# Chapter 30
# Plasmapheresis and Laser Therapy in Complex Treatment of Myasthenia and their Influence on Erythrocytes and Endothelium

**I.M. Baybekov, Sh.Z. Kasimov, J.A. Ismailov, B.A. Saidkhanov, and A.Kh. Butaev**

**Abstract** The main factor in the pathogenesis of myasthenia gravis is the antibody mediated autoimmune response to acetylcholine receptors (AChR). Myasthenia is accompanied by significant increase in pathological forms of erythrocytes in peripheral blood. Plasmapheresis is the most effective way to eliminate antibodies from blood. However, plasmapheresis significantly increases the level of pathological forms of erythrocytes. Endovascular laser blood irradiation (ELBI) is one of the most effective methods of erythrocyte preservation and restoration. ELBI is conducted to decrease the level of pathological forms of erythrocytes and increase the level of discocytes – the normal form of erythrocytes. The investigation of low intensive laser irradiation (LILI) effect on blood vessel walls revealed that ELBI lasting longer than one hour causes alteration in endothelial cells. But endothelial cells recover very soon post ELBI. Laser therapy in combination with efferent methods of detoxification such as plasmapheresis allows elimination of antibodies from blood and restores the optimal ratio between discocytes and pathological forms of erythrocytes.

**Keywords** Plasmapheresis • Laser therapy • Myasthenia • Ultrastructure

## 30.1 Introduction

Myasthenia gravis is an autoimmune disease resulting from production of autoantibodies against AChR at the motor end plate, causing defects in neuromuscular transmission. Depending on the muscles affected a patient may develop dysphagia or respiratory failure [1]. The appearance of pathological forms of erythrocytes such as stomatocytes, echinocytes etc., in peripheral blood causes microcirculation disorders [2].

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Efferent methods of detoxification such as haemosorption (haemoperfusion), plasma sorption (plasma perfusion) and plasmapheresis are the most effective ways to eliminate antibodies from the blood at myasthenia. Among these methods plasmapheresis is the preferred choice [3].

It is known that any sorption method used for blood purification increases the quantity of pathological forms of erythrocytes. Endovascular laser blood irradiation (ELBI) is the most effective method of preserving and restoring erythrocytes however, application of ELBI in the clinic is largely based on empirical evidence. The influence of ELBI on the main blood vessel's walls through which the irradiation is performed has not been clarified.

On the other hand, low intensive laser irradiation (LILI) was used for endovascular treatment of blood for different pathological conditions including myasthenia [4]. However the influence of the LILI on blood cells and particularly on erythrocytes has not been investigated.

In this work we have studied the endothelial microrelief alteration of main arteries and veins in the zones where the waveguide was placed. The variation of erythrocyte forms influenced by ELBI, their morphological changes induced by LILI using He-Ne laser (HNL) and UV Nitrogen laser (UVL) in vitro and in small animals (rabbits), and erythrocyte structure in patients with myasthenia before and after plasmapheresis have been investigated. The complex usage of ELBI together with plasmapheresis has been studied.

## 30.2 Material and methods

Chinchilia rabbits were used to study ELBI effect on microrelief of endotheliocytes [3, 4]. The waveguide connected to HNL –LG -75 (Russia) was inserted into the aorta or femoral vein under ether anaesthesia. The irradiation power on the end of the waveguide was 8 mW. The vessels underwent perfusion fixation after 10 min, 30 min, 1 h, 6 h and 1–2days following the completion of irradiation with 2.5% glutaraldehyde in phosphate buffer for 15 min. The vessel segments after perfusion were cut and fixed with 2.5% glutaraldehyde in phosphate buffer for 24 h. The segments were dehydrated using a series of water-acetone mixtures with progressively increasing acetone content and critical point dried using $CO_2$ in HCP-2 (Hitachi). Dried specimens were mounted on a metal brass stub with conductive paint and coated with gold in Eiko IB-3. Samples were examined in a Hitachi S-405A scanning electron microscope operated at 15 kV.

ELBI effect on the morphology of erythrocytes was studied in seven rabbits. ELBI was performed by introducing the waveguide into the lumen of the femoral vein under ether anesthesia. He-Ne laser LG-75 (HNL), Russia, and nitrogen ultraviolet laser LGI-21 (UVL) were used. The power of radiation at the end of waveguide was 2.5 mW, and irradiation was applied for one hour.

HNL and UVL direct effect on human erythrocytes was studied using fresh donor blood. The blood was placed into a quartz flask, and the end of the waveguide

connected to the corresponding laser or white light source was immersed into the blood. The irradiation of 2.5 mW was applied for 30 min and samples were taken each 5 min for analysis. For SEM investigation erythrocytes were placed on metal plates covered with a thin layer of gelatin. After 30 s the plate was immersed into 2.5% glutaraldehyde solution for 1 h and was processed as described above.

SEM is an informative method of studying erythrocyte forms [5–8]. However this method needs special equipment and time associated with sample preparation and analysis. The "Thick Drop Express Method" - TDEM developed in our group, has been used for the evaluation of erythrocyte detoxification before and after haemoperfusion. Briefly, one or two drops of blood incubated in glutaraldehyde was immediately placed on a glass slide and covered with a cover glass. Erythrocytes number was counted using light microscopy (this procedure takes 10–15 min). The peripheral blood of 43 patients with myasthenia was analysed using this method. Eight patients underwent ELBI using the apparatus "Matrix –VLOK" (Russia) with special needles. Plasmapheresis was carried out in all 8 patients using a "Hemophonix" device. Complex effect of ELBI and plasmapheresis was studied in 6 patients. The control group comprised 21 patients. Erythrocytes with different forms were counted in a portion of blood with 1,000 red cells. Statistical analysis of the data was performed.

## 30.3 Results and Discussion

The luminal surface of aorta is folded, and the folds reflect the goffers of the inner elastic membrane. The aorta's intima is covered with an entire layer of endotheliocytes. The surface of these endotheliocytes is fine folded (Fig. 30.1a). The luminal surface of the femoral vein is covered by endotheliocytes, whose plasma membrane is also fine folded. The bounds which separate endotheliocytes appear as irregular lines (Figs. 30.1a–30.2a).

ELBI for 15 min causes alteration of endotheliocytes of the luminal surface of the aorta. The fine folds of their surface become smooth. The endotheliocytes swell and intercellular space is increased denuding the basal membrane. These changes have been noticeable within 1 h after irradiation and completely disappeared after 6 h.

Prominent changes in the cells have been revealed after 30 min of irradiation. Besides the oedema of the cytoplasm and nuclei swelling, crater-like erosions appeared on the luminal surface of the endotheliocytes (Fig. 30.1b). Intercellular spaces became wider, and fibrin thread and single erythrocytes were found in these spaces. In some zones the longitudinal folds of the intima were smoothed, but the endothelial layer remained intact. Such zones may correspond to the sub-endothelial oedema. These changes remained for 6 h after irradiation. One day later the microrelief of the aorta's intima returned to normal.

Irradiation for 60 min caused more damage. Partial detachment and desquamation of endotheliocytes from the basal membrane took place (Fig. 30.1c). Thrombocytes, fibrin threads and erythrocytes were found on the surfaces of denudated zones.

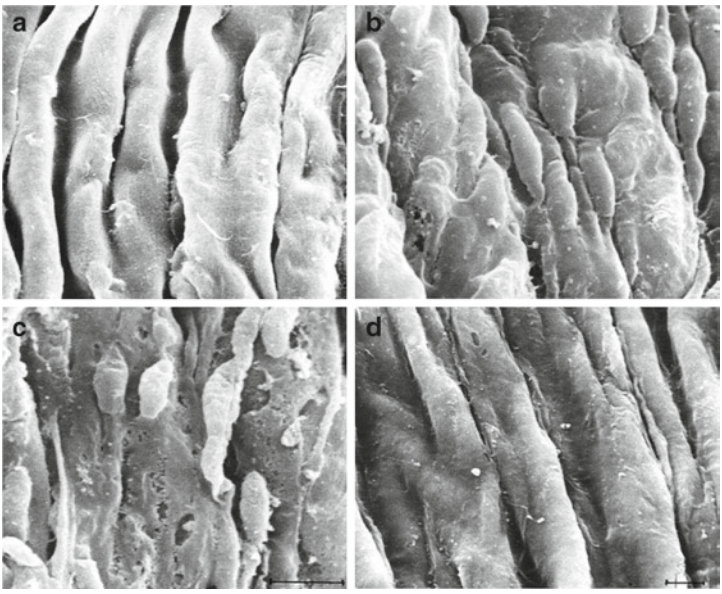


**Fig. 30.1** Luminal surface of the rabbit's aorta intima after ELBI with HNL. (**a**). Microrelief of the luminal surface of intact aorta. (**b**). Microerosions and swellings of supranuclear parts of endotheliocytes after 30 min irradiation. (**c**). Desquamation of some endotheliocytes after 60 min irradiation. (**d**). Microrelief of the aorta intima two days after 60 min irradiation

These changes were considerably less noticeable 6 h after the irradiation and completely disappeared after two days (Fig. 30.1d). The formation of thrombi did not take place.

The irradiation of the vein for 15 min caused smoothing of the folds and wrinkles on the endotheliocyte surface. The cell swelling influenced the intercellular space which became wider (Fig. 30.2b).

Irradiation for 30 min resulted in an increase in the intercellular space and led to the formation of crater-like defects on the cell surface. The microrelief normalised 6 h later (Fig. 30.2c).

Irradiation for 60 min resulted in the desquamation of some cells from the basal membrane and the formation of denudated areas of the intima. The affected areas were covered with thrombocytes, erythrocytes and fibrin threads. But 1–2 days after irradiation the intima microrelief of the vein returned to normal. There were no thrombi formations either in the aorta or the vein.

Thus the effect of ELBI on the aorta and vein endotheliocytes depends on the duration of irradiation. Irradiation for 15 and 30 min caused reversible changes which were expressed in changes of the normal cell forms, appearance of crater-like depressions and surface defects, swelling of nuclei and oedema. Irradiation for 60 min had a more pronounced effect on the inner surface of blood vessels resulting in detachment of endotheliocytes from the basal membrane and their desquamation. Restoration of the endothelial structure after 15 and 30 min of laser irradiation

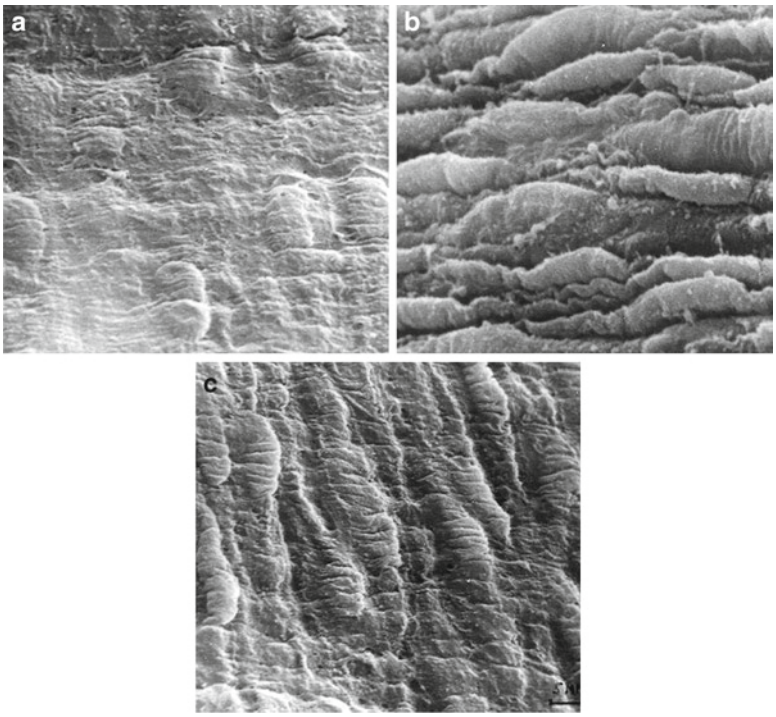


**Fig. 30.2** Luminal surface of the rabbit's femoral vein after ELBI with HNL. (**a**). Microrelief of the luminal surface of intact vein. (**b**). Swelling of the supranuclear parts of endotheliocytes after 15 min irradiation. (**c**). Microrelief of the luminal surface of vein two days after 60 min irradiation

occurred within 6 h. After 60 min of irradiation the microrelief of the endothelial layer returned to normal one day later.

SEM is a more informative method of studying erythrocyte form. It allows examining changes in the erythrocyte shape and the surface microrelief [4–8].

Normal erythrocytes must retain their shape and elasticity, which is very important for the microcirculation. In normal conditions most erythrocytes have the form of discocytes (concave-concave disc) (Fig. 30.3a). The ability of discocytes to change their form depends on physical and chemical properties of their membranes. Discocytes transform into echinocytes when the volume of calcium ions in the cell increases and the volume of ATP decreases, or the level of bile acid in blood increases. The transformation of discocytes into stomathocytes is caused by increase of ATP concentration [4–8]. A study of erythrocytes after 60 min irradiation did not reveal any significant changes in the erythrocyte form. The dominant form of erythrocytes remained discocytes (Figs. 30.3b,c). Thus ELBI *in vivo* did not affect the erythrocyte form. Moreover, LILI prevented appearance of pathological forms of erythrocytes and may restore pathological forms into discocytes.

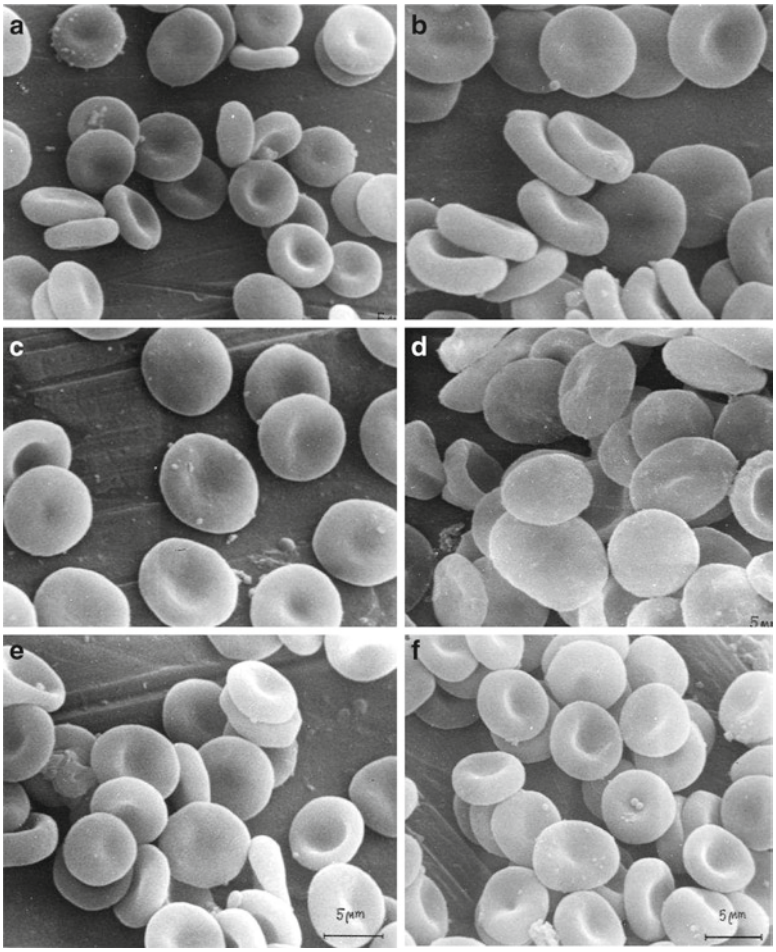


**Fig. 30.3** Erythrocyte forms after irradiation with HNL and UVL. (**a**). Erythrocytes of control rabbit. (**b**). Erythrocytes after 60 min ELBI with HNL. (**c**). Erythrocytes after 60 min ELBI with UVL. (**d**). Erythrocytes after *in vitro* irradiation with white light. Transformation into stomatocytes. (**e**). Erythrocytes after *in vitro* irradiation with HNL. (**f**). Erythrocytes after *in vitro* irradiation with UVL

SEM investigations have shown that irradiation of blood with white light caused an increase in the number of stomatocytes transformed from erythrocytes. Before irradiation the volume of discocytes was 89.3 ± 1.6%, echinocytes 8.2 ± 0.5% and stomathocytes 1.9 ± 0.06%, and after 15 min irradiation the volume of discocytes decreased to 72 ± 2.4%, but the volume of stomathocytes increased to 16.4 ± 0.6%. The volume of echinocytes remained unchanged. Irradiation for 30 min caused an increase in the number of stomathocytes to 27.5 ± 2.8% (Fig. 30.3d).

HNL irradiation of blood did not cause any changes in volume of all above mentioned forms of erythrocytes. The same results have been obtained after the irradiation of blood with UVL (Figs. 30.3e,f). Thus LILI of blood resulted in better conservation of erythrocytes *in vitro*.

Myasthenia is accompanied by a significant increase in the number of pathological forms of erythrocytes such as echinocytes and stomatocytes, in peripheral blood, and so does plasmapheresis. Thus ELBI was conducted to decrease the number of pathological forms of erythrocytes and to increase the number of discocytes, which are normal erythrocytes. Treatment of patients with myasthenia by ELBI and plasmapheresis revealed significant differences in the ratio of discocytes, echinocytes and stomatocytes. ELBI decreased the level of pathological forms of erythrocytes and increased the level of discocytes (Figs. 30. 4 and 30. 5).

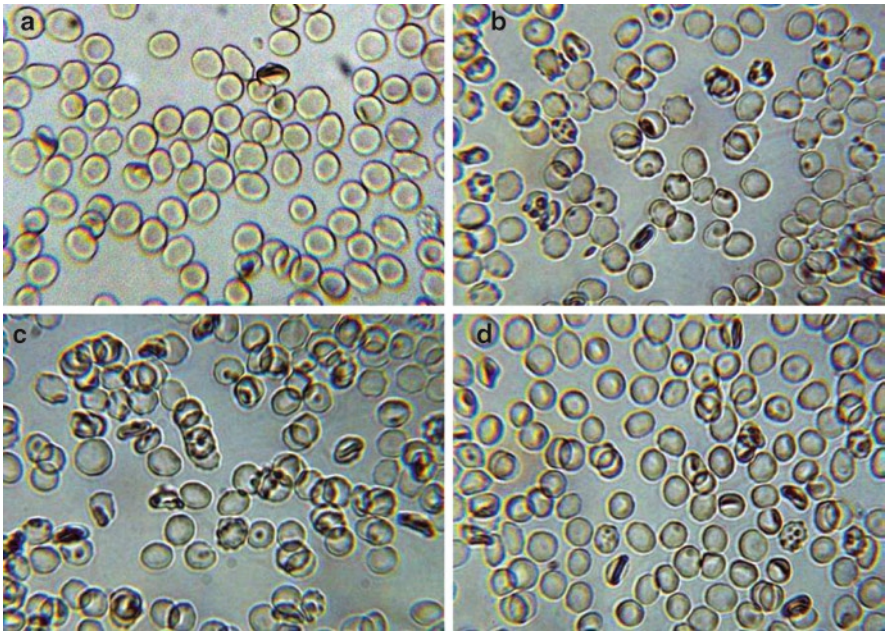


**Fig. 30.4** Various forms of erythrocytes in "Thick Drop" at different conditions. (1). Control. Domination of discocytes in peripheral blood. 10×40. (2). Myasthenia. Numerous pathological forms of erythrocytes. 10×40. (3). Increase of the number of pathological forms of erythrocytes after plasmapheresis. 10×40. (4). Decrease of the number of pathological forms of erythrocytes after ELBI. 10×40

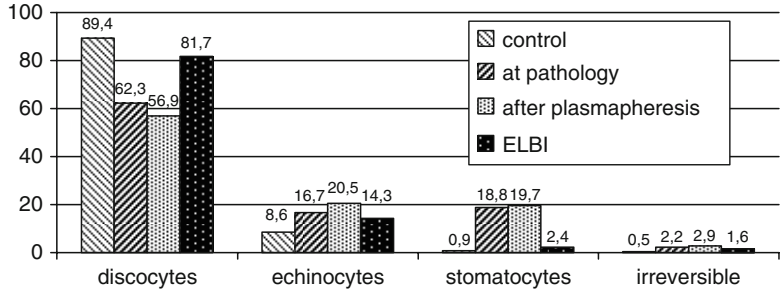


**Fig. 30.5** The ratio of different forms of erythrocytes in the blood of patients with myasthenia after plasmapheresis and ELBI

## 30.4 Conclusion

Laser therapy in combination with efferent methods of detoxification effectively eliminates AChR antibodies from blood and restores an optimal ratio between discocytes and pathological forms of erythrocytes. On the basis of our data, plasmapheresis can be recommended for use together with the endovascular laser blood irradiation, ELBI in treatment of myasthenia. The duration of ELBI should not exceed 30 min.

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