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Thermovaccination – thermoheliox as a stimulator of the immune response. Kinetics of the synthesis of antibodies and C-reactive protein in coronavirus infection

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ABSTRACT

Clinical trials of thermoheliox application (inhalation with a high-temperature mixture of oxygen and helium, 90 °C) in the treatment of the acute phase of coronavirus infection were conducted. Dynamics of disease development in infected patients (PCR test for the virus) and, dynamics of changes in blood concentration of C-reactive protein, immunoglobulin M, specific immunoglobulin G were studied. High efficiency of thermoheliox in releasing the organism from the virus and stimulating the immune response (thermovaccination effect) was shown. The kinetic model of the process is proposed and analyzed.

1. Introduction

The fight against coronavirus infection is an extremely acute problem, requiring the development of new methods that expand the range of possibilities of modern therapy and prevention. Currently used antiviral and general strengthening therapeutic agents are often not sufficiently effective and lead to deep damage to many body systems and often death of the patient. High hopes are placed on the development of synthetic vaccines that act at the level of antibody synthesis and specifically interact with certain virus proteins.

We have developed a new method to fight coronaviral lesions based on the use of thermoheliox (inhalation with a high-temperature mixture of helium and oxygen). Clinical trials were conducted at the intensive care unit of N.V. Sklifosovsky Federal Research Institute of Emergency Medicine. Patients with medium and high severity coronaviral lesions (50–75% of lung lesions) were admitted to the clinic. The age of the patients ranged from 35 to 75 years old, and most of them were over 55 years old. The vast majority of patients had concomitant diseases: arterial hypertension of varying degrees, chronic obstructive bronchitis, chronic obstructive pulmonary disease, coronary heart disease, atherosclerosis of the lower extremities, bronchial asthma, stomach ulcer, and others. The results of the clinical trials are extremely positive. As a rule, on 2–3 days of thermoheliox application with the medical apparatus "Heliox Extreme" (LLC "MedTechInnovations"), the virus detected by standard PCR-methods, is destroyed in most patients, and a stable antibody response appears. These effects have been predicted on the basis of a kinetic model that includes the dynamics of virus growth and reproduction in the body, the dynamics of the virus affecting recipient cells, the effects of thermal destruction of viruses and the dynamics of an antibody response. Theoretical analysis predicts the potential effects of thermovaccination, i.e., production of antibodies to proteins of destroyed viral particles [1–3].

On the basis of the study of the protein composition of the patient's exhaled air condensate, the safety of the thermoheliox application has

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been shown [4,5]. We conducted an experimental study of the kinetics of specific antibody accumulation in patients affected by coronavirus. The study included 65 patients divided into two equal groups. Both groups were treated using a standard approved protocol. ELISA testing helps to identify the disease when approximately 2 weeks have passed since the moment of infection and the virus has penetrated into the lungs. In combination with CT diagnostics, ELISA allows quick identification of changes in the lungs and application of the necessary therapy.

2. Materials and methods

Swabs from the nasopharynx and oropharynx for the detection of coronavirus RNA SARSCoV-2 by polymerase chain reaction (PCR) testing (detection amplifier CFX-96 "REAL TIME" (Bio-Rad, USA)) [6–8], computed tomography (CT) of lungs and venous blood sampling for the content of immunoglobulins IgG (quantitative) and IgM (quantitative) to coronavirus SARS-CoV-2 according to standard enzyme-linked immunosorbent assay (ELISA) technique (immunochemiluminescent analyzer, Mindray 6000, USA) [8] were carried out in all patients.

The kinetic model is based on the kinetic equations describing the growth and evolution of microbial and viral populations [9–12]. The ordinary differential equation system was integrated using a specially developed program (in Delphi Community Edition).

3. Results and discussions

Thermoheliox as a therapeutic agent in the treatment of an acute form of coronaviral infection. Clinical application of thermoheliox in the N.V. Sklyfosovsky Clinic (Moscow) showed the effective influence of inhalations with a high-temperature thermoheliox on disease development. Fig. 1 shows data on virus detection using a standard PCR test in patients with thermoheliox treatment and in control group patients (without thermoheliox). The use of a thermoheliox significantly accelerates the release of the organism from the virus. For some patients, a single inhalation is enough to completely destroy the virus in the body.

Two types of kinetic behavior of a system of the antibody and C-reactive protein (CRP) synthesis – two stages of immune response development. Of interest is the study of the kinetics of the immune response development in patients in the control (reference) group. Traditional antiviral therapy without the use of inhalation of a high-temperature mixture of helium and oxygen was used for treatment. The kinetic regularities that we observed for patients in this group are basic for the treatment of patients affected by respiratory viral infection.

The dynamics of IgG and IgM accumulation as well as the dynamics

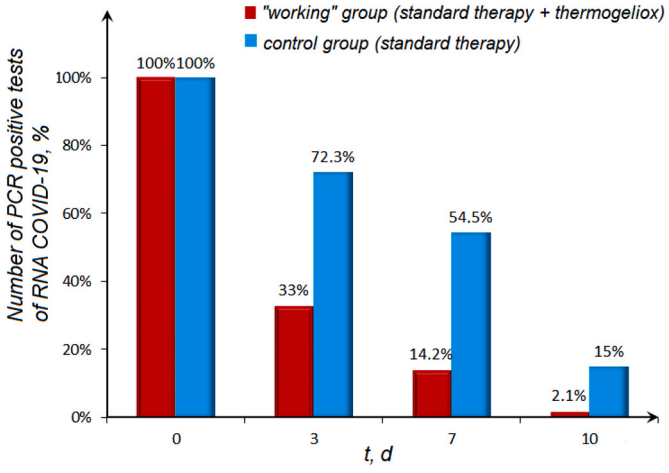

Fig. 1. The number of PCR positive tests in the control and "working" groups (*p ≤ 0.05).

of growth and elimination of CRP, are complex and are personalized for each patient. This reflects the complex nature of the immune response mechanisms and the polymorphism of the proteins involved in this process [12]. A simplified scheme of the processes during the development of the immune response can be presented in Fig. 2.

However, a detailed analysis of the process kinetics reveals two fundamentally different types of kinetic behavior. The large share of kinetic curves (the greater share of patients, approximately 60%) has a complicated characterization of IgG synthesis with an induction period on the kinetic curve of antibody accumulation (Fig. 3).

In the initial period of time, the rate of antibody accumulation was close to zero, and only for 3–4 days did the system gain a high rate of IgG concentration growth (behavior of type A, Fig. 3). The other group of patients is characterized by zero levels of IgG at the initial time. However, the rate of antibody accumulation at the initial time is very high (behavior of type B, Fig. 4). The share of patients with kinetic behavior type B is approximately 40%.

Similarly, two types of kinetic behavior are traced in the analysis of the dynamics of changes in the concentration of C-reactive protein in the blood. Kinetic curves of changes in the blood of patients and the concentration of CRP with kinetic behavior of type A are presented in Fig. 5. At a relatively low level of CRP at the initial moment of time the system dynamically gains a high protein concentration. Furthermore the concentration of CRP decreases rather quickly to almost zero. The share of patients with dynamics of CRP of this type (type A) is 60–65%.

A significant proportion of the patients (35–40%) are admitted to the clinic with already high levels of CRP. On kinetic curves, the dynamics of protein concentration growth are not observed, and the process is characterized by an exponential decrease in its concentration (Fig. 6). For patients with this type of kinetic behavior, the process of CRP accumulation was passed rather quickly and they were admitted to the clinic at the stage when the process of accumulation had already ended.

Comparison of Figs. 3 and 5, 4 and 6 shows that the period of induction of IgG accumulation over time correlates with the time of CRP accumulation, and the IgG concentration growth process correlates with the CRP consumption process (Figs. 5 and 6).

Influence of thermoheliox on IgG synthesis kinetics and kinetic behavior of CRP. For patients for that use thermoheliox (inhalations 4 times a day for 15 min with 15 min breaks, 40% oxygen, 60% helium, 90 °C), the kinetic responses of the immune system are fundamentally different. Thermoheliox accelerates the synthesis of CRP and IgG. Fig. 7 shows the kinetic curves of IgG accumulation during therapy with thermoheliox. The induction period disappears for all patients based on IgG growth kinetics. A single thermoheliox procedure is enough for antibody synthesis to start at an initial high rate.

Similarly, the effect of thermoheliox on the kinetics of a decrease in CRP concentration in the patient's blood, i.e., the process of CRP accumulation is accelerated, and the slow process of its accumulation at the initial stage disappears (Fig. 8). For more than 80% of patients on the kinetic curves of changes in CRP concentration the process of its accumulation is not fixed, and the initial concentration of CRP is high. However, it should be noted that for 15–20% of patients, the process of CRP accumulation is observed.

It is also interesting to note that approximately 20–30% of patients with already high levels of antibodies are admitted to the clinic. The IgG level is maintained at a high level during the whole period of the patient's stay in the clinic.

Kinetics of immunoglobulin M synthesis. We investigated the kinetic regularities of IgM synthesis in the "working" and control groups (Figs. 9 and 10).

Thermoheliox also stimulates the formation of IgM. The differences in the kinetics of IgM accumulation in the "working" and control groups are not as significant as in the case of IgG and CRP. Thermoheliox noticeably stimulates IgM accumulation in the initial stage of the process. For example, the average value of IgM(3)/IgM(0) for all patients who have inhaled thermoheliox is 4.1, and for the control group, it is

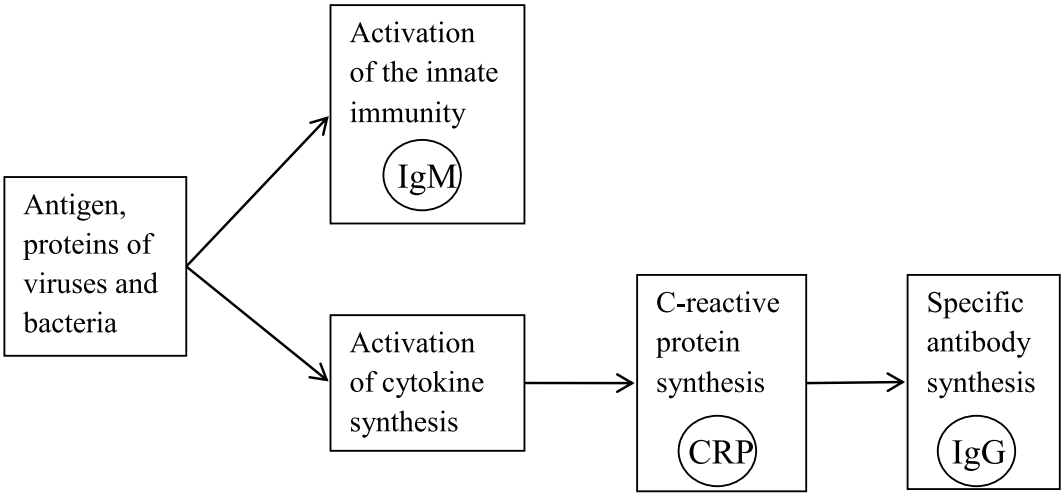

Fig. 2. Scheme of the processes during the development of the immune response. The circles mark the components, the concentrations of which were measured in this study.

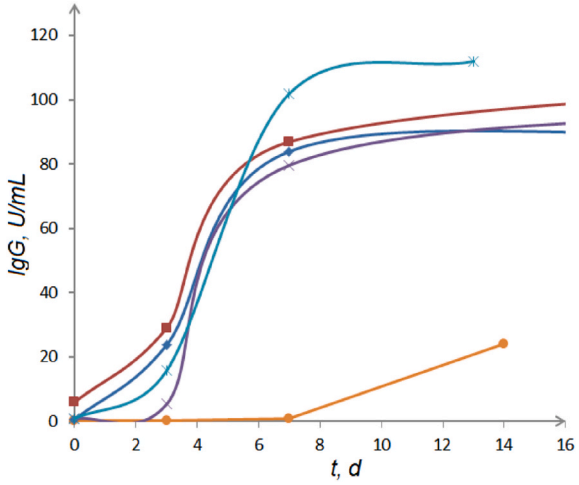

Fig. 3. Dynamics of the synthesis of IgG antibodies (type A) for the control group with traditional treatment.

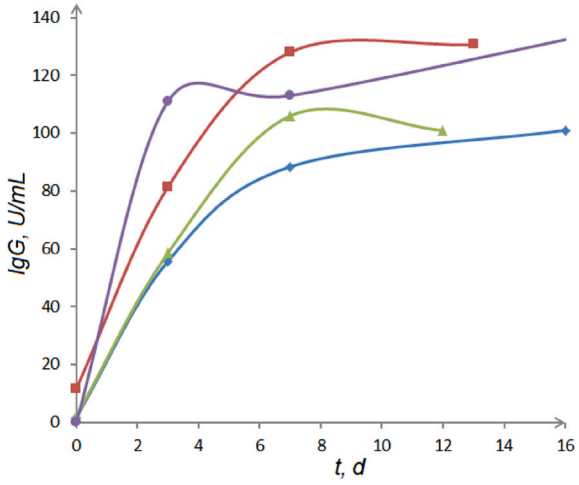

Fig. 4. Dynamics of the synthesis of IgG antibodies (type B) for the control group with traditional treatment.

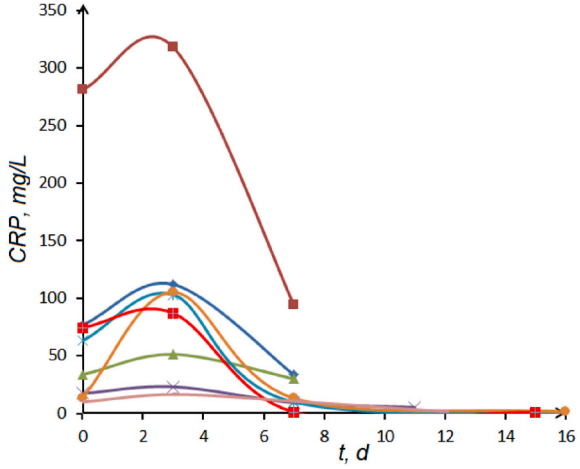

Fig. 5. Curves of changes in the concentration of CRP in the blood of patients in the control group (type A).

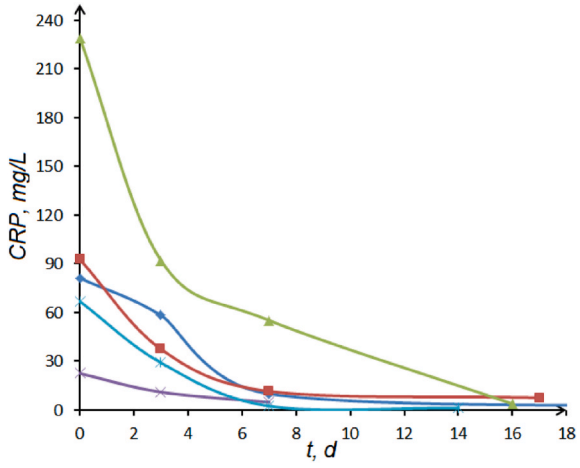

Fig. 6. Changes in the concentration of CRP in the blood of patients in the control group (type B).

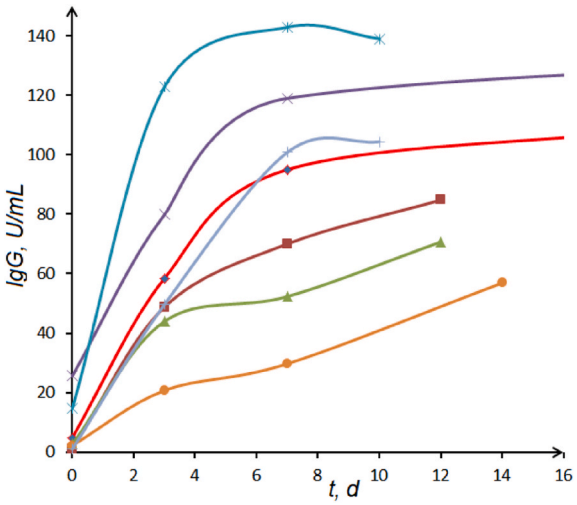

Fig. 7. Dynamics of accumulation of IgG antibodies for the "working" group during treatment with thermoheliox.

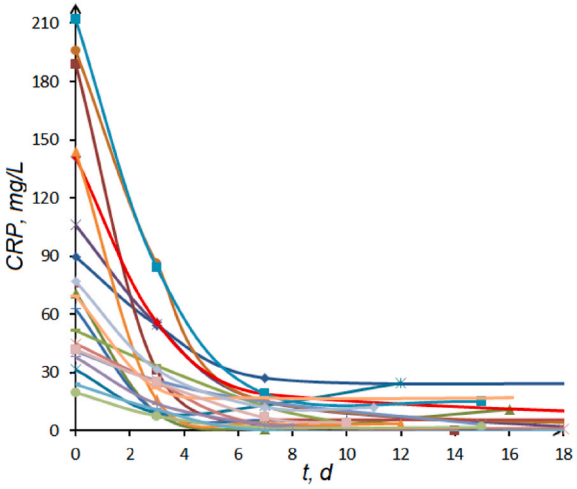

Fig. 8. Curves of changes in the concentration of CRP for the "working" group during treatment with thermoheliox.

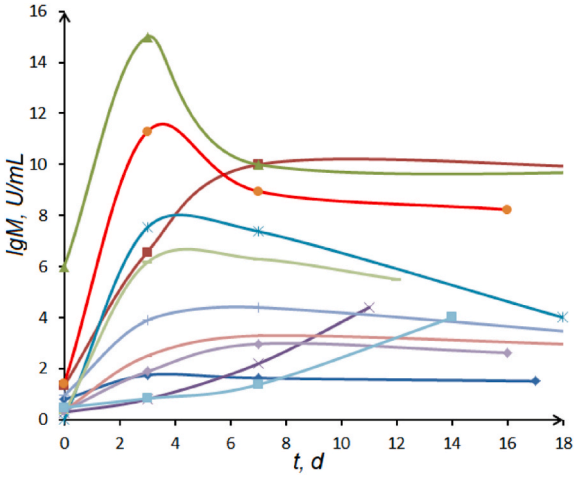

Fig. 9. Dynamics of the synthesis of immunoglobulin IgM in the control group.

3.2.

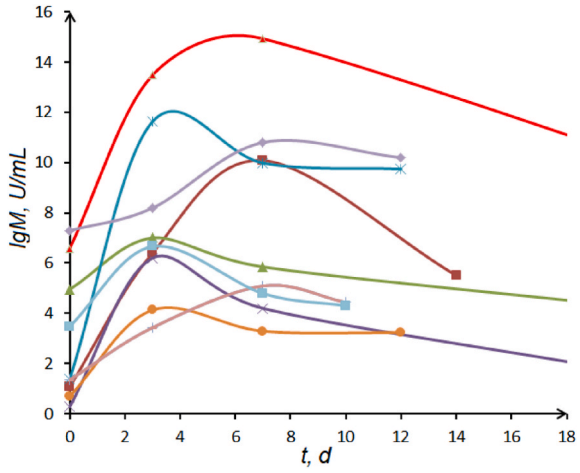

Fig. 10. Dynamics of the synthesis of immunoglobulin IgM in the "working" group in therapy with thermoheliox.

Formal kinetic description of the immune response kinetics. A quantitative estimation of time parameters for the synthesis of antibodies and CRP within the framework of formal kinetic empirical approximation seems expedient. Visual analysis of the kinetic curves of growth, synthesis and elimination of IgG and CRP shows that the process kinetics can be described by a combination of exponential functions. Therefore, the kinetics of IgG accumulation when using thermoheliox is adequately described by the function:

$$G_B(t) = A_0\left(1 - e^{-k_g t}\right) \quad (1)$$

where A_0 is the limit of IgG accumulation and k_g is the empirical rate constant of synthesis (see Fig. 7).

The elimination process of CRP (type B) is well described by the function

$$C_B(t) = B_0 e^{-k_c t} \quad (2)$$

where k_c is the empirical rate constant for CRP elimination (see Fig. 8).

The more complex dynamics of the process, including the stage of increasing the CRP concentration, can be represented by the function:

$$C_A(t) = C_0\left(e^{-k_m(t+\Delta t)} - e^{-k_c(t+\Delta t)}\right) \quad (3)$$

where k_c is the characteristic of the rate of CRP accumulation and k_m is the characteristic of protein elimination. $t + \Delta t$ is the time of development of CRP synthesis, including period Δt, which reflects the time until

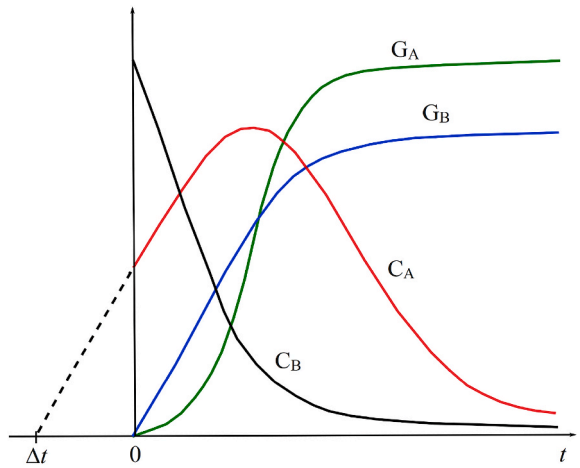

Fig. 11. Kinetic curves of the main process components (C_A, C_B, G_A, and G_B).

the patient is admitted to the clinic (see Fig. 5).

Fig. 11 shows the kinetic curves that reflect the dynamic behavior of the main experimentally determined process components.

The experimentally obtained set of kinetic data allows the estimation of the kinetic parameters of the process, despite the complex, polymorphic nature of patients participating in the therapeutic process. With a limited number of experimental points in time (0, 3, 7, and discharge), we used the difference method for determining constants (Guggenheim method) [13,14]. The rate constant (the parameter characterizing the exponent) is determined by the equation

$$k = \frac{\ln(y_j/y_i)}{t_j - t_i} \tag{4}$$

where t_i and t_j are the times at which the values of the studied variables y_j and y_i were determined. Therefore, for fixed times 0, 3, and 7 and the time of discharge, the number of defined values of constants is 6, which allows us to calculate the average value and the standard deviation (dispersion) from the average value. This operation was performed for all G_B and C_B kinetic curves. The values of the antibody accumulation rate constants and CRP elimination rate constants are almost the same:

$$\begin{aligned} k_g &= 0.25 \pm 0.08 s^{-1} \\ k_c &= 0.24 \pm 0.09 s^{-1} \end{aligned} \tag{5}$$

The same parameter characterizes the exponential antibody accumulation process for type B patients without using thermoheliox:

$$k_g = 0.19 \pm 0.08 s^{-1} \tag{6}$$

The data obtained show that the stages of IgG synthesis and CRP elimination are limited by the same kinetic process.

Experimental data allow us to determine the period Δt in the analysis of the CRP accumulation kinetics (equation (3), describing $C_A(t)$). The decomposition of the first exponential term into the series using the first decomposition term (linear function) makes it possible to determine the value of Δt (a segment cut off by a derivative at point 0) on the t axis (see Fig. 11).

$$\Delta t = \frac{y(0)(t_2 - t_1)}{y_2 - y(0)} d$$

In the first approximation we can take $t_2 - t_1 = 3$ days. In this case

$$\Delta t = \frac{y(0) \times 3}{y(3) - y(0)} d$$

Using this equation to analyze the data presented in Fig. 5, we have

$$\Delta t = 8.7 \pm 3.5 d$$

Thus, the analysis of kinetic data on the synthesis of IgG and CRP based on empirical approximation suggests that the inflammatory immunological process with CRP synthesis for many patients begins on average 8 days before the patient is admitted to the clinic.

The obtained experimental data and the analysis performed demonstrate the following facts:

1) The kinetics of IgG-specific antibody synthesis has an induction period (lag phase) during which the rate of antibody synthesis increases from zero to the maximum possible rate. This induction period is observed for 2–4 days for most patients undergoing traditional antiviral therapy.
2) The use of therapy with thermoheliox activates the IgG synthesis process and eliminates the induction period.
3) Thermoheliox activates CRP synthesis, accelerating the stage of accumulation of this protein, which plays a crucial role in the inflammatory and immune response of the body.
4) The processes of synthesis and elimination of CRP and synthesis of specific IgG antibodies are kinetically correlated. The dynamics of these parameters are limited by the same biochemical stages.

Kinetic model for the synthesis of antibodies and C-reactive protein. Experimental studies of antibody synthesis dynamics and formal-kinetic descriptions of the process serve as a basis for kinetic modeling of the dynamic behavior of the system within the framework of mathematical models, the basis of which is described in [1–3].

Let us consider the simplest kinetic scheme and the corresponding system of differential equations connecting all main measured variables of the system. The discussed kinetic model allows us to include all main processes in the description:

- Dynamics of virus concentration growth in the body and inhibition of growth by specific IgG antibodies.
- Dynamics of antigen accumulation (or a group of antigens), including a description of the "jump" in the antigen concentration caused by the thermal destruction of the virus. Thermoheliox clearly destroys the virus and this follows from a PCR analysis of the dynamics of virus elimination from the body. When the virus is destroyed (or inactivated), the products of its thermal inactivation (partially destroyed RNA and thermally destroyed protein) should be formed, that is, "weakened forms of pathogen" should be formed in the terms of Louis Pasteur.
- The kinetic model must describe the accumulation and elimination of C-reactive protein.
- Dynamics of IgG accumulation as a response to antigen growth. In the simplest case, the rate of IgG accumulation should be related to the CRP concentration, since the kinetics of changes in the concentration of IgG and CRP are correlated (see the section "Formal kinetic description of the immune response kinetics").

The rate of change in virus N concentration can be represented by the equation:

$$\frac{dN}{dt} = k_1 N - \alpha(T) N - \xi N G, \tag{7}$$

where the first term reflects the exponential nature of virus reproduction. The rate of virus inactivation, including thermal destruction, describes the term (–α(*T*)*N*). The virus destruction by interaction with antibodies is described by the term (– ξ*N*·*G*), where *G* is the concentration of antibodies.

The dynamics of the change in antigen concentration is described by the equation

$$\frac{da}{dt} = \alpha(T) N - \chi \cdot a \tag{8}$$

The synthesis and elimination rate of C-reactive protein includes the stage of synthesis dependent on antigen concentration and the stage of its elimination by interaction with antibodies.

$$\frac{dc}{dt} = k_c a - k_g c \cdot G \tag{9}$$

The introduction of a negative term ($- k_g c \cdot G$) into equation (1) does not mean that C-reactive protein is removed by forming complex with IgG. It means that the accumulation of antibodies indirectly increases the rate of CRP elimination.

The above analysis shows that the dynamics of antibody synthesis (*G*) and the dynamics of changes in the concentration of CRP (*c*) are correlated. This is reflected in

$$\frac{dG}{dt} = \sigma \cdot c, \tag{10}$$

and in equations (7) and (9).

The kinetic model adequately describes the dynamics of the observed processes and allows us to trace the dynamics of processes in the variation of various parameters, including α(*T*), χ, k_c k_g and σ. A fundamentally important parameter that characterizes the rate and depth of

the immune response is the parameter σ. As the experimental data demonstrate (see Figs. 3 and 10), the specific kinetics of the immune response is quite variable, and while maintaining the law of development, t differs in rate and depth of response. The computational calculations of the kinetic model (7)–(10) were performed with the following values of the parameters: $k_1 = 0.021\ h^{-1}$, $\alpha = 0.006\ h^{-1}$, $\xi = 0.08\ h^{-1}$, $\chi = 0.02\ h^{-1}$, $\sigma = 0.02\ h^{-1}$, $k_c = 0.24\ s^{-1}$, $k_g = 0.25\ s^{-1}$ (using thermoheliox), $N(0) = c(0) = 10^{-3}$ mM/L, and $a(0) = G(0) = 0$ mM/L. This is a reflection of the molecular polymorphism of human proteins [12]. Figs. 12–15 illustrate the dynamics of the process with variation in the rate of antibody synthesis (the parameter σ, possibly different in different patients). Fig. 12 presents the theoretical dependences of changes in the CRP concentration, which are solutions of equations(7)–(10).

For patients of type A, the process of CRP accumulation is deployed in time. Theoretically and experimentally maximums on kinetic curves of changes in CRP concentration are observed. The "jump" of antigen concentration (Fig. 13), caused by the action of the high temperature of the thermoheliox causes accelerated synthesis of CRP, and dynamics of the process passes to category B (exponential decrease of CRP concentration).

The dynamic behavior of CRP leads to the unambiguous behavior of the IgG synthesis system.

In the absence of therapy with thermoheliox, IgG synthesis develops over time and has a well-identifiable induction period (Fig. 14, type A). Thermal jump antigen concentration significantly accelerates CRP synthesis. IgG accumulation curves are exponential functions with saturation (Fig. 15, type B). It is important to note that in the framework of the mathematical model, the half-transformation times in the reaction of IgG synthesis and CRP elimination coincide (compare Figs. 13 and 15). This is consistent with the experimental data (see the section "Formal-kinetic description of the immune response kinetics"). Thermoheliox and the "jump" of the antigen concentration remove the induction period in IgG synthesis and accelerate CRP synthesis.

4. Conclusions

The experimental materials presented in this article and the conducted analysis reveal the nature of the therapeutic effect of thermoheliox in the treatment of coronaviral infection. High-temperature thermoheliox (90 °C) intensifies thermodestruction of the virus in the lungs and the circulatory system as a whole. This leads to an increase in the concentration of the antigen of destroyed proteins of the virus. This process stimulates the synthesis of CRP and specific IgG antibodies. On its molecular and immunological basis, the use of thermoheliox is similar to the introduction into the organism of destroyed and inactivated cells or viruses ("weakened pathogen", Pasteur vaccination). In the case of thermoheliox, this is achieved by inactivating the virus in vivo.

For the first time, we discovered and described the effects of "thermovaccination" in the treatment of coronaviral lesions – stimulation of the immune response by thermoheliox. This method may be generally applicable for the treatment of lesions caused by other types of viruses.

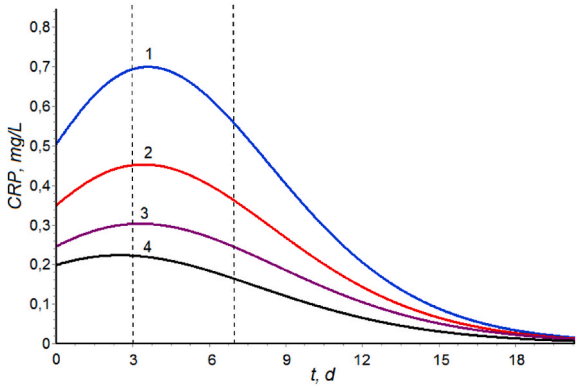

Fig. 12. Kinetic curves of changes in CRP concentration (for the control group) with variation σ: 1–0.002; 2–0.0027; 3–0.0035; 4–0.0047.

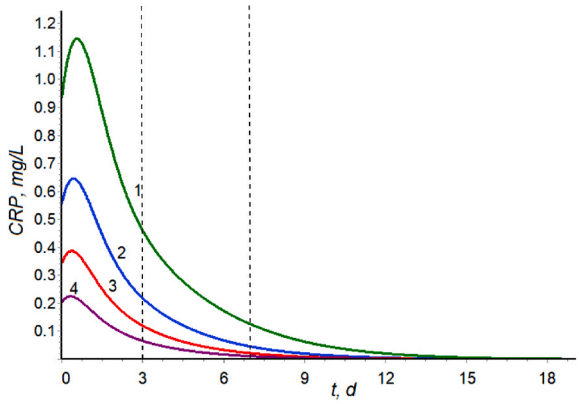

Fig. 13. Kinetic curves of changes in CRP concentration (for the "working" group – therapy with thermoheliox) with variation σ: 1–0.0035; 2–0.007; 3–0.012; 4–0.02.

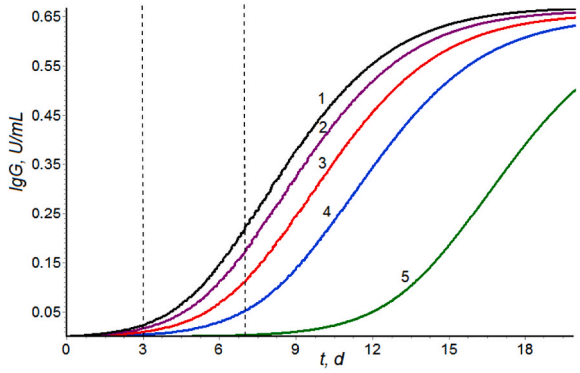

Fig. 14. Calculated curves of changes in the IgG antibodies amount (for the control group) with variation σ: 1–0.13; 2–0.09; 3–0.05; 4–0.02; 5–0.002.

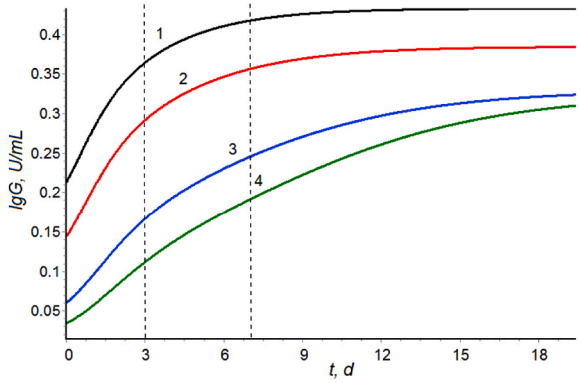

Fig. 15. Calculated curves of changes in the IgG antibodies amount (for the "working" group – therapy with thermoheliox) with variation σ: 1–0.02; 2–0.008; 3–0.002; 4–0.001.

CRediT authorship contribution statement

Sergey D. Varfolomeev: Conceptualization, Methodology, Supervision, Formal analysis, Writing - original draftWriting – original draft,

preparation. **Alexander A. Panin:** Resources, Validation, Writing - review & editingWriting – review & editing. **Valeriy I. Bykov:** Methodology. **Svetlana B. Tsybenova:** Software, Visualization, Writing - review & editingWriting – review & editing. **Sergey V. Zhuravel:** Supervision, Investigation, Validation. **Anna M. Ryabokon:** Formal analysis, Data curation. **Irina I. Utkina:** Investigation. **Pavel V. Gavrilov:** Investigation. **Sergey S. Petrikov:** Project administration, Resources. **Lyudmila V. Shogenova:** Data curation, Formal analysis, Visualization, Validation. **Alexander G. Chuchalin:** Conceptualization, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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