Effect of Bioresonance Therapy on Antioxidant System in Lymphocytes in Patients with Rheumatoid Arthritis

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We measured activities of superoxide dismutase, catalase, and glutathione peroxidase and content of nonprotein thiol groups (reduced glutathione) in blood lymphocytes from patients with rheumatoid arthritis before and during bioresonance therapy. The state of the antioxidant system in lymphocyte from patients receiving standard pharmacotherapy was characterized by activation of the key antioxidant enzymes and decreased content of thiol groups. Bioresonance therapy increased the content of thiol groups and normalized activities of superoxide dismutase and glutathione peroxidase. However, catalase activity remained above the control. Changes in the lymphocyte antioxidant system indicate that bioresonance therapy activates nonspecific protective mechanisms in patients with rheumatoid arthritis.

Key Words: *rheumatoid arthritis; lymphocyte antioxidant system; bioresonance therapy*

The role of reactive oxygen species in the pathogenesis of autoimmune disorders, including rheumatoid arthritis (RA) attracts much attention [5]. Reactive oxygen species (superoxide radical, hydroxyl radical, H_2O_2, peroxynitrite, *etc.*) are involved in the formation of tissue damages during RA [9]. Little is known about the antioxidant system of peripheral blood lymphocytes in patients with RA. It remains unclear whether bioresonance therapy (BRT) can regulate this system [2].

Here we studied the lymphocyte antioxidant system in patients with RA and evaluated the possibility of correcting disturbances by means of BRT.

MATERIALS AND METHODS

We examined 20 women (19-60 years) with RA of stages II-III. All patients received nonsteroid antirheumatic drug diclofenac (daily dose 50-200 mg), 7 patients received prednisolone (n=7, daily dose 5-15 mg) and one of basic preparations (aminoquinoline preparations, methotrexate, and sulfasalazine). BRT was performed on an IMEDIS- OLL complex once a week (20-30 min) for 9 months. Electronic analogues of homeopathic preparations were selected individually [7].

Blood samples were taken before, by the end, and 2-3 months after the therapy.

The control group included 10 healthy women of the same age.

Lymphocytes (mononuclear cells) were obtained by centrifugation of the peripheral blood in a Verografin- icoll density gradient (ρ=1.077) and washed 2 times in phosphate buffer. Activities of antioxidant enzymes were measured in special buffers. The cells were destroyed by repeated freezing and thawing (-20°C). Activities of antioxidant enzymes were measured.

The concentration of reduced thiol groups was measured using 5,5'-dithio-bis(2-nitrobenzoic) acid as described elsewhere [4]. The reaction between 5,5'-dithio-bis(2-nitrobenzoic) acid and acid-soluble SH-groups yields a colored product with an absorption maximum at λ=412 nm.

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Glutathione peroxidase (GSH-Px) activity was estimated by utilization of reduced glutathione using tert-butyl hydroperoxide as the substrate [4]. Total superoxide dismutase (SOD) activity was measured as described elsewhere [10]. Catalase activity was determined by the rate of H_2O_2 degradation [12].

The results were analyzed by nonparametric Van der Warden X test. The differences were significant at $p<0.05$.

RESULTS

Before BRT, activities of SOD, catalase, and GSH-Px in blood lymphocytes from RA patients surpassed the normal by 109, 42, and 92%, respectively. The content of nonprotein thiol groups in these patients decreased by 17% compared to the control (Fig. 1). More than 90% thiol groups were presented by glutathione. During BRT activity of GSH-Px decreased by 37%, but remained 41% above the control. SOD activity decreased by 19% and was 169% of the control. Catalase activity remained unchanged. The content of nonprotein thiol groups increased by 44% and reached 121% of the control level (Fig. 1).

These changes in the content of nonprotein thiol groups and activities of antioxidant enzymes persisted after termination of BRT. The content of nonprotein thiol groups increased by 67% and was 138% of the control. GSH-Px activity approached the control level and was 58% lower than that observed before the therapy. SOD activity progressively decreased, but remained 51% above the control. Catalase activity remained unchanged.

RA is usually associated with decreased intracellular SOD activity [14]. Our observations showed that SOD activity in RA patients is significantly higher than in healthy donors. This is probably related to the therapy with nonsteroid antiinflammatory drugs, hormones, and immunosuppressive preparations and compensatory cell response to continuous production of free oxygen radicals. Unfortunately, most patients received standard medicinal preparations before and du-

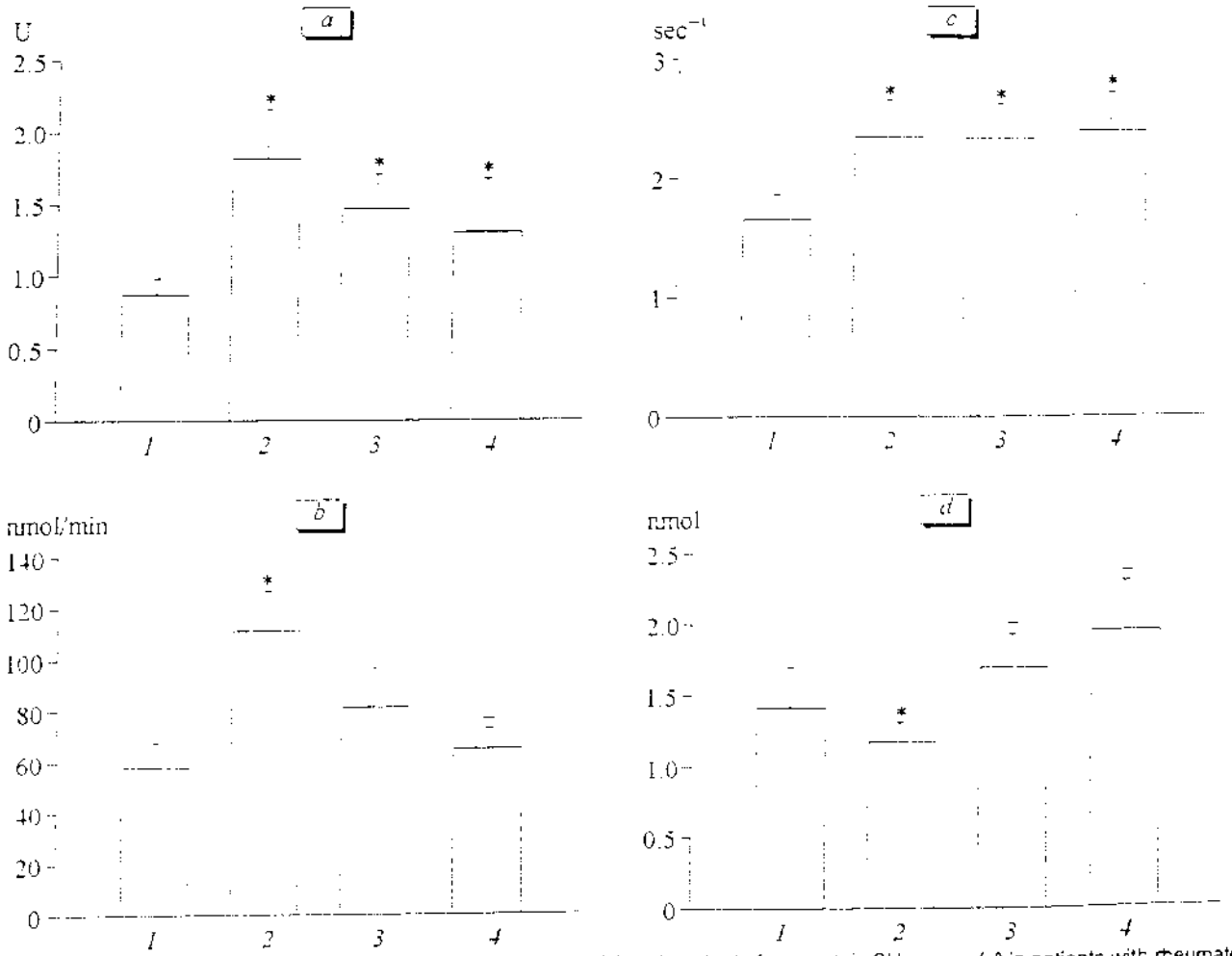

Fig. 1. Activities of SOD (*a*), glutathione peroxidase (*b*), and catalase (*c*) and content of nonprotein SH-groups (*d*) in patients with rheumatoid arthritis (per 10^6 cells): control (*1*) and before (*2*), by the end (*3*), and 2-3 months after bioresonance therapy (*4*). $p<0.05$: *compared to the control; *compared to parameters before the therapy.

ring BRT. In patients with low SOD activity nonsteroid antiinflammatory drugs markedly increase this parameter [13]. Previous studies showed that inflammation and bacterial phagocytosis are suppressed by SOD [11]. Changes in SOD activity improve the state of patients [13].

In our observations clinical state of RA patients improved during BRT despite the decrease in SOD activity. It can be assumed that BRT reduces the inflammatory response and decreases the strain of adaptive reactions.

Catalase is a heme-containing enzyme localized in peroxisomes. This enzyme degrades H_2O_2, which contributes to generation of reactive oxygen metabolites. Published data show that 0.5% O_2 formed after H_2O_2 degradation is in activated singlet state [6]. In our experiments catalase activity remained unchanged. Probably, BRT did not modulate the formation of H_2O_2. It cannot be excluded that catalase is not the key antioxidant enzyme in lymphocytes.

GSH-Px is localized in the cytoplasm and mitochondria. This enzyme catalyzes degradation of various peroxides by oxidizing glutathione with the formation of its conjugates. GSH-Px affinity for H_2O_2 surpasses that of catalase. Therefore, GSH-Px is more effective at low H_2O_2 concentrations. In our observations GSH-Px activity in RA patients markedly surpassed that in healthy donors. Enzyme activity progressively decreased during BRT and did not differ from the control after the therapy.

SH-containing compounds play an important role in the protection of cells from OH radicals formed in the enton reaction. Thiol compounds maintain oxidation-reduction homeostasis in cells and tissues. Stress roduces reversible oxidative modification of thiol groups, which serves as the nonspecific reaction to extreme factors [1]. These changes modify the state of cell membranes, their permeability, and adhesive properties and affect enzymes activities and cell proliferation. Previous studies showed that SH-containing compounds first undergo oxidation. This prevents other functional groups and molecules from oxidation. Changes in the concentration of nonprotein thiol groups and GSH-Px activity during BRT were inversely related. The ratio between reduced and oxidized SH-groups reflects nonspecific resistance of the organisms [7].

Our observations indicate that before BRT the lymphocyte antioxidant system in RA patients receiving standard pharmacotherapy is in the strained state. Activities of key antioxidant enzymes GSH-Px, SOD, and catalase increase, while the content of reduced thiol groups decreases. BRT increases the content of reduced SH-groups and normalizes SOD and GSH-Px activities. The increase in glutathione content and induction of stress-protein synthesis improve protective reserves of the organism [3]. These data show that BRT produces a variety of effects on patients. BRT stimulates nonspecific mechanisms protecting the organism from damaging exogenous and endogenous factors.

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