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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Thermovaccination: Thermoheliox as an Immune Response Stimulant. Kinetics of Antibodies and C-Reactive Protein Synthesis in Coronaviral Infection

Corresponding Member of the RAS S. D. Varfolomeev[a,e], S. V. Zhuravel[b], A. A. Panin[c], L. V. Shogenova[d], V. I. Bykov[e], S. B. Tsybenova[e,*], A. M. Ryabokon[e], I. I. Utkina[b], P. V. Gavrilov[b], and A. G. Chuchalin[d]

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Abstract—The high efficiency of using thermoheliox (inhalation with a high-temperature mixture of helium and oxygen) in the treatment of patients affected by COVID-19 was shown. The dynamics of accumulation of IgG, IgM, and C-reactive protein (CRP) in patients with coronavirus infection in the "working" and control groups was studied experimentally. It was shown that thermoheliox intensifies the synthesis of IgG, IgM, and CRP antibodies, while eliminating the induction period on the kinetic curves of the synthesis of specific antibodies in the IgG form and transfers the synthesis of CRP to a fast phase. The results of experiments confirm the previously obtained data based on the analysis of the kinetic model of the development of coronaviral infection in the human body.

Keywords: coronavirus, kinetic model, thermoheliox, immunoglobulin G, immunoglobulin M, C-reactive protein, thermovaccination

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Combating coronavirus infection is an extremely acute problem requiring the development of new methods that expand the range of possibilities of modern therapy and prevention [1]. Currently used antiviral and restorative therapeutics are often insufficiently effective and lead to deep damage to many body systems and often to the death of the patient [2]. Great hopes are pinned on the development of synthetic vaccines that function at the level of antibody synthesis and specifically interact with certain proteins of the virus.

We have developed a new method for combating coronavirus damage to the body, based on the use of thermoheliox (inhalation with a high-temperature mixture of helium and oxygen). Clinical trials were performed on the basis of the intensive care unit of the N.V. Sklifosovsky. Patients with moderate and high severity of coronavirus disease (20–50% of lung damage) were admitted to the hospital. The vast majority of patients have concomitant diseases: arterial hypertension of varying degrees, chronic obstructive bronchitis, COPD, coronary heart disease, atherosclerosis of the lower extremities, bronchial asthma, gastric ulcer, etc. The results of clinical trials are highly positive. Usually, on days 2–3 of thermoheliox administration using the Heliox-Extreme medical device (MedTechInnovations LLC), the patient does not have the virus detected by the standard polymerase chain reaction (PCR), and a stable antibody response develops. These effects were predicted on the basis of a kinetic model that includes the dynamics of growth and reproduction of the virus in the body, the dynamics of damage of recipient cells by the virus, the effects of thermal destruction of viruses, and the antibody response dynamics. Theoretical analysis predicts the potential effects of thermovaccination—the production of antibodies against the proteins of the destroyed viral particles [3–5].

The study of the protein composition of the air condensate exhaled by the patient showed the safety of the use of thermoheliox [6, 7]. We performed an experimental study of the kinetics of accumulation of specific antibodies (IgG, IgM) in patients affected by

[a] *Department of Chemistry, Moscow State University, Moscow, 119991 Russia*
[b] *Sklifosovsky Federal Research Institute of Emergency Medicine, Moscow, Russia*
[c] *OOO MedTechInnovations, Moscow, Russia*
[d] *Pirogov Russian State National Research Medical University, Moscow, Russia*
[e] *Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia*
**e-mail: s.tsybenova@gmail.com*

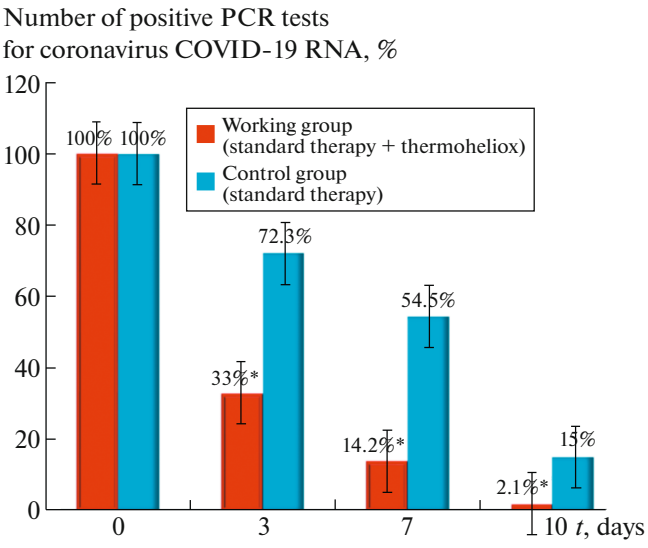

Fig. 1. Number of positive PCR tests in the control and "working" groups (* $p \le 0.05$).

coronavirus. The study included 60 patients with COVID-19, who were divided into two equal groups. The first ("working") group ($N = 30$) included the patients who received thermoheliox therapy in the standard COVID-19 treatment protocol, and the second (control) group ($N = 30$) included the patients who received the standard therapy according to the temporary methodological recommendations of the Ministry of Health of the Russian Federation.

Of the 60 patients included in the study, 28 (46.7%) were medical professionals. The male/female ratio was 17/13 in the "working" group and 16/14 in the control group. In both groups, patients were sex-matched ($p = 0.403$ according to Fisher's exact test). The mean age of patients in the study was 56.7 years (45 years; 61 years). The mean age of patients in the "working" group was 58 years (45 years; 59.5 years); in the control group, 55 years (46 years; 66 years). The patients were comparable in age ($p = 0.537$ according to Mann–Whitney test). The severity of lung damage at the time of inclusion in the study was 25.2% (21%, 42.5%) in the "working" group and 26% (25%, 41.7%) in the control group. The average duration of the disease was 2 days (1 and 4) in the "working" group and 3 days (1 and 5) in the control group. General clinical symptoms of patients in both groups included the feeling of loss of smell and taste, runny nose, feeling short of breath, shortness of breath, weakness, fever, headache, pain in muscles and throat, and dry cough.

All patients included in the study protocol no. 11-20 dated April 20, 2020 were treated for pneumonia caused by the SARS–CoV 2 virus from April 21 to June 2020 inclusive.

All patients underwent swabbing from the nasal cavity and oropharynx mucosa to detect SARSCoV-2 coronavirus RNA by PCR in the CFX-96 real-time PCR detection system (Bio-Rad, United States)) [8–10]; computed tomography (CT) of the lungs [11]; and venous blood sampling for analyzing the content of immunoglobulins IgG (sc) and IgM (sc) to the SARS-CoV-2 spike S protein using the standard enzyme-linked immunosorbent assay (ELISA) with a Mindray 6000 immunochemiluminescence analyzer (United States) [10]. ELISA testing for the presence of IgG helps to detect the contact of the body's immune system with the virus if 2 weeks have passed since the time of infection.

Data were statistically processed using the SPSS 17.0 software package (SPSS Inc., United States). Methods of nonparametric statistics included the Mann–Whitney U test (comparison of two independent variables) and Fisher's exact test. Differences were considered significant at $p < 0.05$.

Figure 1 shows the dynamics of changes in the number of patients in the control and "working" groups with a positive test for SARSCoV-2 RNA. In the "working" group, a significant decrease in the number of patients with a positive test for SARSCoV-2 RNA was observed. According to our observations, in the "working" group of COVID-19 patients who received inhalations with thermoheliox, a negative PCR result was obtained on the third day, and in some patients a negative result was detected as early as on the first day after the start of therapy. Against the background of the standard therapy in the control group, a positive reaction to coronaviral RNA was detected from 7 days to 4 weeks after the onset of the disease, in some cases even longer (Fig. 1).

The results obtained demonstrate that the use of thermoheliox effectively stimulates the synthesis of antibodies of both types (immunoglobulin G and immunoglobulin M). Some of the patients were admitted to the hospital with already formed high levels of IgG and IgM. Both in the "working" and in the control group, the proportion of such patients was approximately 25%. For kinetic analysis, data with a complete set of antibody level measurements (four measurements: at admission, on days 3 and 7, and at discharge) were suitable. Figures 2 and 3 show experimental data on the dynamics of IgG accumulation in the control (Fig. 2) and "working" (Fig. 3) groups. A fundamental difference in the kinetics of the immune response can be seen.

When the patients of the control group were treated without inhalations with Thermoheliox, the synthesis of IgG in the first three days was practically blocked (the induction period on the kinetic curve of IgG accumulation). The use of thermoheliox (four 15-min inhalation procedures with a 15-min break) by the patients of the "working" group eliminated the induction period and triggered the synthesis of immunoglobulin G starting from the first procedure. It should be noted that approximately 30% of patients are

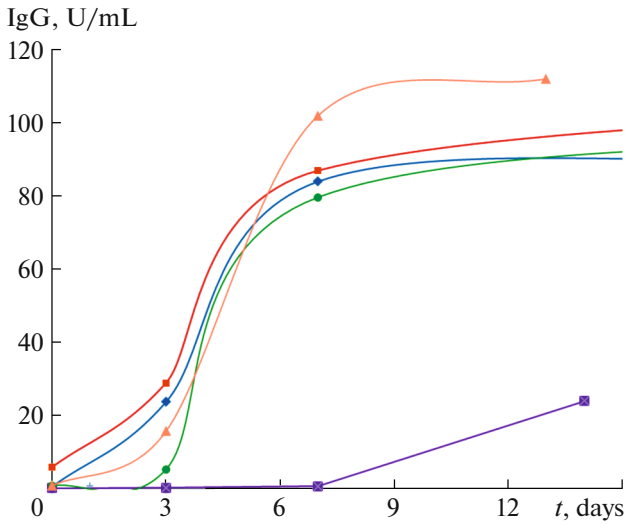

Fig. 2. Dynamics of accumulation of IgG to the SARS-CoV-2 spike S protein in the control group that received the standard treatment (typical curves are shown).

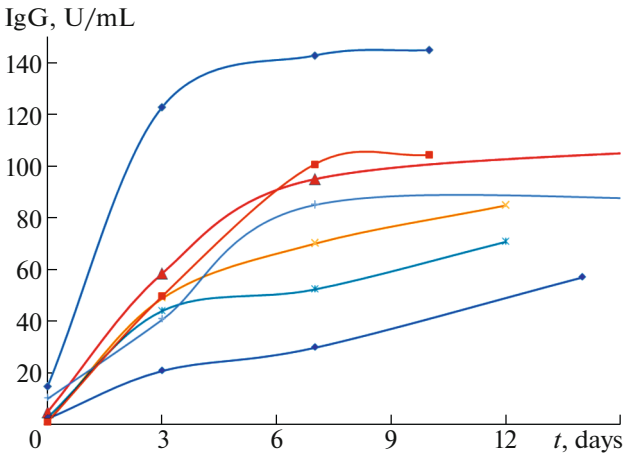

Fig. 3. Dynamics of accumulation of IgG to the SARS-CoV-2 spike S protein in the "working" group during the treatment with thermoheliox (typical curves are shown).

admitted to the hospital with already completed stage of the immune response activation (the induction period on the kinetic curves of IgG accumulation is not observed).

Thermoheliox also stimulates the production 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. Thermoheliox markedly stimulates the accumulation of IgM at the initial stage of the development of the process. For example, the mean IgM(3)/IgM(0) value for the entire population of data for the patients who underwent inhalations with Thermoheliox was 4.1 (in the control group, 3.2).

The results on the dynamics of the synthesis of IgG and IgM clearly demonstrate that the use of Thermoheliox in the treatment of coronavirus infection leads to activation of the immune system and stimulates the production of specific antibodies.

The immune response of the body is complex and involves switching on various biochemical systems of the body [12, 13]. In particular, the C-reactive protein (CRP) is considered as one of the components of the complex chain of biochemical processes, one of the first to respond to bacterial and viral infections. CRP synthesis is induced by cytokines and by lung tissue destruction [14, 15]. We investigated the comparative dynamics of the accumulation and reduction of CRP in the course of the standard treatment and the treatment with the thermoheliox inhalations. A fundamental difference in the response dynamics was observed (Fig. 4).

It can be seen that, during the "normal" course of the disease in the control group, in most cases, CRP accumulation was relatively slow and reached a maximum on days 2–4 of treatment (Fig. 4, curves above the dotted line). The subsequent process of treatment in dynamics is characterized by a decrease in CRP to zero. Thermoheliox inhalations in the "working" group stimulated a rapid accumulation of CRP with the transition of the system from the very initial period to an exponential decrease in the CRP level (Fig. 4, curves below the dotted line). On average, the use of thermoheliox reduces the patient's stay in hospital by 2–2.5 days.

Stimulation of the immune response by thermoheliox can be defined by the term "thermovaccination." We have shown experimentally that, on days 2–3 of

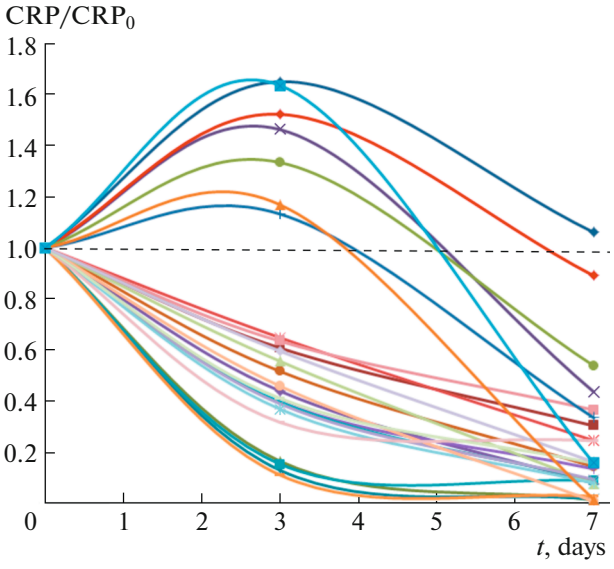

Fig. 4. Dynamics of changes in the CRP content in the blood of patients in the control (curves with a maximum) and "working" (exponential fall) groups (the experimental results were normalized to the CRP_0 index determined at the admission of the patient ($t = 0$)).

using thermoheliox, in most cases, the body is freed from viral particles, judging by the results of PCR analysis. We assume that the accelerated production of antibodies takes place, apparently, against the proteins that are the products of thermal destruction of viral particles in the lungs of patients. Thus, in the case we are discussing, we deal with the "classical" vaccination with a weakened or destroyed antigen. A fundamentally positive difference lies in the fact that the process takes place in vivo with the involvement of natural viral proteins, and "thermovaccination" can have a wide range of specificity.

The mechanism of the observed effect of the stimulation of immune response by thermoheliox requires further investigation. The kinetic model developed and investigated by us [3–5] explains the observed effects by an increase in the antigen concentration during the thermal destruction of the virus.

The effects of "thermovaccination" in the treatment of coronavirus lesions (stimulation of the immune response by thermoheliox), which were discovered and described by us for the first time, may have a general nature and can be used in the treatment of lesions by viruses of another nature.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. Study protocol no. 11-20 dated April 20, 2020 was approved by the Ethical Committee on Biomedical Ethics of the Sklifosovsky Research Institute for Emergency Medicine. Informed consent was obtained from all individual participants involved in the study.

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