHg	60–85	Day 1	79.15	5.58	70	90
		Day 6	78.8	7.61	65	94
		Day 11	77.6	7.88	64	94
		Day 24	77.1	6.82	64	93
HR, bpm	60–90	Day 1	75.6	11.55	64	89
		Day 6	73.05	8.15	49	102
		Day 11	73.95	8.89	56	90
		Day 24	74.3	8.93	55	92
RR, respirations/min	up to 22	Day 1	16.05	0.94	14	18
		Day 6	16.2	1.06	14	18
		Day 11	15.9	0.85	14	18
		Day 24	16.4	1.05	14	18
BT, °C	35.5–36.9	Day 1	36.19	0.24	35.8	36.7
		Day 6	36.26	0.33	35.7	36.8
		Day 11	36.26	0.36	35.5	36.8
		Day 24	36.16	0.42	35.0	36.7

Note. M – mean value; σ – standard deviation; min – minimum; max – maximum.

cal activity should be considered effective in the prevention and treatment of respiratory viral infections of any etiology, and especially those whose immunopathogenesis is accompanied by inhibition of endogenous interferon synthesis, in particular COVID-19.

The study reveals that serum levels of IFN-α and IFN-γ remained at background levels throughout the study period.

The results obtained in healthy volunteers are consistent with the results of previous studies, in which it was revealed that Thymogen does not have the ability to stimulate a significant amount of endogenous interferon, which is associated with its mechanism of action, and is rather a regulator of this process, and to a greater extent against the background of primary antigenic induction [18].

Other authors note that Thymogen is an inducer of the production of endogenous interferon, mainly alpha and beta fractions. Specifically, in an *in vivo* study, Thymogen was administered subcutaneously to white mice and interferon levels in serum, lungs, and brain were assessed [19]. According to the results obtained, after already 2 hours the concentration of IFN in serum reached 10–20 IU/ml, persisted up to 4 h and decreased to zero a day after the drug administration.

Most likely, the different findings are related to the difference in the assessment time of endogenous interferon production – in the first day and in the distant period which are subject to significant changes in the normal course of the infectious process, as well as to the type of ob-

ject studied – animals or humans. In a number of drug study observations, the effects in animals often do not replicate the effects in humans.

Statistically significant changes in bactericidal activity of neutrophil granulocytes and phagocytic activity of neutrophils in healthy volunteers were not observed during the study, which suggests that in the absence of an infectious agent the drug does not change the background values, does not cause hyperactivation of protective factors of immunity, being activated only in the presence of a pathogen.

It should be noted that there are scientific studies confirming high local antiviral activity of Thymogen spray due to antiseptic action in suppressing the infectivity of SARS-CoV-2 virus [20] in patients with coronavirus infection. Besides, in earlier scientific studies devoted to examining the dynamics of immunological parameters in patients it was revealed that against the background of acute infectious diseases, i.e. in conditions of pre-formed pathogen exposure or other acute conditions, the use of Thymogen contributed to a faster recovery of reduced bactericidal activity of neutrophils determined by NBT-test. And this has been demonstrated for patients with severe mechanical trauma compared to patients receiving conventional treatment [21], as well as for patients with chronic pyoderma [22]. As concerns phagocytosis, previous studies have provided data about the stimulating effect of Thymogen in patients with chronic generalised periodontitis [23].

In the course of the study, it was also revealed that prophylactic use of Thymogen in the form of spray 2 times a day over a period of 10 days had no significant effect on the main vital signs of the organism. The diagnosed AEs were of mild severity and resolved independently. No allergic reactions to the administration of the drug were observed. Neither were there any episodes of study drug withdrawal initiated by the surveyors and study subjects. The data obtained confirm good tolerability and safety of Thymogen during its prophylactic use.

CONCLUSION

A statistically significant increase in virus-induced INF-α production by daily culture of peripheral blood cells in healthy volunteers was revealed by the study. The observed effect of the drug Thymogen, nasal dosed spray was persisted during the whole period of its administration – 10 days, and 14 days after the completion of the course. No statistically significant changes in neutrophil granulocyte bactericidal activity (including spontaneous and induced bactericidality) and neutrophil phagocytic activity (including neutrophil phagocytosis, phagocytosis completion and phagocytosis index) were observed during the study. The obtained data about the ability of the drug Thymogen to activate the reactions of innate immunity, involving the IFN system, deserve attention and further study of the mechanisms of its action.

Conflict of interest

The authors of this article declare no conflicts of interest.

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Information about the authors

Anna A. Ruleva – Cand. Sc. (Med.), Research Officer at the Department of Vaccinal Prevention and Postvaccinal Pathology, Pediatric Research and Clinical Center for Infectious Diseases, Federal Medical Biological Agency, e-mail: ruleanna@yandex.ru, https://orcid.org/0000-0002-5888-6313

Alexey A. Krasnov – Dr. Sc. (Med.), Associate Professor at the Department of the Medical Valeological Subjects, Herzen State Pedagogical University, e-mail: dr.krasnov_28@mail.ru, https://orcid.org/0000-0002-8732-6390

Sergey V. Petlenko – Dr. Sc. (Med.), Leading Research Officer, Golikov Research Center of Toxicology, e-mail: petlenko@mail.ru, https://orcid.org/0000-0002-2752-4598

Vasiliy A. Zaplutanov – Research Officer at the Laboratory of Peptide Chemistry of the Department of Biological Gerontology, St. Petersburg Institute of Bioregulation and Gerontology, e-mail: vasiliy@zaplutanov.ru, https://orcid.org/0000-0001-5294-6533

Vera A. Apryatina – Cand. Sc. (Med.), Senior Research Officer at the Laboratory of Neurobiology and Molecular Pharmacology, Institute of Translational Biomedicine, St. Petersburg State University, e-mail: vera1577@mail.ru, https://orcid.org/0000-0002-9819-6835