Water Status and LPO in Rat Tissues during Massive Blood Loss and Irradiation with He-Ne Laser

A. A. Roshchina, S. S. Nikolaeva, V. L. Kozhura*, Yu. O. Teselkin, I. V. Babenkova, L. V. Yakovleva, V. A. Bykov, I. S. Novoderzhkina*, D. A. Basarab*, L. B. Rebrov, E. Yu. Parshina*, and A. K. Kirsanova*

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We revealed a relationship between water balance and LPO in the myocardium, liver, and blood plasma during massive blood loss and irradiation with He-Ne laser. Low-intensity laser irradiation of the plasma inhibits LPO and normalizes water balance in rat tissues during massive blood loss.

Key Words: *blood loss; myocardium; liver; water; LPO; laser irradiation*

Massive blood loss is a leading cause of death in the large-scale and man-caused accidents. It ranks third after asphyxia and crush syndrome by the number of victims in accidents. Hypoxia and ischemia are the leading pathogenic mechanisms triggered by massive blood loss and hemorrhagic shock. They are accompanied by considerable circulatory disturbances providing conditions for changes in biochemical indices, shift of water-electrolyte balance, and the development of oxidative processes [9,13]. According to current views, water is a necessary component of all biological systems directly affecting the formation and stabilization of native structure of biopolymers, biological membranes, and more complex supramolecular structures in norm and pathology [1,5,6,8,11]. LPO plays an important role during ischemic pathology, where it produces damage to all components of cell membranes [12,14]. Therefore, it is vitally important to inhibit LPO and support water balance in tissues at critical states. At present, low-intensity laser irradiation is widely used in clinical practice as cytoprotective therapy in critical and terminal states [2,3].

Our aim was to study the state of water and intensity of LPO in the myocardium, liver, and blood plasma in rats subjected to massive blood loss and treated with He-Ne laser irradiation.

VILAR Research, Training, and Methodological Center of Biomedical Technologies; Research Institute of General Resuscitation, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

The study was carried out on male albino random-bred rats weighing 230-330 g. In series I, the rats (n=21) were sacrificed 10 min after sham operation or experimental blood loss, which decreased mean arterial pressure to 40 mm Hg. In series II, the rats (n=18) were sacrificed 2 h after the same procedures. In each series the rats were subdivided into 3 groups: the first (control) group comprised sham-operated rats (n=6-9), the second (untreated) group was subjected to arterial hypotension only (AH, n=6-7), and the third (treated) group was composed of rats subjected to AH and irradiated with He-Ne laser (n=6-7).

AH was produced by bloodletting via a catheter inserted into the caudal artery. The mean blood loss was 20 ml/kg.

The light guide from an ALOK-1 He-Ne laser (λ=633 nm, 1 mW beam power) was placed in the region of the caudal vein and artery. Irradiation was performed for 2 min, and it was started on minutes 5-8 of bloodletting [4].

Myocardium and liver biopsy specimens and blood plasma were examined. Total water was determined by the method of Fisher, free water was assessed by

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the amount of frozen water measured by differential scanning calorimetry, and bound water was calculated as the difference between total and free water [7]. LPO intensity in organs and blood plasma was assessed by the content of 2-thiobarbituric acid metabolites reactive substances (TBARS), which were determined by fluorimetry [15].

RESULTS

The total water content in the myocardium did not change 10 min after blood loss (Fig. 1); the content of free water decreased, while the content of bound water increased 1.4-fold ($p<0.05$). After a 2-h AH the relative content of various water forms in the myocardium returned to normal (initial) values. Blood loss induces a compensatory reaction, which modifies the functional state of the myocardium: the rate of myocardium contractions decreases and hypoxia develops, which results in acidosis. These processes disturb water-electrolyte balance and lead to accumulation of osmotically active components in cells, thus promoting water redistribution from the extracellular to the intracellular space. During AH period (10 min or 2 h) myocardial level of TBARS did not differ from the control. Probably, thickened multilayer hydrate shell covering the active groups in myocardial biopolymers protects these groups from oxidation. Preliminary irradiation produced no changes in TBARS content and proportion of various water forms in the myocardium. These data corroborate the views on high antioxidant activity in the myocardium [10].

No changes in the total water content in the liver and myocardium were observed after 10-min AH, although the proportion between the bound and free water changed (Fig. 2). In contrast to the myocardium,

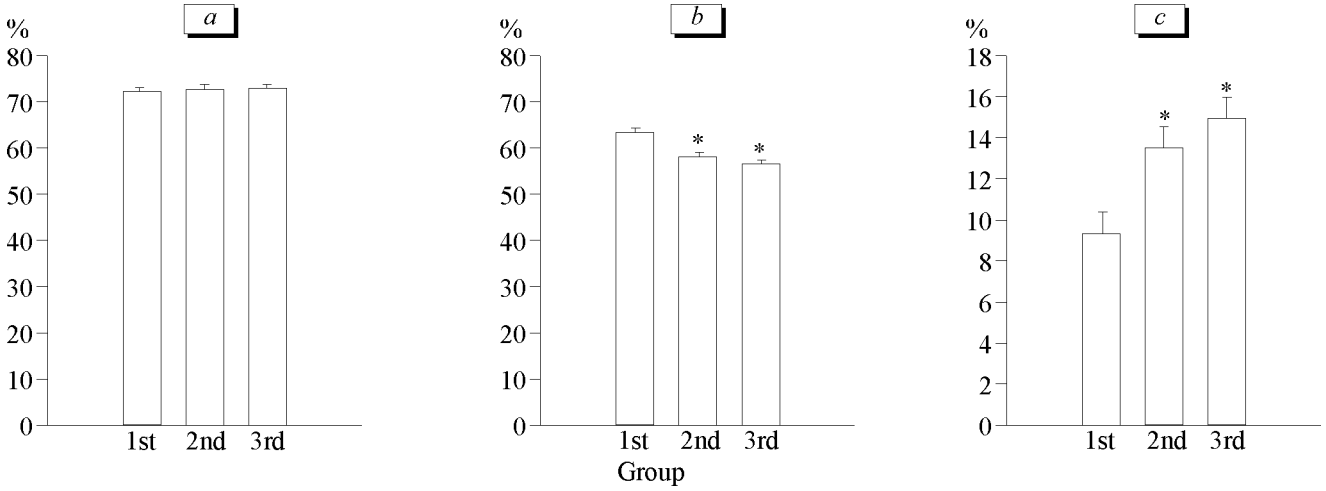

Fig. 1. Effect of blood loss (10 min) and laser irradiation on the content of total (*a*), free (*b*), and bound (*c*) water in rat myocardium. Here and in Figs. 2 and 3: *$p<0.05$ compared to the first group.

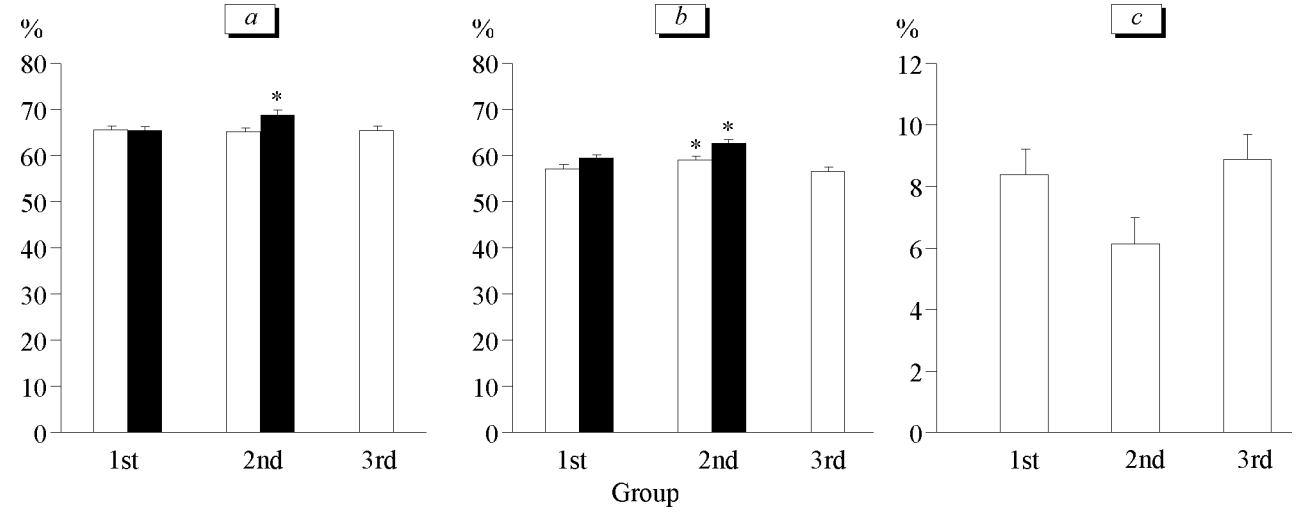

Fig. 2. Effect of blood loss and laser irradiation on the content of total (*a*), free (*b*), and bound (*c*) water in rat liver. Duration of arterial hypotension 10 min (light bars) or 2 h (solid bars).

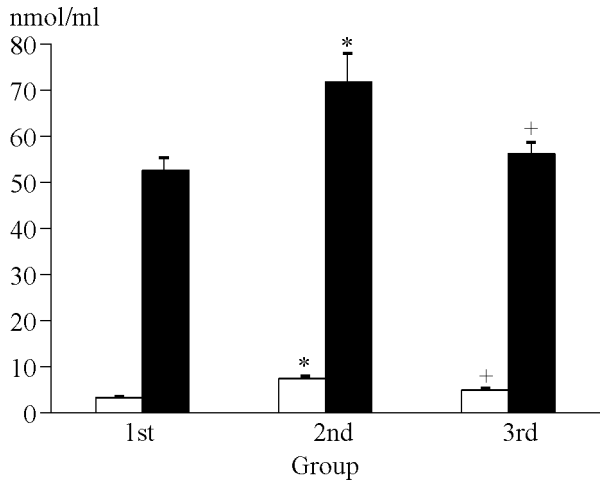

Fig. 3. Effect of blood loss and laser irradiation on TBARS content in rat plasma (light bars) and liver (dark bars). $^{+}p<0.05$ compared to the second group.

the content of free water in the liver increased, and the content of bound water decreased 1.5-fold ($p<0.05$). During the subsequent 2-h AH, the increase in the content of free water led to a significant increase in total water content by 5% ($p<0.05$), which attests to hyperhydration of hepatocytes. The content of bound water remained unchanged. These changes in the content of total, bound, and free water suggest that the liver is most susceptible to ischemic damage. It is known that acute blood loss decreases blood volume and blood velocity in the terminal hepatic vessels. It can be hypothesized that the increase in free water content at the expense of bound water during blood loss is a compensatory mechanism stabilizing systemic hemodynamics and regional macro- and microcirculation. When preliminary laser irradiation was carried out, the content of free and bound water in the liver approached the control values.

No significant changes in TBARS content in the liver and blood plasma were observed (Fig. 3). LPO in the liver and blood plasma increased only after 2-h AH, which led to accumulation of TBARS in the liver and blood plasma by 1.4 and 2.3 times, respectively ($p<0.05$). We hypothesize that the decrease in bound water content observed in this period leads to exposure of active centers of biopolymers in cell membranes and facilitates their oxidation with free-radical oxygen derivatives. Since water redistribution between fractions was preceded by the increase in TBARS content, the oxidative processes induced by AH are probably preceded by the breakage of hydration shells around the active centers in biopolymers, which trigger LPO processes.

After preliminarily irradiation of blood plasma the content of LPO metabolites in liver cells remained at the control level, while the level of these metabolites in the plasma significantly decreased but remained above the control. It can be hypothesized that laser irradiation prevents LPO activation. The fact that laser irradiation not only inhibits the development of LPO, but also prevents changes in the proportions of water fractions in tissues probably attests to interdependence of these processes. It is known that oxidative processes destroy biopolymer macromolecules, expose active groups of these biopolymers, and change their structure, which can induce further changes in water balance in blood and tissues. It cannot be excluded that at some stage of blood loss oxidation processes pass ahead and/or go in parallel with dehydration of protein molecules and promote this process. Therefore, in some cases the procedures preventing oxidative processes during acute and massive blood loss are necessary.

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