

# Antioxidant Status of Erythrocytes after Acupuncture Treatment

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A course of acupuncture therapy in patients with locomotor, peripheral nervous system, gynecological, and bronchopulmonary diseases led to complete or partial normalization of nonspecifically changed MDA content, catalase and glutathione peroxidase activities in erythrocytes. SOD activity increased after therapy and did not differ from the control. Acupuncture stabilized disordered LPO processes and improved the antioxidant status of erythrocytes.

**Key Words:** *acupuncture; erythrocytes; superoxide dismutase; glutathione peroxidase; catalase*

Acupuncture is now used in many therapeutic institutions as an effective nonmedicamentous method of treatment. However, the main mechanisms underlying the effect of acupuncture on the health status of patients with various diseases remain unclear.

Oxidative stress resulting from imbalance between the formation and neutralization of active oxygen species is the main mechanism of cell damage in various pathologies, including rheumatoid arthritis, neurodegenerative, bronchopulmonary, and gynecological diseases, diabetes, atherosclerosis, and many other conditions [1-5]. Numerous antioxidants (among them antioxidant enzymes SOD, catalase, and glutathione peroxidase) protect biomembranes from the cytotoxic action of excessive oxygen metabolites. SOD catalyzes transformation of superoxide radicals into  $H_2O_2$ , which then transforms (under the effect of catalase) into water. Glutathione peroxidase reduces  $H_2O_2$  to water in the presence of glutathione and thus plays an important role in erythrocyte protection from oxidative damage.

According to published data, SOD activity in the plasma or serum of experimental animals decreases under pathological conditions, while LPO is nonspecifically intensified, but acupuncture normalized these parameters [6,7]. Biochemical mechanisms underlying the effects of acupuncture and its regulatory effect on the antioxidant system are little studied. It is not clear, for example, whether this treatment modality induces changes in erythrocytes contacting (through blood plasma) with all body cells. Erythrocyte membranes are highly sensitive to oxidative stress *in vivo*. These cells are most easily available under clinical conditions (are easily isolated and retain their functional activity *in vitro* for a long time).

We studied the antioxidant status of erythrocytes during acupuncture treatment.

## MATERIALS AND METHODS

Clinical studies were carried out at the Department of Rehabilitative Treatment, Hospital of Pushchino Research Center of Russian Academy of Sciences. A total of 198 patients aging 22-75 years with locomotor, peripheral nervous system, gynecological, bronchopulmonary diseases and asthenodepressive states were examined over 4 years.

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Acupuncture was carried out daily in an outpatient setting. Round needles of stainless steel (0.35 mm in diameter) were used. The duration of exposure was 15-20 min, the depth of the needle insertion depending on the emergence of specific sensations (dull pain, bulging out, pressure, heaviness, warmth, sensation of current). Of the general action points, GI11 (tsui-chi), E36 (Tzu-san-li), RP6 (san'-in'-tszyai), G14 (he-gu), MC6 (nei-guan'), TR5 (van-guan'), V60 (kun-lun'), etc., were used most often. The peripheral points were selected individually. The treatment of gynecological patients was supplemented by auriculopuncture. In this case microneedles in acupuncture points 55 (CNS), 22 (endocrine glands), 23 (ovaries), 28 (pituitary) were used. The patients received 2 courses of acupuncture, each course consisted of 9-11 sessions. The intervals between the courses were as a rule 1-1.5 months.

The treatment efficiency was evaluated by subjective sensations (sleeping, presence of pain, organ function, working capacity, appetite, and mood) and by objective parameters (pulse, BP, ultrasonic data, and laboratory analysis of the blood). Special attention was paid to clinical blood analysis as an informative and sufficiently available objective method of examination. The peripheral blood was collected after over-

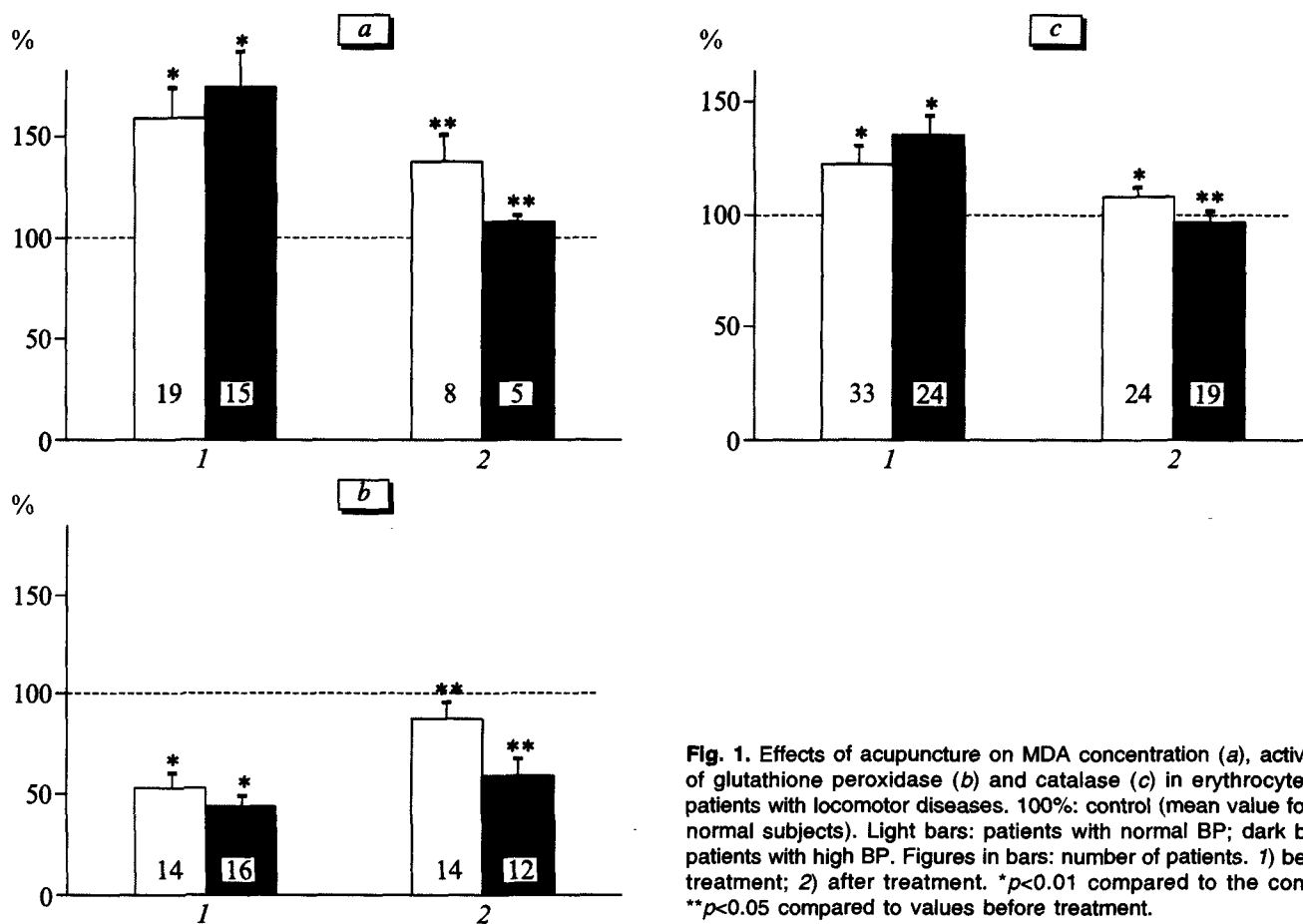
night fasting during the first and last days of each course of therapy.

For biochemical analysis the erythrocytes were isolated by centrifugation. Heparin (200  $\mu$ l, 10 U/ml) and 200  $\mu$ l blood were put into tubes, placed on ice, and the mixture was centrifuged at 1000g for 10 min. Erythrocytes were separated from the plasma and washed twice in cold 0.9% NaCl by centrifugation at 1000g for 10 min. Precipitated erythrocytes were suspended in 0.9% normal saline. In order to prepare lysate, 0.25 ml suspension of fresh or frozen erythrocytes were added to 0.5 ml buffer containing 50 mM triethanolamine (pH 7.4) and 0.02% saponine. MDA content, SOD, catalase, and glutathione peroxidase activities were measured in the lysates by routine methods.

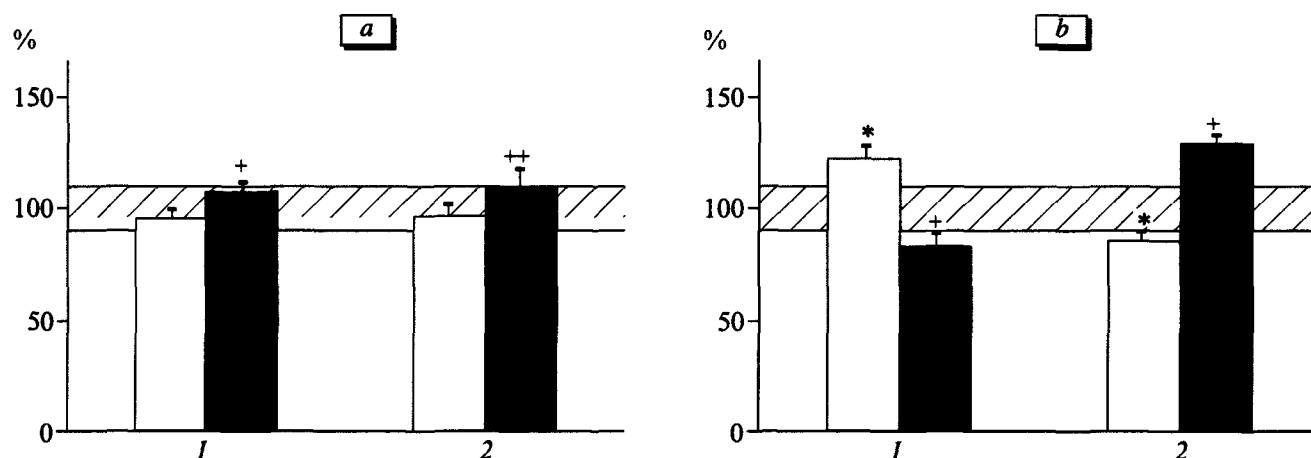
The results were statistically processed using Student's *t* test.

## RESULTS

A negligible BP decrease was observed in some patients during the course of acupuncture. After treatment BP generally did not change. The total count of leukocytes and lymphocyte/segmented neutrophil ratio in the peripheral blood normalized; the erythrocyte



**Fig. 1.** Effects of acupuncture on MDA concentration (a), activities of glutathione peroxidase (b) and catalase (c) in erythrocytes of patients with locomotor diseases. 100%: control (mean value for 30 normal subjects). Light bars: patients with normal BP; dark bars: patients with high BP. Figures in bars: number of patients. 1) before treatment; 2) after treatment. \* $p < 0.01$  compared to the control; \*\* $p < 0.05$  compared to values before treatment.



**Fig. 2.** Effects of acupuncture on erythrocyte SOD activity in patients with locomotor diseases. Light bars: before treatment; dark bars: after therapy. Cross-hatched area: control levels of enzyme activities in erythrocytes of 16 healthy volunteers; mean value 100%. *a:* 1) patients with normal BP; 2) patients with high BP. *b:* 1) patients with high BP, subgroup 1; 2) patients with high BP, subgroup 2. \* $p < 0.01$  compared to the control; + $p < 0.01$ , ++ $p < 0.05$  compared to parameters before therapy.

sedimentation rate decreased, and eosinophil count normalized (decreased in asthmatics). Heart rate did not change as a rule. Ultrasonic examination revealed no disorders in the viscera. Objective improvement of the health status after the course of acupuncture was observed in all patients. The general efficiency of this treatment was 79-91%.

Changes in the erythrocyte concentrations of MDA and activities of antioxidant enzymes were similar in patients with different diseases after a course of acupuncture. This paper presents quantitative data recorded in patients with locomotor diseases. These patients were divided into 2 groups: with normal and high BP. Twenty-seven patients had high BP before treatment and it remained high in 14 after a course of acupuncture.

The concentration of MDA (indicator of the intensity of membrane lipid peroxidation) in erythrocytes increased by 60-75% in patients of both groups in comparison with healthy subjects (Fig. 1, *a*). Activity of glutathione peroxidase, decreased 2-fold in both groups, increased after treatment. This parameter returned almost to the control level in patients with normal BP (Fig. 1, *b*). High catalase activity virtually normalized in both groups after treatment (Fig. 1, *c*). Changes in all three parameters of erythrocytes were similar in patients with normal and high BP.

The mean SOD activities in erythrocytes of patients with normal and high BP were similar and did not differ from the normal. After the course of acupuncture activity of this enzyme in cells of patients of both groups increased negligibly (by about 10%; Fig. 2, *a*).

Analysis of individual changes in SOD activities in patients with high BP showed that these patients can be divided into 2 subgroups. In subgroup 1 erythrocyte SOD activity before therapy was 23% higher

and in subgroup 2 patients it was 14% lower in comparison with the control (Fig. 2, *b*). After therapy erythrocyte SOD activity, increased in subgroup 1, decreased by 47% and SOD activity, decreased in subgroup 2, increased by 50% (Fig. 2, *b*).

Changes in erythrocyte antioxidant enzymes caused by acupuncture were never studied before. In general, our results confirm published reports that this treatment modality improves the physiological status of patients and promote normalization of some biochemical parameters of erythrocytes. We first described changes in activities of human erythrocyte antioxidant enzymes after acupuncture therapy and showed that the antioxidant defense system in erythrocytes is involved into biochemical mechanisms underlying the effects of acupuncture. Initially high SOD activity in erythrocytes of patients with high BP decreased, while initially low SOD activity increased after acupuncture therapy (Fig. 2). This phenomenon cannot be explained by the data available by today and requires further investigation.

Hence, acupuncture therapy led to subjective and objective improvement of the health status in the absolute majority of patients; none of 198 patients reported deterioration. Biochemically the treatment results manifested in adaptive changes in the quantitative parameters characterizing the oxidative stress (MDA) and antioxidant status (enzymes) of erythrocytes.

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