Bioresonance Therapy of Rheumatoid Arthritis and Heat Shock Proteins

B. I. Islamov, V. A. Funtikov, R. V. Bobrovskii, and Yu. V. Gotovskii*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 11, pp. 525-528, November, 1999
Original article submitted November 11, 1998

Bioresonance therapy normalized protein synthesis in lymphocytes inhibited by 60% in patients with rheumatoid arthritis, restored impaired synthesis of heat shock proteins at rest (73 and 65 kD proteins) and after heat shock induction (120, 87, 73 and 72, 65, 55, 32 kD, respectively), and provided high induction level for 70 and 32 kD proteins compared to healthy subjects. It is assumed that therapeutic effect of bioresonance therapy is partially determined by recovery of functional activity of lymphocytes due to normalization of heat shock protein synthesis.

Key Words: *heat shock proteins; rheumatoid arthritis; bioresonance therapy*

Despite of numerous studies, the role of stress proteins in the etiology and pathogenesis of rheumatoid arthritis (RA) remains unclear [10]. Experimental modeling of RA in animals suggests the key role of heat shock proteins (HSP) of the 60 kD family (HSP60) in triggering autoimmune processes [6,7]. However, examination of peripheral blood cells, serum, and synovial fluid components showed the presence of autoreactive T-cell clones and autoantibodies to HSP60 in healthy subjects [8], which raises doubt about the specific role of stress proteins in initiating this disease.

Previous *in vitro* studies [3] showed that synthesis of some HSP (120, 95, 70, and 65 kD) in lymphocytes is inhibited during RA; this inhibition depends on the duration and severity of the disease. Similar inhibition of protein synthesis is typical of all lymphocyte proteins suggesting that impaiment of HSP synthesis during RA is unspecific and reflects general disfunction of immunocompetent cells accompanied by disturbances of unspecific protection against damaging factors of RA, in particular hypoxia, immune complexes, *etc.*

Here we present the study of protein synthesis by lymphocytes from RA patients receiving bioresonance therapy (BRT) routinely used for correction of energetic disturbances in the meridian system of the organism. The BRT device was constructed at IMEDIAS Center in 1993 [2]. Therapy was performed by electromagnetic waves in a frequence range of 10-500,000 Hz. Oscillations recorded from patient's skin were processed and used for the therapy [1]. During therapy, a patient and device form a closed circuit of adaptive regulation, which potentiates the organism capacities for restoration of physiological homeostasis.

MATERIALS AND METHODS

Six patients aged 33-50 with 10-15-year history of RA were examined. Before BRT course, biologically active points characterizing the state of joints and immune system were determined by Foll electroacupuncture method [1]. Phenomenon of pointer drop was observed in all patients.

Five subjects of the same age group with normal electroacupuncture parameters (50 units) served as the control.

A course of BRT consisting of 10-15 sessions (30-min each) was performed under the control of electroacupuncture parameters and terminated after their normalization.

Lymphocytes were isolated from 10 ml heparine-stabilized peripheral blood by centrifugation in Ficoll-

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences; *IMEDIS Center, Moscow

0007-4888/99/0011-1112$22.00 ©2000 Kluwer Academic/Plenum Publishers

Verografin gradient (1.077 g/cm^3) for 40 min at 400*g*. The cells were washed twice with Dulbecco phosphate buffer (Sigma) and then with RPMI-1640 medium (Sigma) by centrifugation at 400*g* for 20 min. Isolated lymphocytes (2×10^6/ml) were incubated in RPMI-1640 containing ^{35}S-methionine (10 μCi/ml) with gentle shaking (rest condition). After incubation the cells were sedimented and used for protein extraction.

Heat shock in lymphocytes was induced by 15-min heating at 44°C in ^{35}S-methionine-containing (10 μCi/ml) RPMI-1640 followed by 3-h incubation at 37°C in the same medium.

The cells were stained with 0.2% trypan blue and viable and dead cells were counted in a Goryaev chamber (hemocytometer). Cell death did not exceed 3% at all stages of lymphocyte isolation and incubation.

Polypeptide composition of *de novo* synthesized proteins was analyzed by electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulfate [9], quantitative autoradiography, modified two-dimensional electrophoresis [5], and autoradiography (for visual estimation).

Standard molecular weight markers for electrophoresis with sodium dodecyl sulfate (Sigma) were used. Isoelectric points (pI) on electrophoreograms for test proteins were determined by pI of standard proteins [5].

For autoradiography analysis, the gels were stained with 0.25% Coomasse R-250 (50% alcohol : 7% acetic acid) and dried in a Gel-1 device (Ukraine) with a RT-1V X-ray film (Svema) for 15 days. The films were developed, dried, and scanned on an automatic double-beam densitometer (Institute of Biophysics, Pushchino). Densitograms were analysed with a Chromatopac C-R3A integrator (Shimadzu).

Statistical analysis was performed by Fisher test. The ratio between the amount of ^{35}S-methionine-labeled newly synthesized protein (area under the peak corresponding to test protein on densitogram) and total protein content in the sample (sum of all peaks on densitogram obtained by scanning of the same Coomasse R250-stained gel) was analyzed. Differences were considered significant at $p<0.05$.

RESULTS

In RA patients, a significant (by on average 60%) inhibition of *in vitro* protein synthesis in lymphocytes was observed (Fig. 1, *a*). Heat shock further suppressed synthesis of constitutive proteins (to about 30% of normal) and enhanced synthesis of 95, 70, and 65 kD proteins due to induction of the corresponding HSP identified by two-dimensional electrophoresis, the level of their induction being significantly below normal.

Biochemical tests of the blood showed high activity of C-reactive protein (+++), uric acid concentration (612±31 μmol/liter), and erythrocyte sedimentation rate (ESR, 25±12 mm/h).

After BRT the intensity of protein synthesis in resting lymphocytes from RA patients returned practically to normal (Fig. 1), though synthesis of 120, 111, 95, and 50 kD proteins remained low. Heat shock (Fig. 1, *b*) enhanced synthesis of 95, 70, 65, 54, and 33 kD proteins due to induction of the corresponding HSP identified by two-dimentional electrophoresis (Fig. 2). It should be noted that the induction of 70 and 33 kD HSP significantly exceeded the normal level. Synthesis of constitutive proteins was more stable, though synthesis of 170, 111, 50 kD proteins and actin (43 kD) was inhibited.

Comparative analysis of protein synthesis in resting lymphocytes before and after BRT (Fig. *a, b*) showed that therapy 2-fold enhanced this synthesis. This was accompanied by a significant enhancement of the synthesis of 170, 70 and 65 kD HSP and 56 (probably, tubuline) and 54 kD proteins. Heat shock induced similar changes: synthesis of 80, 54, 50, 38, 33, 30, and 27 kD proteins significantly (3-fold) increased after BRT course.

Two-dimentional electrophoresis and autoradiography of lymphocyte proteins showed that after BRT polypeptide composition of *in vitro* synthesized proteins corresponds to that in the control at rest and after heat shock (Fig. 2) [3]. In resting lymphocytes constitutive synthesis of 73 and 65 kD HSP was observed, while heat shock dramatically enhanced this synthesis and induced 120, 87, 72 and 55 kD HSP, as well as 33 (possibly, 32 kD HSP) and 27 kD proteins (pI 4.9 and 5.1, respectively). Synthesis of 56-54 kD constitutive proteins from the tubuline family was more stable. This was accompanied by inhibition of synthesis of 111 kD protein (pI=5.3), actin, and acid 63 kD protein.

In all patients treatment resulted in stable clinical improvement manifested as normalization of ESR (5.3±0.5 mm/h), decrease of uric acid concentration to 275±63 μmol/liter and attenuation of C-reactive protein activity to +-.

RA is associated with the inhibition of synthesis of constitutive and stress proteins in lymphocytes indicating impairment of protective mechanisms in the organism. BRT produced an immunomodulatory effect by normalizing protein synthesis in lymphocytes and improving biochemical parameters of the blood. We assume that therapeutic effect of BRT is associated with recovery of unspecific resistance of immune cells against RA damaging factors (hypoxia, immune complexes, *etc.*) via normalization of HSP synthesis. Thus, BRT induced constitutive 73 and 65 kD HSP

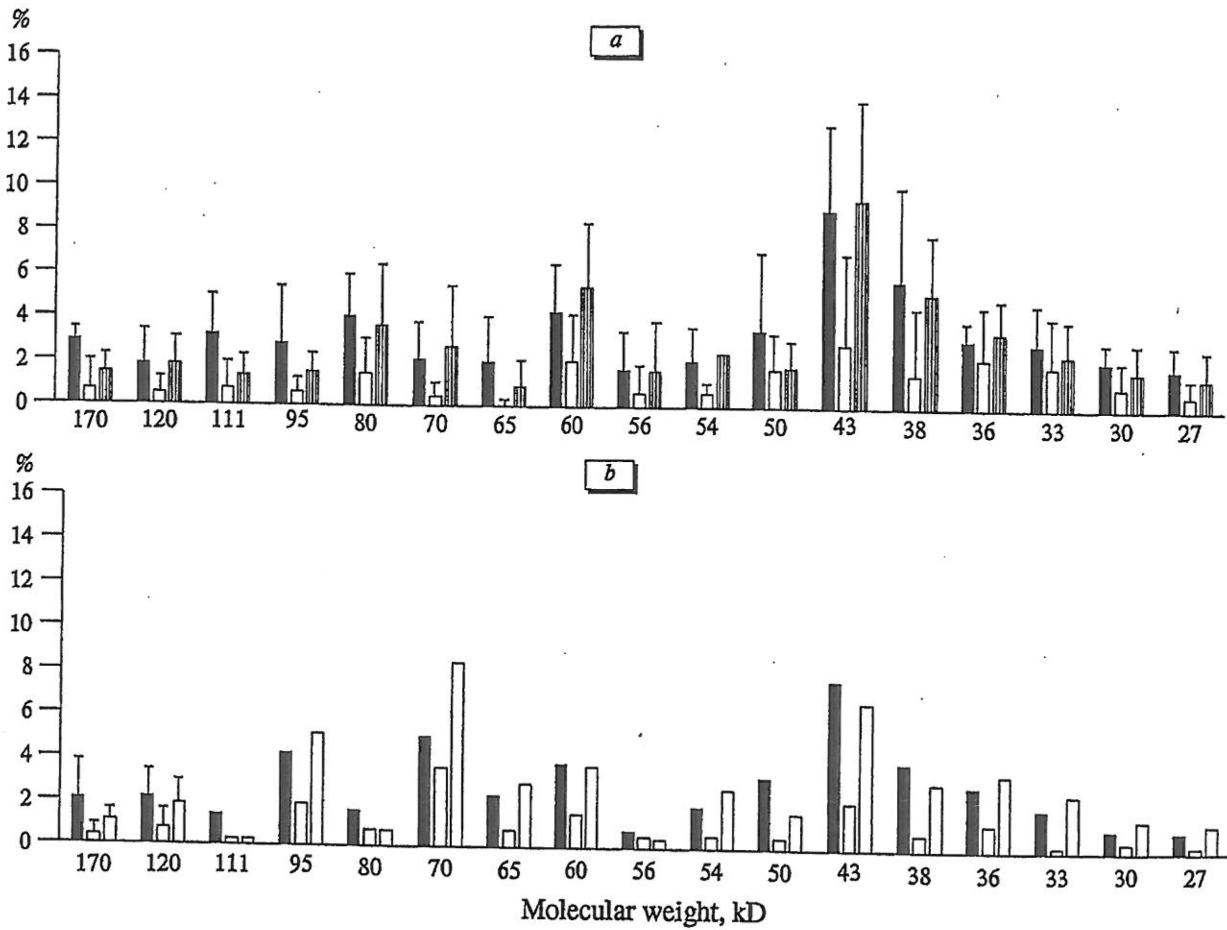

Fig. 1. Initial (*a*) and heat shock-induced (*b*) *in vitro* protein synthesis in resting lymphocytes from patients with rheumatoid arthritis (n=6) before and after bioresonance therapy in relation to the control (n=5). Ordinate: newly synthesized protein/total protein.

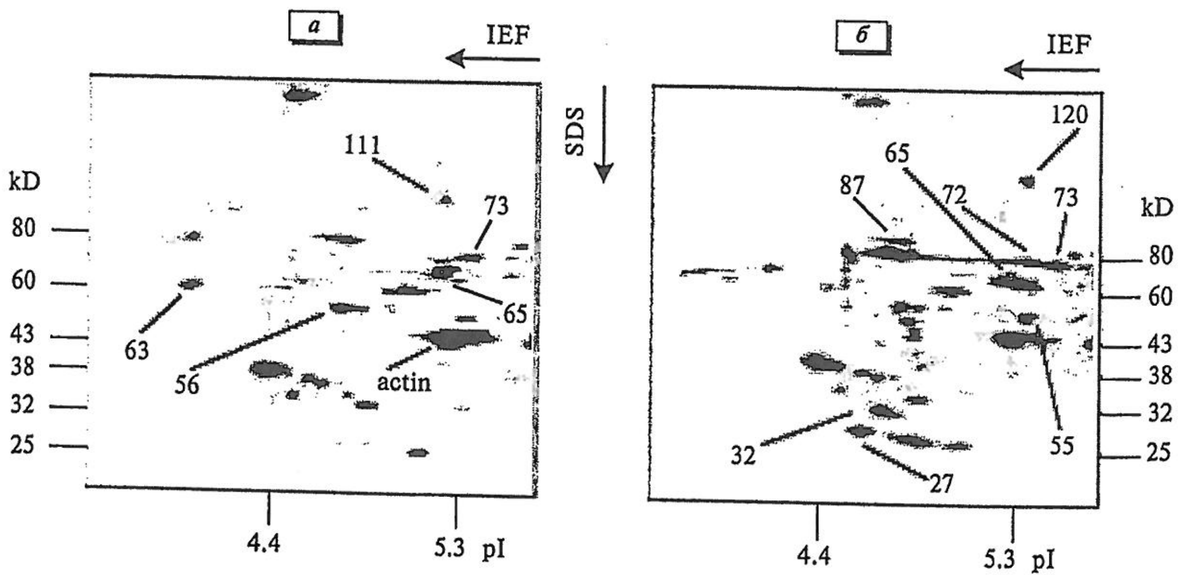

Fig. 2. Typical two-dimentional fluorograms (computer scanning) of polipeptide composition of proteins synthesized in lymphocytes from patients with rheumatoid arthritis after bioresonance therapy at rest (*a*) and after heat shock (*b*). IEF: direction of isoelectric focusing, SDS: direction of electrophoresis in polyacrylamide gel with sodium dodecyl sulfate, pI: isoelectric points of tropomyosin and actin.

synthesis, heat shock stimulated synthesis of almost all HSP typical of lymphocytes from healthy people, synthesis of some proteins (70 kD HSP) surpassed the control level. BRT stabilized synthesis of constitutive proteins both at rest and after heat shock except 170, 111 (pI=5.3), and 50 kD proteins and actin (43 kD); however, the role of these proteins in the pathogenesis of RA remaines poorly understood.

Thus, BRT represents a perspective method for treating RA, which contributes to existing therapeutic approaches to this disease. It remains unclear whether HSP serve as a direct target for low-frequency electromagnetic oscillation [4] underlying the effect of BRT [1]. It is possible that BRT activates the genome of immune cells, including HSP genes, via regulation of other components of cell activation (antioxidant system, cytokines and others).

REFERENCES

1. Yu. V. Gotovskii and A. V Samokhin, *Electropuncture Diagnostics and Therapy According to R. Foll Method*, Moscow (1995).
2. Yu. V. Gotovskii, Patent No. 35, p. 144 (1996).
3. V. A. Funtikov, B. I. Islamov, R. V. Bobrovskii, and Yu. V. Gotovskii, *Dokl. Ross. Akad. Nauk.*, **364**, No. 1, 126-129 (1999).
4. P. Cairo, B. Greenebaum, and E. Goodman, *J. Cell Biochem.*, **68**, 1-7 (1998).
5. J. E. Celis, B. Gesser, H. H. Rasmussen, *et al.*, *Electrophoresis*, No. 11(12), 989-1071 (1990).
6. I. R. Cohen, *Adv. Intern. Med.*, **37**, 295-311, (1992).
7. J. Holoshitz, A. Matitau, and I. R. Cohen, *J. Clin. Invest.*, **73**, 211-215 (1984).
8. M. Jaattela and D. Wissing, *Ann. Med.*, **24**, 249-258 (1992).
9. U. K. Laemmly, *Nature*, **227**, 680-685 (1970).
10. J. B. Winfield and W. N. Jarjour, *Curr. Top. Microbiol. Immunol.*, **167**, 161-187 (1991).