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Effect of Plasmapheresis and Laser Radiation on Red Blood Cells in the Complex Treatment of Myasthenia

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Abstract The main factor of myasthenia pathogenesis is the presence of autoantibodies to acetylcholine receptors in the blood. Myasthenia is accompanied by a significant increase the pathological forms of erythrocytes in peripheral blood. Plasmapheresis is the most effective way to remove antibodies from the blood. It has been found that plasmapheresis significantly increases the level of pathological forms of erythrocytes. Intravascular laser irradiation of blood is one of the most effective methods for preserving and restoring red blood cell forms. The authors investigated the effect of low-intensity laser radiation on erythrocytes in combination with plasmapheresis. Laser therapy in combination with efferent detoxification methods allows to eliminate antibodies from the blood, and on the other hand restores the optimal ratio among discocytes and pathological forms of erythrocytes.

Keywords Myasthenia, Erythrocytes, Laser radiation

1. Introduction

Myasthenia is an autoimmune disease due to the production of autoantibodies against acetylcholine receptors of efferent endings resulting in a defect in neuromuscular transmission. Depending on the involvement of various muscles the patient may have dysphagia or respiratory failure [8]. There are various methods of efferent detoxification such as hemosorption, plasmasorption and plasmapheresis which are effective ways of removing antibodies from the blood during myasthenia. Plasmapheresis is preferable among these methods [1]. All methods of modern medicine associated with the use of various devices with which the blood contacts are inevitably associated with mechanical and other effects on its cells. First of all, with effects on the most numerous cells that form its basis - red blood cells. Laser effects on the blood, both outside the body and with intravascular laser irradiation of blood, contribute to the reduction of pathological forms of red blood cells and an increase in the proportion of discoocytes. Low-intensity laser radiation (LILR) is used for intravascular blood irradiation in various pathological conditions including myasthenia [2]. Scanning electron microscopy is an indispensable method for assessing the effect of LILR on changing red blood cell forms [4-7]. But this method is laborious and expensive, requires special equipment.

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The "Rapid Thick Drop Method" (RTDM) is used to evaluate the effect of detoxification on red blood cells before and after plasmapheresis. This method was developed in the laboratory of our Center and takes only 10-15 minutes. [2].

This research presents the results of studying the change in the shape of red blood cells in patients with myasthenia before and after plasmapheresis, as well as using intravascular laser irradiation of blood (ILIB) in combination with plasmapheresis.

Objective: to study the effect of laser radiation and plasmapheresis on erythrocytes in the complex treatment of myasthenia.

2. Materials and Methods

The condition of 23 patients having received inpatient treatment was clinically examined and analyzed. There were 9 males and 14 females. Males' age varied from 21 to 50 years, females' age – from 20 to 55 years. In all patients the diagnosis was previously confirmed by clinical tests, including prozerin test, electromyographic data, CT / MRI investigations of the anterior mediastinum. In 21 cases a generalized form of myasthenia according to the distribution of muscle weakness was detected: with a predominance of movement disorders – 9, with respiratory function disorders – 2 cases. A bulbar form of myasthenia was detected in 2 patients. Comparison of myasthenia forms in men and women showed the following: in men a generalized form with a predominance of movement disorders (4 cases) and with respiratory function disorders (5 cases) was observed. A generalized form of myasthenia was detected in 12 women: in 7 cases – with respiratory function disorders, in 5 women –

with a predominance of movement disorders, and in 2 cases – a bulbar form of myasthenia (Tab.1).

Table 1. The distribution of patients by sex and myasthenia forms

№	The form of myasthenia	Male	Female	Total
1	Generalized form with a predominance of movement disorders	4	5	9
2	Generalized form with respiratory function disorders	5	7	12
3	A bulbar form	-	2	2
4	Total	9	14	23

Patients with a generalized form of myasthenia with a predominance of movement disorders complained of the limbs, the body muscles weakness and inability to climb the steps after exercise. Patients with a generalized form of myasthenia with respiratory function disorders complained of breathing difficulty after exercise which weakened after resting or taking anticholinesterase drugs. Patients with the bulbar form of myasthenia complained of hoarseness, "nasal" tone of voice, its "fading", slurred speech and swallowing difficulty.

Patients (n = 34) were divided into the following groups:

I control group – healthy people (n = 11)

Group II – patients with a diagnosis of myasthenia before treatment (n = 23)

Group III – patients after thymectomy (n = 23)

Group IV – patients after surgery who underwent plasmapheresis with laser irradiation "IVA" and without laser therapy "IVB" (the blood of the IVB group was studied after the 1st and the 3rd plasmapheresis sessions) (n = 12).

Group V – patients after surgery were performed a plasmapheresis session with simultaneous blood irradiation through the incoming plasma filter circuit (n = 11). It should be noted that patients of groups II and III are the same patients before and after thymectomy which were subsequently divided into two groups.

8 patients were performed intravascular blood irradiation (IVBI) using the "Matrix-VLOK" device (Russia) (output power 1.5 MW, wavelength 0.63 μm, exposure 20 min.) with special needles. 8 patients were undergone plasmapheresis using the "Hemophenix" device. 6 patients were performed IVBI and plasmapheresis.

For the "Rapid Thick Drop Method" (RTDM), 1-2 drops of blood were fixed in glutaraldehyde and immediately placed on glass and covered with a cover glass. Red blood cells were counted and photographed using a light microscope. The duration of the procedure took 10-15 minutes. To calculate the proportion of erythrocyte forms by RTDM, 1000 erythrocytes were taken in each portion of blood. The data were subjected to statistical processing.

The standard scale of muscle strength of the limbs proposed by A. Szobor (1976) was used for the clinical assessment of the severity of movement disorders:

0 point – no movement in the muscle;

1 point – there are minimal movement in the muscle, but the patient does not hold the weight of his own limb;

2 points – the patient holds the weight of his own limb, but the resistance to the researcher is minimal;

3 points – the patient resists efforts to change the position of the limb, but it is insignificant;

4 points – the patient resists well the efforts to change the position of the limb, but there is some reduction in strength;

5 points – muscle strength corresponds to the age and constitutional norm.

The clinical efficacy of treatment tactics for inpatient patients was estimated by the dynamics of reducing myasthenic deficiency, changes in the range of motion in the affected muscles, as well as changes in the basic doses of anticholinesterase drugs, prednisolone obtained before inpatient treatment. At the time of admission to the hospital, the average total assessment of the state of muscle strength in patients in all groups was 2.4 ± 1.7 points. All 23 patients were performed timectomy.

The scheme of G. Keynes (1949) in the following modification was used for the analysis of the obtained results:

A – excellent effect (full restoration of motor functions, availability without medical support);

B – good effect (significant improvement, almost complete restoration of motor function and availability with a significant decrease in the daily dose of anticholineesterase drugs compared to preoperative (2 times or more) and with virtually no need for immunosuppressive therapy;

C – satisfactory effect (improvement of motor function against the background of the previous number of anticholinesterase drugs and sometimes prednisolone, the absence of disease progression);

D – no effect in improving the state;

E – lethality.

3. Results and Discussion

After surgery in the early stages, 10 days after surgery, the "B" effect was observed in 5 patients: in 2 patients with bulbar form and in 3 patients with generalized form of myasthenia with a predominance of movement disorders. A satisfactory effect "C" was observed in 5 patients with a generalized form with a predominance of movement disorders and in 1 patient with a generalized form with respiratory function disorders. The effect "D" was observed in 12 patients: 1 patient with a generalized form with a predominance of movement disorders and 11 patients with a generalized form with respiratory function disorders.

Patients with severe myasthenia and no effect after timectomy (n = 12) were performed membrane plasmapheresis in 3 sessions (session duration 60-90 minutes; 1000-1200 ml of plasma was removed in one session), with and without irradiation by an infrared laser. Comprehensive inpatient treatment gave a significant improvement in the form of increasing in strength in all muscle groups by 1.5-2.5 points, in 3 patients with a generalized form with dysfunction of breathing - a slight improvement, with an increase in

strength of not more than 1 point.

In the study of red blood cells after plasmapheresis, the following results were obtained:

In normal conditions most erythrocytes are in the form of discocytes (biconcave disc) (Fig. 1).

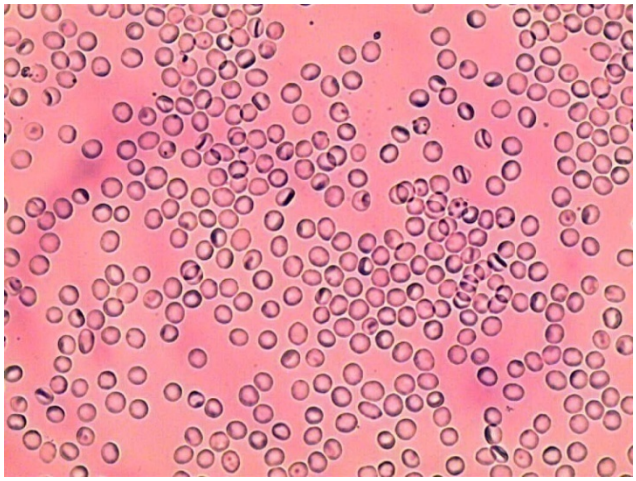

Figure 1. Control. The predominance of discocytes in the peripheral blood. RTDM 10x40

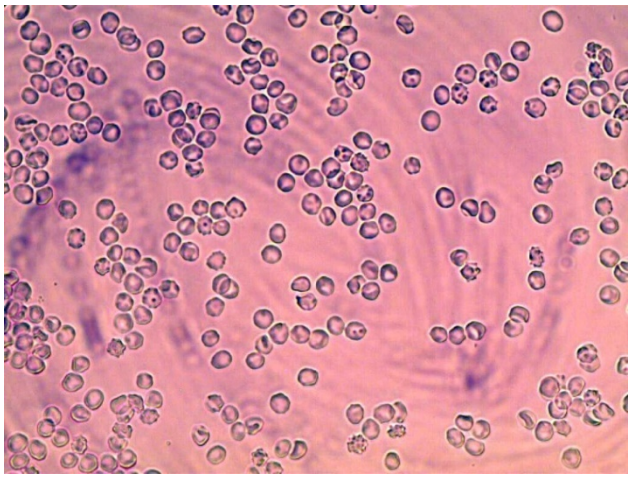

Figure 2. A large number of pathological forms of erythrocytes. Blood of a patient with myasthenia without treatment. RTDM 10x40

For normal functioning red blood cells must retain their shape and elasticity which allows them to change shape for passing through arterioles, where gas exchange takes place, the lumen of which is smaller than the size of the red blood cell. The potential of discocytes to change their shape depends on the physical and chemical properties of their membranes. Discocytes turn into echinocytes when the volume of calcium ions in the cells increases, and the volume of ATP decreases, or the level of bile acids in the blood rises. The transformation of the discocytes into stomatocytes is caused by an increase in ATP concentration [2, 4-7]. Myasthenia is accompanied by a significant increase of erythrocytes pathological forms (echinocytes, stomatocytes, etc.) in peripheral blood (Fig. 2.).

The calculation of the ratio of various forms of erythrocytes in patients with myasthenia showed that they have significantly reduced proportion of discocytes in the peripheral blood (Tab.2).

Counting the number of discocytes and pathological forms of erythrocytes in patients with myasthenia after ILIB, plasmapheresis and a combination of these methods revealed significant differences in the proportion of discocytes, echinocytes and stomatocytes. ILIB leads to a decrease of pathological forms of erythrocytes and an increase of discocytes- erythrocytes. Plasmapheresis increases the level of pathological forms of red blood cells (Fig. 3).

Laser therapy in combination with efferent detoxification methods lead not only to the elimination of antibodies from the blood, but also restores the optimal ratio among erythrocytes (discocytes and erythrocytes pathological forms) (Fig. 4.).

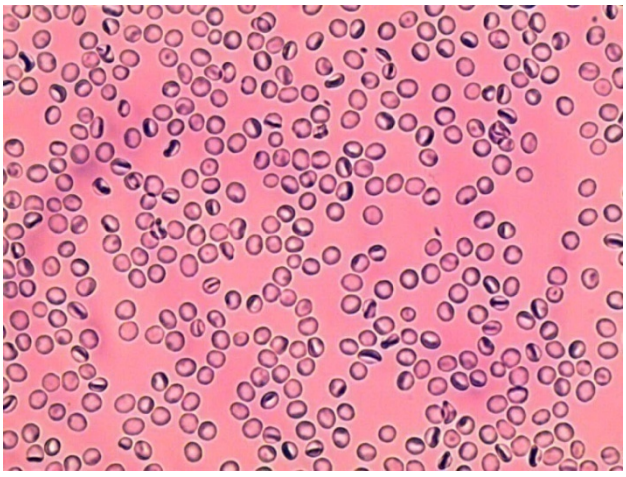

Figure 3. Patient's blood with myasthenia after plasmapheresis. The prevalence of pathological forms of erythrocytes is preserved. RTDM 10x40

Table 2. Ratio of erythrocyte forms in patients with myasthenia before, after plasmapheresis and efferent laser therapy (%)

Erythrocytes form	Norm	Before thymectomy	After surgery without laser therapy	1 plasmapheresis session	3 plasmapheresis sessions	3 plasmapheresis sessions with efferent laser therapy
Discocytes	89±0.5	62±1 %*	72.5±2.0 %,	70±1**	68±1**	82±1**
Echinocytes	9±0.3	17±0.5*	11.6±1.6 %	12±0.6**	12±0.1**	6±0.01**
Stomatocytes	1±0.05	16.0±0.4*	14.7 ±1.8	15 ±0.04**	14 ±0.02**	6 ±0.04**
Erythrocytes with crest	0.5±0.02	3±0.03*	2±0.01	2±0.01	5 ±0.01	5 ±0.02
Irreversible forms	0.5±0.05	2±0.01*	1.2±0.1%.	1±0.01	1±0.01	1±0.01

Note: *reliable to the control;

** reliable to Group I (thymectomy without laser therapy) ($p<0.05$).

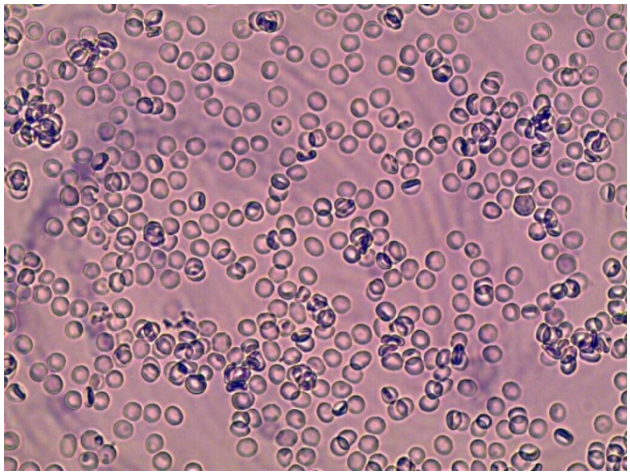

Figure 4. Patient's blood with myasthenia after plasmapheresis with blood irradiation by LILR. An increase in the number of discocytes is observed. RTDM 10x40

Uneven absorption of low-intensity laser radiation and light by various structures causes thermal disequilibrium in biological tissues and can lead to deformations of cell membranes, changes in their electrical potential which affects the rate of vital processes in biological tissues. This multistep process can be represented as follows: absorption of light quanta, the primary photophysical act, intermediate stages, including the formation of photosensitive products in tissues or energy transfer on cell membrane components, the formation of physiologically active compounds in tissues, the inclusion of neurohumoral reactions, the final photobiological effect. Laser therapy is almost universal and is a fundamental component of the complex therapy of myasthenia due to the effect on the subcellular and cellular levels of the organization of living matter. Our data allow us to recommend plasmapheresis and intravascular laser irradiation of blood in the treatment of myasthenia.

4. Conclusions

The proportion of pathological forms of red blood cells in the peripheral blood increases at myasthenia. Plasmapheresis increases the proportion of pathological forms of red blood cells in peripheral blood. Irradiation of blood with a laser (wavelength of 0.63 microns) simultaneously with plasmapheresis significantly reduces the number of pathological forms of red blood cells.

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