The dynamics of protein synthesis in blood mononuclear cells of patients with rheumatoid arthritis with bioresonance therapy

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Rheumatoid arthritis (RA) is the most common disease among chronic inflammatory diseases of the joints; its prevalence is approximately the same in different countries. According to V.A. Nasonova et al. [1], from 1 to 2% of the world's population suffer from RA, which clearly demonstrates the relevance of the search for effective methods of its therapy.

Previous studies have shown violations of protein synthesis in blood lymphocytes of patients with rheumatoid arthritis [2], which leads to functional insufficiency of immunocompetent cells, which develops as a result of external and internal unfavorable circumstances. Consequently, neither antibiotics, nor non-steroidal anti-inflammatory drugs, nor hormones, nor immunosuppressants can solve the problem, so we tried to restore the violation of protein synthesis in the blood lymphocytes of patients with alternative methods of exposure.

Materials and research methods

The study involved 40 people (most women), of whom 30 with rheumatoid arthritis (13 - at the age (B) 32 (4 years, the duration of the disease (P) 2-5 years, the degree of activity (A) I-II), at the stage of rheumatoid process (C) I-II, functional insufficiency (FN) I-II; 17 - B = 45 (12 years old, II = 5-15 years, A = II-III, C = II-III, FN = II) The control group consisted of practically healthy people (10 people) of the same age group.

Most patients with rheumatoid arthritis were seropositive, had systemic manifestations of RA in the form of polyneuropathy, lymphadenopathy, cutaneous rheumatic nodules, etc. Before contacting us, all patients underwent drug therapy (corticosteroids, basic drugs, etc.) in hospitals.

The course of treatment was carried out in a complex manner. We used individually selected homeopathic medicines, sessions of exogenous and endogenous bioresonance therapy (BRT) - only 10-15 sessions, 1-2 sessions per week for up to 30 minutes. (Bioresonance therapy: Methodological recommendations of the Ministry of Health of the Russian Federation No. 2000/74. - M .: SPC TMG Ministry of Health of the Russian Federation, 2000. - 26 p.). The control of the effectiveness of treatment was carried out according to the indicators of electrical conductivity of biologically active points by the method of R. Voll and known objective (biochemical blood tests and anthropometric data) and subjective (complaints) assessment methods.

Lymphocyte preparations were obtained from 10 ml of heparinized peripheral blood by centrifugation in a ficoll-verographin gradient (1.077 g / cm3) at 400 g for 40 min. Cell suspensions were washed twice with Dulbecco's phosphate buffer (FBD, Sigma) and once with RPMI 1640 culture medium (Sigma) by centrifugation at 400 g for 20 min. Further lymphocytes

incubated at 37 ° C for 3 hours in RPMI 1640 culture medium containing ^{35}S-methionine with an activity of 10 μCi / ml, in the mode of gentle shaking ("state of rest"). The concentration of cells in the medium was 2 x 10^6 cells / ml. After incubation, cells were pelleted and used for protein extraction.

Heat shock for lymphocytes was performed at 44 ° C for 15 min. in RPMI 1640 environment containing^{35}S-methionine (10 μCi / ml), and then the cells were incubated in the same medium at 37 ° C for 3 hours under the conditions indicated above.

At all stages of the isolation and incubation of lymphocytes, cell death, which was monitored by staining cells with a 0.2% trypan blue solution and counting living and dead cells in the Goryaev chamber, did not exceed 3%.

The polypeptide composition of proteins synthesized de novo in lymphocytes was analyzed using polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (DSP) according to Laemmli [3] and autoradiography for quantitative analysis and using two-dimensional electrophoresis modified by Celis et al. [4] and autoradiography for visual assessments.

As markers of molecular weight, we used standard sets of protein markers for chip electrophoresis from Sigma. The isoelectric points (pI) of proteins on two-dimensional electrophoretograms were determined by the pI of known proteins [4].

Autoradiography was performed by exposure of gels pre-stained with a solution of 0.25% Coomassie R-250: 50% ethanol: 7% acetic acid and dried on a special device "Gel-1" (Ukraine), with an X-ray film RT-1V (Svema) for 15 days. X-ray films were developed in accordance with their instructions, dried and scanned on an automatic two-beam densitometer (IBF, Pushchino). Densitograms were analyzed using a Chromatopac C-R3A integrator (Shimadzu).

Statistical analysis was performed using Fisher's test. The ratio of the amount of newly synthesized protein labeled^{35}S-methionine, (the area of the peak corresponding to the protein under study on the densitogram from the radio autograph) to the total protein content in the sample (the total area of the peaks of all proteins on the densitogram obtained by scanning the same protein sample stained with Coomassie R250). Differences were considered significant at $P < 0.05$.

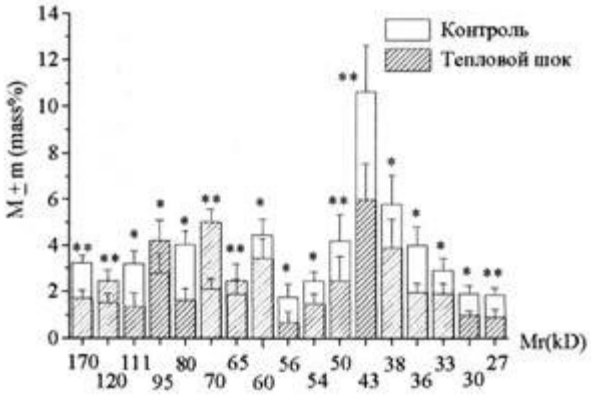
Clinical and biochemical analyzes of the blood were carried out v relevant laboratories of medical institutions.

Statistical analysis of the data obtained was carried out using the parametric Student's t-test for pairwise related samples. Values at $P < 0.05$ were taken as statistically significant.

Research results and their discussion

Heat shock (HS) was used as a classical inducer of stress proteins or heat shock proteins (HSP) [5, 6]. At the same time, cell death did not exceed 3% of the value characteristic of nonspecific cell death during their isolation from the blood. In response to HS, an increase in the synthesis of proteins with molecular weights of 120, 95, 70, and 65 kDa is observed in the lymphocytes of conventionally healthy individuals.

Two-dimensional electrophoresis were identified as HSPs corresponding to molecular weight families (for 70 kDa HSPs 72 and 73 kDa). In this case, the synthesis of other proteins (constitutive) decreases by 30% relative to synthesis at physiological temperature (Fig. 1).



Rice. 1. Synthesis of proteinsde novo in blood lymphocytes of healthy people (n = 10).
Designations: * - p <0.05, ** - p <0.001

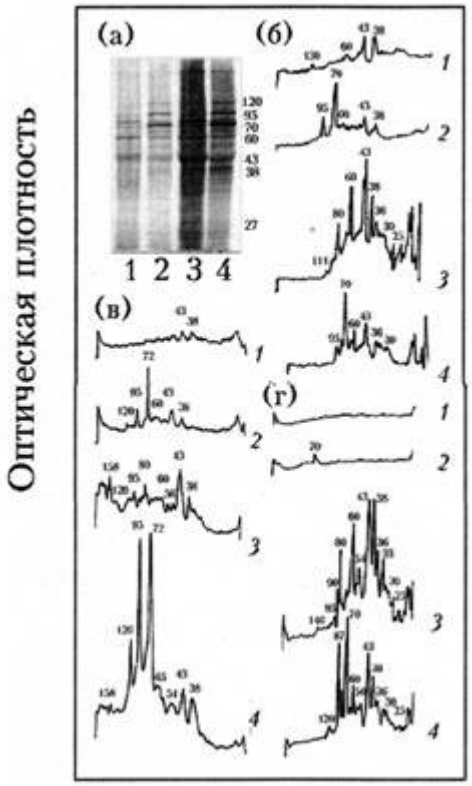
In RA patients, the ability of lymphocytes to synthesize HSP and other cellular proteins has a pronounced depressed character and depends on the severity and duration of the disease (Fig. 2). At the I degree of activity of the rheumatoid process, initially there is a reduced level of constitutive protein synthesis by lymphocytes, which in response to HS is slightly inhibited and HSPs of 95.70 and 65 kD are induced (Fig. 4 A, B). The II degree of RA activity is characterized by markedly reduced protein synthesis at physiological temperature, weak induction of HSPs of 70 and 65 kDa, and inhibition of the synthesis of constitutive proteins in response to HS (Fig. 2C). In the case of severe forms of PA manifestation (II-III degree of activity), protein synthesis is pronounced inhibited both in resting and in lymphocytes shocked by heating, while induction of HSP synthesis is not observed (Fig. 2D).

Statistical analysis of the densitograms of the spectrum of de novo synthesized proteins in the lymphocytes of patients with RA showed that the level of protein synthesis in the lymphocytes of these patients in the initial state is suppressed by approximately 60% relative to the control group (Fig. 4), the suppressed nature of synthesis extends to the entire spectrum of proteins studied (from 170 to 27 kDa) and is statistically significant. HSP inhibits the synthesis of constitutive proteins even more and reliably induces HSPs of 95, 70, and 65 kDa, induction of the synthesis of 120 kDa HSPs is barely noticeable (Fig. 3). However, the level of expression of their synthesis is significantly inferior to that in the control group (Fig. 5).

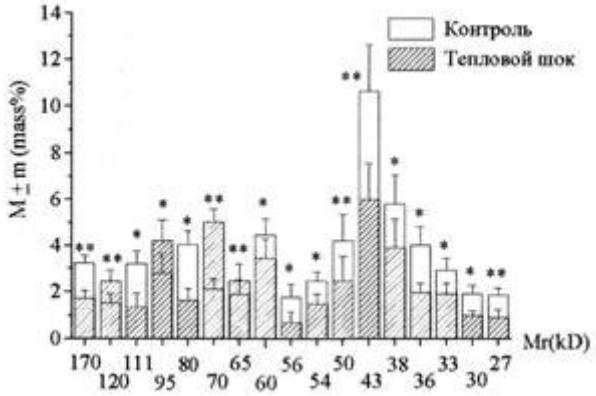
The ability of lymphocytes to synthesize proteins in vitro in response to heat shock also depends on the severity and duration of the disease. In the case of moderate RA (II degree of activity), the synthesis of constitutive proteins is even more suppressed, and HSPs of 72 and 73 kDa, rarely 65 kDa, are induced. In milder cases (I degree of activity), against the background of induction of the above HSPs, only some inhibition of the synthesis of constitutive proteins is observed. There are severe cases (activity of RA III degree), when in response to HS

the synthesis of cellular proteins in the lymphocytes of patients is completely suppressed.

After therapy, the level of protein synthesis in resting lymphocytes in RA practically reaches the level in the control group (Fig. 6). Only the synthesis of proteins with molecular weights of 170 and 111 kDa remains significantly reduced. In response to HS, an increase in the synthesis of stress proteins with molecular weights of 120, 95, 70, and 65 kDa is observed in RA lymphocytes (Fig. 7), and the synthesis of HSP has a higher level relative to conventionally healthy individuals (significant for 70 and 33 kDa). The synthesis of constitutive proteins is also more stable.



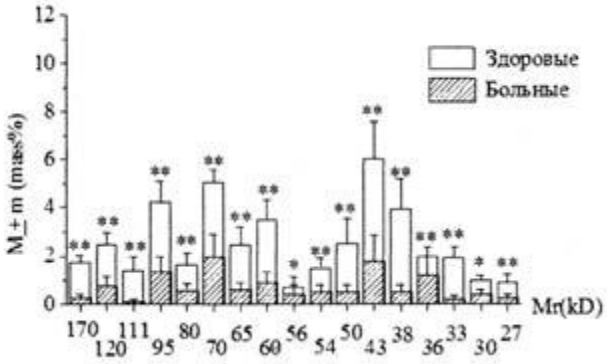
Rice. 2. Radio autograph and densitograms of autoradiographic researchpolypeptide composition of proteins synthesized de novo in blood lymphocytes of patients with RA I (a, b), II (c), and III (d) degrees of activity up to (1 - before and 2 - after HS) and after (3 - before and 4 - after HS) therapy



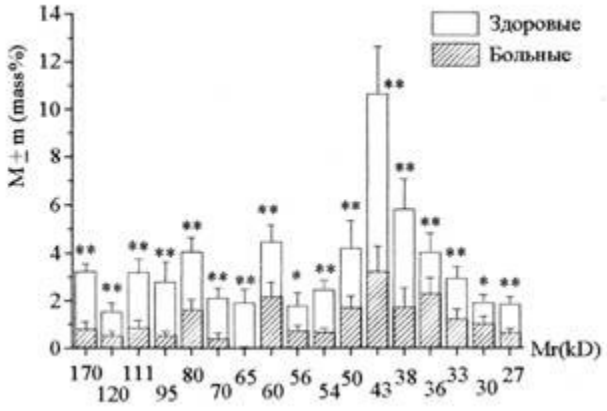
Rice. 3. Protein synthesisde novo in blood lymphocytes in RA patients (n = 12) before the course BRT.

Designations: * - P <0.05, ** - P <0.001

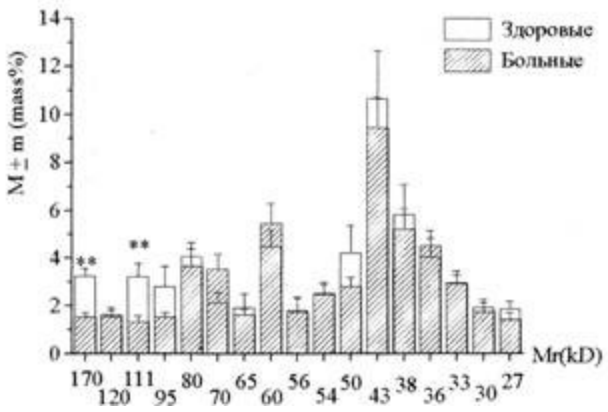
Comparative analysis showed that from the moment of treatment of patients as a result of the therapy in lymphocytes there is an increase in the level of protein synthesis by about 2 times. In response to HS, a similar trend is observed: the level of protein synthesis after the course of therapy increases 3 times compared to that before the course of treatment, a significant increase in the level of synthesis is found for proteins 170, 120, 95, 80, 70, 65, 60, 54, 43, 38 , 36, 33, 30 and 27 kDa.



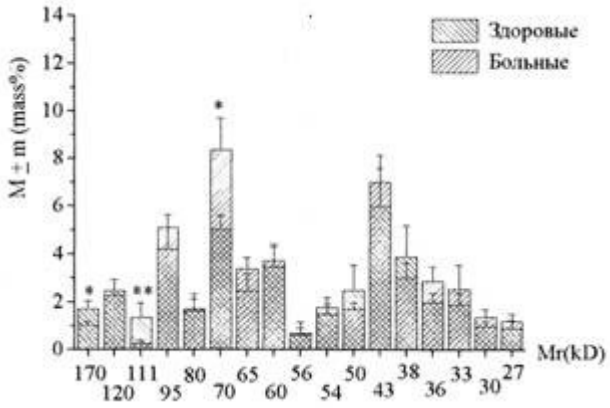
Rice. 4. Protein synthesisde novo in blood lymphocytes at rest in patients RA (n = 12) before the BRT course in relatively healthy people (n = 10).
Designations: * - p <0.05, ** - P <0.001



Rice. 5. Protein synthesisde novo in blood lymphocytes in response to heat shock in patients with RA (n = 12) before the course of BRT relatively healthy people (n = 10).
Legend: P <0.05



Rice. 6. Synthesis of proteins in blood lymphocytes at rest in patients with RA (n = 12) after the BRT course in relatively healthy people (n = 10).
Designations: * - p <0.05, ** - p <0.001

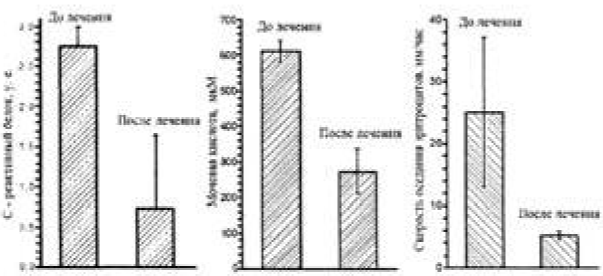


Rice. 7. Synthesis of proteins in blood lymphocytes in response to heat shock in patients with RA (n = 12) after the BRT course in relatively healthy people (n = 10).
Designations: * - P <0.05, ** - P <0.001

The lack of confirmation of the infectious origin of RA, the nature of changes in humoral and cellular immunity in patients with this disease, the systemic nature of its manifestations of RA give the right to consider rheumatoid arthritis as a disease with an autoimmune genesis. However, the unsatisfactory search for a specific autoantigen that initiates and maintains autoimmune processes in patients with RA does not allow us to name rheumatoid arthritis as a purely autoimmune disease. In the process of development of rheumatoid inflammation, a breakdown of physiological mechanisms takes place, affecting the state of all levels of the functional organization of the immune system. It is now generally accepted that RA is based on the formation of a stable pathological condition, which, often under the condition of the patient's genetic predisposition,

internal environment: secondary infections, trauma, psychogenic factors, drugs, etc. This multifactorial nature of RA allows us to consider it as a maladjustment disease with a primary violation of the central mechanisms of regulation of the body's homeostasis [7]. And in this vein, already in the new (and in fact, the "old") light, the role of HTS appears. Modern rheumatology makes the ability of lymphocytes to synthesize HSPs as one of the probable conditions for their normal immune activation. Nevertheless, HSPs are synthesized constitutively and in response to a stressful effect of a very different nature in all cells and tissues of the body and have a more general physiological function than as chaperones. Induced in response to heat, heavy metals, poisons, reactive oxygen species, etc., HSPs stabilize the DNA-protein synthesizing apparatus and the cytoskeleton of cells, thereby providing cells, tissues and organs with mechanisms of adequate response to adverse changes in the environment [8]. And the violation of the body's ability to induce stress proteins under conditions of emotional stress, infection by extreme physical exertion cannot but affect the functional activity of cells, organs and systems of the body, including the immune system and specifically lymphocytes. Therefore, an important task of modern medicine is the regulation of one of the fundamental functions of life support and the integrity of the body as a whole - the ability of body cells to synthesize stress proteins. And the violation of the body's ability to induce stress proteins under conditions of emotional stress, infection by extreme physical exertion cannot but affect the functional activity of cells, organs and systems of the body, including the immune system and specifically lymphocytes. Therefore, an important task of modern medicine is the regulation of one of the fundamental functions of life support and the integrity of the body as a whole - the ability of body cells to synthesize stress proteins. And the violation of the body's ability to induce stress proteins under conditions of emotional stress, infection by extreme physical exertion cannot but affect the functional activity of cells, organs and systems of the body, including the immune system and specifically lymphocytes. Therefore, an important task of modern medicine is the regulation of one of the fundamental functions of life support and the integrity of the body as a whole - the ability of body cells to synthesize stress proteins.

Recovery of HSP synthesis observed in blood lymphocytes of patients with RA as a result of treatment ensures the therapeutic efficacy of BRT, which is also confirmed by the results of biochemical blood tests (Fig. 8).



Rice. 8. Dynamics of some biochemical parameters of blood before and after the course of BRT

Thus, the studies carried out allow us to determine the important role in the development of hyporeactivity of lymphocytes in RA, inhibition of the synthesis of both constitutive and stress proteins in them. Our treatment is fundamentally new, it almost completely restores the regulatory mechanisms of the nonspecific defense system of the body. However, almost all patients before contacting us took immunosuppressants, hormonal drugs, non-steroidal anti-inflammatory drugs, so we cannot unequivocally exclude the role of these drugs in the development of lymphocyte hyporeactivity. One thing is clear - our treatment restores synthesis as

constitutive and stress proteins in blood lymphocytes.

Literature

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