EVALUATION OF THE FEATURES OF MICROCIRCULATORY REACTIONS UNDER THE ACTION OF DINITROSYL IRON COMPLEXES IN THE NORM AND ON THE MODEL OF THERMAL TRAUMA

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Abstract. The actuality of the problem of thermal trauma is determined by its high prevalence. Severe disorders of central, regional and peripheral hemodynamics are developing with a predominant violation of microcirculation and metabolic processes in the body during burn disease. Promising NO donors suitable for biomedical use are dinitrosyl iron complexes (DNIC). The aim of the study was to evaluate the effect of DNIC on the state of microcirculation in normal conditions and on the model of combined thermal trauma (CTT). The experiments were carried out on white male rats of the Wistar line. CTT (contact burn on the area of 20% of the body surface and thermal inhalation exposure to hot air and combustion products for 20-30 seconds) was applied under anesthesia. Animals with CTT were treated daily with intraperitoneal injections of a 10% DNIC solution (1 ml; 0.3 micromol/l). Laser Doppler flowmetry was used to assess the dynamics of the microcirculation state. The physiological and biochemical mechanisms of microcirculation disorders in CTT are revealed in the work. There was an increase in the endothelial, neurogenic and respiratory components of microcirculation, microhemodynamics of the border area of the burn, and the index of the microcirculation bypass. It was shown that when using 0.3 micromol/l of DNIC in rats under normal conditions, there is a decrease in the neurogenic and respiratory components, an increase in perfusion and the myogenic component of the microcirculatory channel. The role of 0.3 micromol/l of DNIC in the normalization of microcirculation in CTT was established.

Keywords: combined thermal injury, nitric oxide, dinitrosyl iron complexes, microcirculation, microblood bypass rate, active and passive factors of microcirculation regulation.

List of Abbreviations

NO – nitric oxide
DNIC – dinitrosyl iron complexes
CTT – combined thermal trauma
IM – index of the microcirculation
LDF – laser Doppler flowmetry
C – cardiac fluctuations of the microcirculation
R – respiratory fluctuations of microcirculation
M – myogenic fluctuations of microcirculation
N – neurogenic fluctuations of microcirculation
E – endothelial fluctuations of microcirculation
IMB – index of the microcirculation bypass
iNOS – inducible NO-synthase
Hb – hemoglobin

Introduction

Currently, burns are one of the global health problems (Lekmanov *et al.*, 2018). The actuality of the problem of thermal trauma is determined by its high prevalence among all age groups of the population (Ostrovskij *et al.*, 2006). Burn stress leads to endogenous intoxication, catabolic reaction of the body, pronounced metabolic disorders (Shulaeva *et al.*, 2013). Severe disorders of central, regional and peripheral hemodynamics develop during burn disease with a predominant violation of microcirculation and metabolic processes in the body (Gol'dzon & Dolgih, 2011).

The microcirculation system is the most important smallest structural and functional unit of the circulatory system. Violations in it can be the root cause of many diseases, determining their outcome in the future. The relevance of studying the processes in the microcirculatory bed is due to the implementation of the transport function of the cardiovascular system and transcapillary metabolism in it. This is necessary for tissue homeostasis and normal vital activity of the body as a whole.

One of the key aspects in the treatment of thermal damage is the preservation of metabolic

processes at the necessary quantitative and qualitative levels, as well as predicting the direction of their response to a burn injury in order to achieve optimal adaptation of physiological systems to trauma. To date, nitric oxide (NO) is widely used in medical practice in the treatment of various diseases. NO regulates the most important physiological processes in the body, participating in maintaining the tone of the smooth muscles of blood vessels, the activity of the central and autonomic nervous system (Herbert *et al.*, 2006), tissue differentiation and apoptosis, immune response, regulation of the expression of a number of genes (Martusevich *et al.*, 2014; Kuznecova & Soloveva, 2015). A promising type of NO donor is dinitrosyl iron complexes (DNIC). They are suitable for biomedical applications, and also do not have the disadvantages inherent in organic nitrates. DNIC can be a form of NO stabilization in both normal and pathological conditions of the body, a large number of which are associated with various disorders in the microcirculatory bed. However, at present, NO donors are rarely used as medicines, due to the lack of understanding of the metabolic pathways of NO in animals and humans. And the role of DNIC in the pathogenesis of thermal trauma has not been studied. In this regard, it is relevant to study their impact on the state of microcirculation. The aim of the study was to evaluate the effect of dinitrosyl iron complexes on the state of microcirculation in normal conditions and on the model of combined thermal trauma.

Materials and Methods

The experiments were carried out on white male rats of the Wistar line (body weight 200–250g) in accordance with the requirements of bioethics and the rules of laboratory practice (GPL), the Geneva Convention for the Protection of Animals «International Guiding Principles for Biomedical Research Involving Animals» (Geneva, 1990), Order of the Ministry of Health of the Russian Federation № 267 of 19.06.2003 «On approval of the rules of laboratory practice». The study was approved by the Local Ethics Committee of the Privolzhsky Research Medical University of the Ministry of Health of the Russian Federation, in accordance with the provisions of the Helsinki Declaration of 1975, revised in 2008. The rats were received from the «Stolbovaya» branch (Moscow). All animals were kept in standard vivarium conditions in cages with free access to food and water on a diet, according to GOST standards «Maintenance of experimental animals in the nurseries of the Research Institute» (Karkishchenko & Gracheva, 2010).

After a 14 – day adaptation to the conditions of the local vivarium and quarantine, 6 groups of animals of equal numbers were formed from 60 rats: 1 – intact group (n = 10) – animals without manipulation; 2 – 1 experimental group (n = 10) – animals that received 1 ml of saline solution daily intraperitoneally; 3 – 2 experimental group (n = 10) – animals that received daily intraperitoneal injections of DNIC (1 ml; 0.3 micromol/l); 4 – 3 experimental group – animals with combined thermal trauma (CTT) without treatment (n=10); 5 – 4 experimental group – animals with CTT that received 1 ml of saline solution daily intraperitoneally (n = 10); 6 – 5 experimental group – animals with CTT who received daily treatment in the form of intraperitoneal injections of a DNIC solution (1 ml; 0.3 micromol/l) (n = 10).

CTT (contact burn on the area of 20% of the body surface and thermal inhalation exposure to hot air and combustion products in the conditions of the inhalation chamber) was applied under anesthesia («Zoletil-100» («VirbacSante-Animale», France) at a dose of 60 mg/kg and «Xylavet» («Interchemie», Netherlands) at a dose of 6 mg/kg) (Vorob'ev *et al.*, 2009). DNIC with glutathione was obtained by the method of A.F. Vanin (2015), mixing 300 millimol $NaNO_2$, 200 millimol reduced glutathione and a solution of FeSO4. The concentration of DNIC was determined by the spectrophotometric method on a spectrophotometer Power Wave XS (Bio-Tek, USA) in the wavelength range of 410–700 nm.

The animals were removed from the experiment on the 10th day after CTT by decapitation with preliminary ligation of the carotid artery under anesthesia (Zoletil-100 + Xylavet). The state of microcirculation was evaluated in animals before excretion.

Laser Doppler flowmetry (LDF) was used to assess the dynamics of the microcirculation state (Krupatkin & Sidorov, 2013). The laser analyzer «LAKK-M» was used. The analyzer probe was installed perpendicular to the studied area, the recording lasted 3 minutes. In the study of microcirculation, the index of the microcirculation (IM) was evaluated. IM reflects the average level of perfusion (the average flow of red blood cells) in a unit of tissue volume per unit of time (measured in perfusion units) and allows us to give an integral assessment of the state of microcirculation in the studied tissue area.

«Wavelet analysis» (amplitude-frequency spectrum) was performed using software tools. It allowed, after a 3-minute recording of the LDF-gram, to calculate slow and high-frequency fluctuations of blood flow in order to assess the role of passive (cardiac fluctuations – C, respiratory fluctuations – R) and active factors (myogenic fluctuations – M, neurogenic fluctuations – N, and endothelial fluctuations – E) regulation of microcirculation, as well as the index of the microcirculation bypass (IMB).

Statistical processing of the results was carried out using a computer program «Statistica 6.0». The Shapiro-Wilk criterion was used to test the hypothesis about the nature of the distribution. Descriptive statistics are given by the mean and mean square deviation (M±σ). The comparison of two independent groups was performed using the Student's t-test, depending on the fulfillment of the applicability conditions. When calculating the Student's t-test, we used the Bonferroni correction, which allows us to eliminate the first-kind error that occurs when comparing more than two samples by this method (Glanc, 1998).

Results

Study of the state of microcirculation in healthy rats under the influence of DNIC. The level of microcirculation on the 10th day of administration of saline solution in rats of the 1st experimental group did not significantly differ from the intact values (Fig. 1). The use of DNIC in the 2nd experimental group led to an increase in perfusion.

IMB in 1 experimental group of animals after a 10-day course of intraperitoneal administration of saline solution relative to intact animals increased by 9% (Fig. 2).

Enrichment of the saline solution with exogenous sources of NO after 10 days of use in rats contributed to an increase in IMB by 40% and 29% of the intact and control values (1 experimental group).

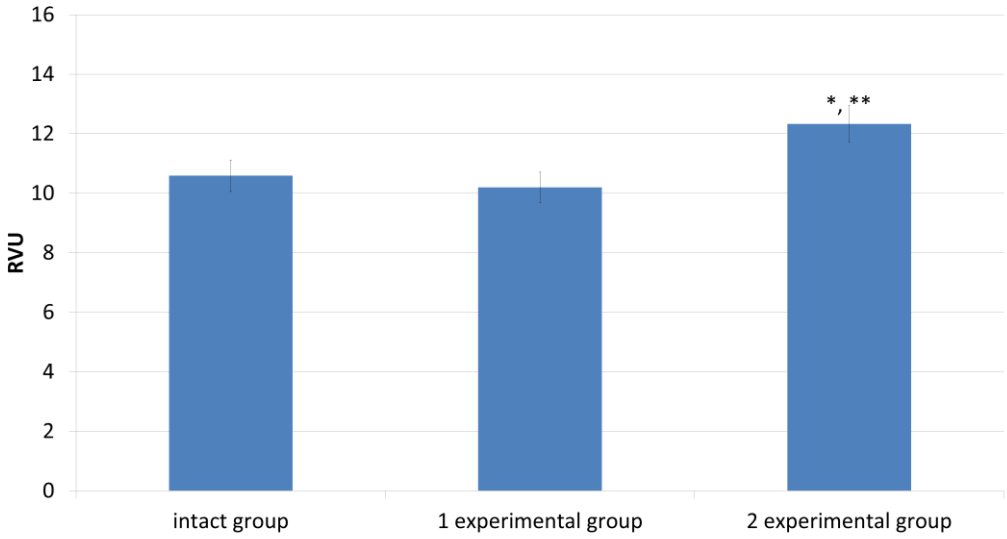

Fig. 1. Index of the microcirculation in rats under the influence of DNIC
Note: * – differences are statistically significant compared to intact group ($p < 0.05$);
** – differences are statistically significant compared to rats of 1 experimental group ($p < 0.05$)

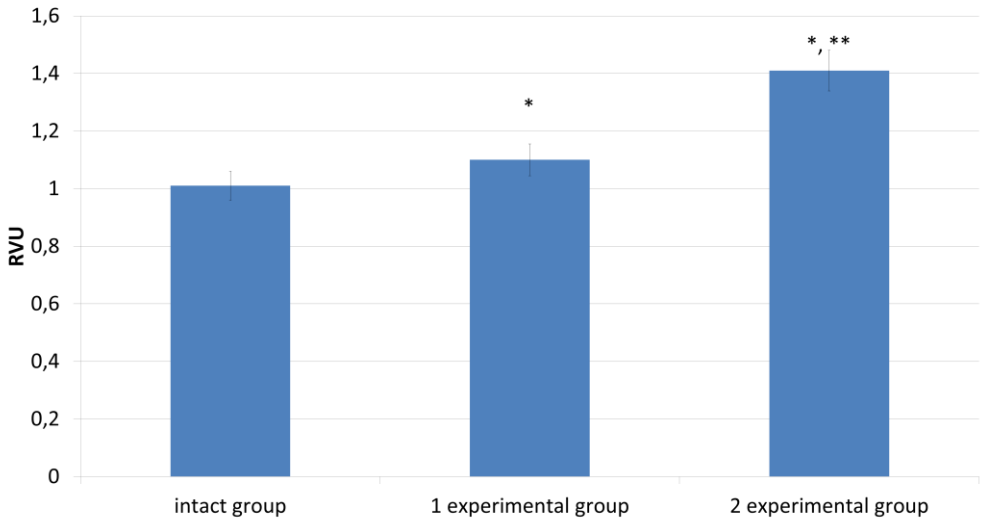

Fig. 2. The level of the rat index of the microcirculation bypass under the influence of DNIC
Note: * – differences are statistically significant compared to intact group ($p < 0.05$);
** – differences are statistically significant compared to rats of 1 experimental group ($p < 0.05$)

The endothelial component in 1 experimental group of animals with saline solution infusions for 10 days did not significantly differ from the intact group (Fig. 3). DNIC in the composition of the intraperitoneal mixture administered by the 10th day contributed to the growth of the endothelial component by 8% from the intact and control (1 experimental group) data.

Neurogenic fluctuations during intraperitoneal administration of saline solution to rats of the 1st experimental group on the 10th day were without significant differences relative to the intact data (Fig. 3). The use of DNIC in animals of the 2nd experimental group contributed to a decrease in the neurogenic component by 17% and 20% from the intact and control (1 experimental group) values, respectively.

The myogenic component in 1 experimental group of animals with saline solution infusions by the 10th day decreased by 6% relative to the intact values (Fig. 3). For the group of rats with DNIC, an increase in myogenic fluctuations was revealed at the same time.

Respiratory fluctuations in 1 experimental group of animals with saline solution on the 10th day were without significant differences from the intact values (Fig. 4). When using DNIC in the composition of the solution administered to animals for 10 days, the respiratory component decreased by 46% and 49% relative to the intact and control (1 experimental group) results, respectively.

Fluctuations in the cardiac range in 1 experimental group of animals with infusion support on the 10th day increased by 78% relative to the intact values (Fig. 4).

The introduction of DNIC at similar periods contributed to the growth of the cardiac component by 93% and 9% of the indicators of the intact and 1 experimental groups, respectively.

Assessment of the state of microcirculation in rats with combined thermal trauma under the influence of DNIC. Microhemodynamics of the boundary region of thermal trauma on the 10th day decreased by 19% compared to the intact data (Fig. 5). Saline solution infusions during CTT contributed to a decrease in microcirculation by 54% relative to the group with trauma without treatment. The addition of CTT to the treatment regimen of DNIC on the 10th day contributed to an increase in perfusion by 9%, 34% and 65% relative to the groups of intact animals, as well as with CTT and the use of saline solution for CTT, respectively.

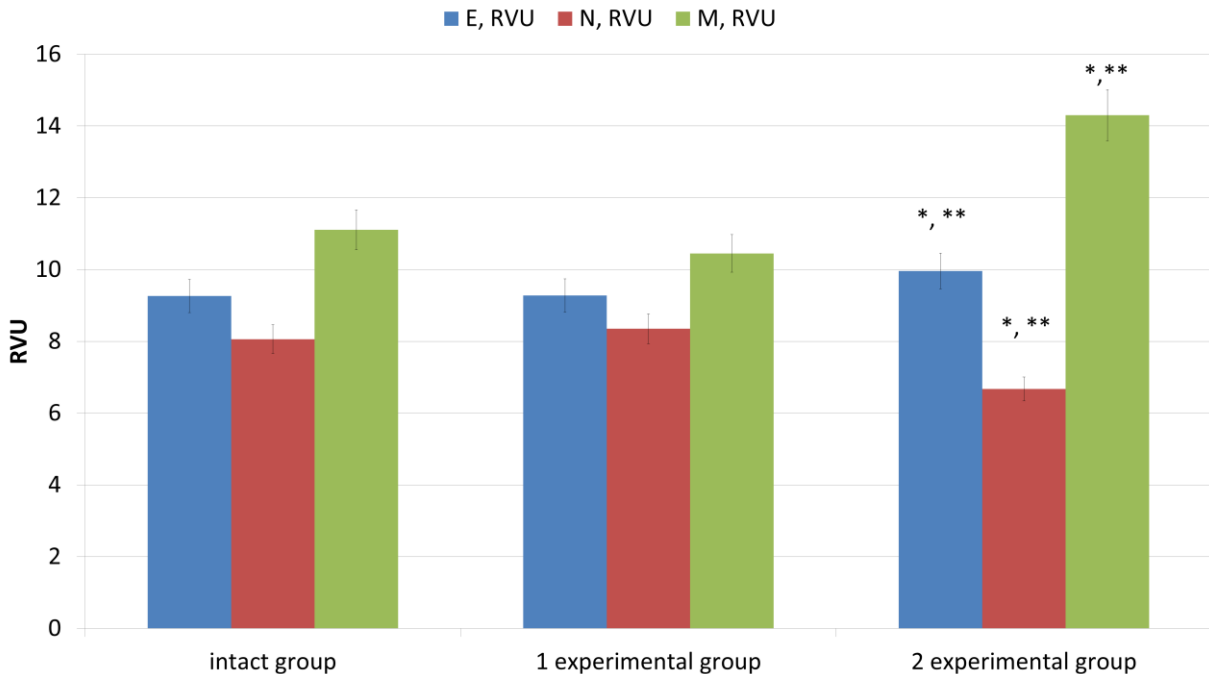

Fig. 3. Active factors of microcirculation regulation (E – endothelial fluctuations of microcirculation, N – neurogenic fluctuations of microcirculation, M – myogenic fluctuations of microcirculation) in rats under the influence of DNIC
Note: * – differences are statistically significant compared to intact group ($p < 0.05$); ** – differences are statistically significant compared to rats of 1 experimental group ($p < 0.05$)

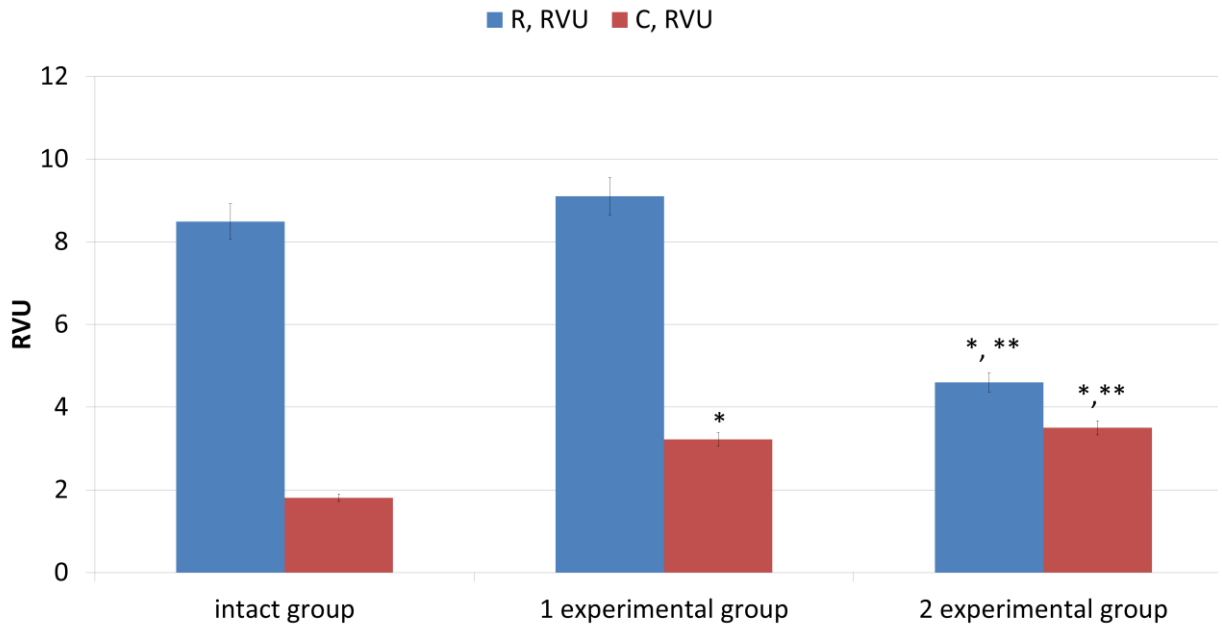

Fig. 4. Passive factors (R – respiratory fluctuations of microcirculation, C – cardiac fluctuations of the microcirculation) of local regulation of microcirculation in rats under the influence of DNIC
Note: * – differences are statistically significant compared to intact group ($p < 0.05$); ** – differences are statistically significant compared to rats of 1 experimental group ($p < 0.05$)

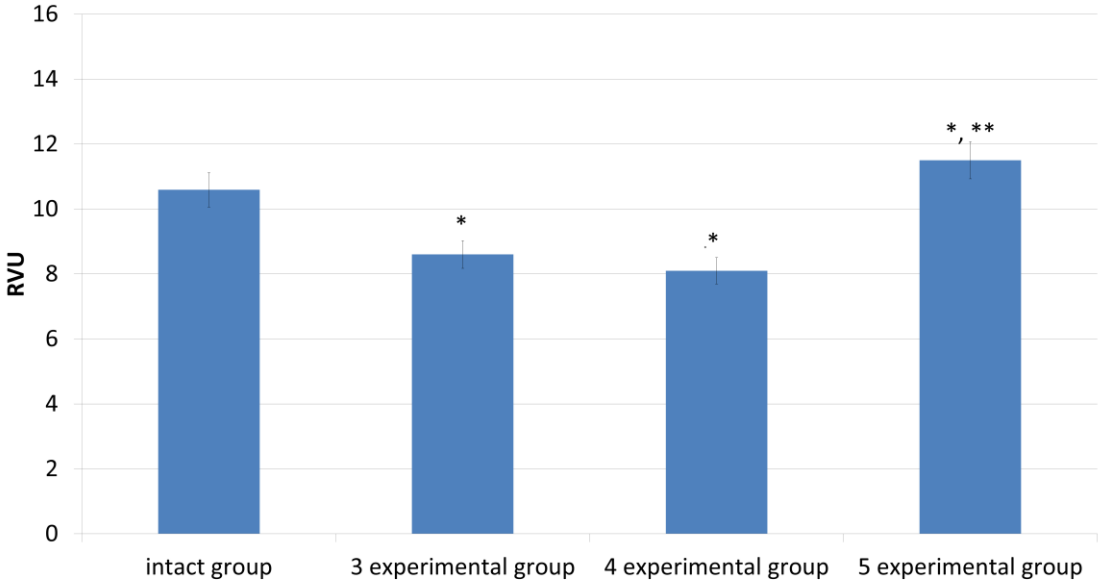

Fig. 5. Index of the microcirculation in rats with CTT under the influence of DNIC.
Note: * – differences are statistically significant compared to intact group ($p < 0.05$); ** – differences are statistically significant compared to rats of 3 experimental group ($p < 0.05$)

The involvement of collateral vessels in CTT increases. Thus, the IMB in the 3 experimental group with CTT without treatment on the 10th day was 20% higher than the intact values (Fig. 6).

Infusion therapy of CTT on the 10th day led to a decrease in IMB by 9% and 24% in relation to the intact and control (3 experimental group) values. The use of DNIC as part of the solution administered to animals after 10 days of CTT treatment allowed to reduce the index of the microcirculation bypass by 62%, 68% and 57% relative to the intact, 3 and 4 experimental groups.

In the 3 experimental group with CTT without treatment, on the 10th day, an increase in the endothelial component was observed by 23% relative to the intact values (Fig. 7).

Group 4 with infusion support of burned animals for 10 days showed an increase in the endothelial component by 13% of the intact values, along with a decrease by 9% relative to the indicators of the 3 experimental group.

The use of DNIC in the scheme of 10-day treatment of experimental CTT contributed to a decrease in endothelial fluctuations to the level of intact values, while 19% and 12% lower than the indicators of the 3 and 4 experimental groups.

Experimental CTT in the 3rd experimental group on the 10th day led to an increase in the neurogenic component by 50% relative to the intact group (Fig. 7). The introduction of saline solution to animals with CTT by the 10th day led to a decrease in neurogenic fluctuations by 17% and 44% of the values of the intact and 3rd experimental groups, respectively. The introduction of DNIC 10 days after CTT led to a decrease in the parameter by 12% and 40% from the indicators of the intact and 3 experimental groups, but caused an increase of 6% relative to the 4 experimental group.

In the border area of the burn wound, myogenic fluctuations on the 10th day were comparable in level with intact values (Fig. 7). The use of infusion support with saline solution caused a decrease in the myogenic component by 24% relative to the indicators of the intact and 3 experimental groups. The introduction of DNIC to animals with CTT for 10 days caused the normalization of this indicator.

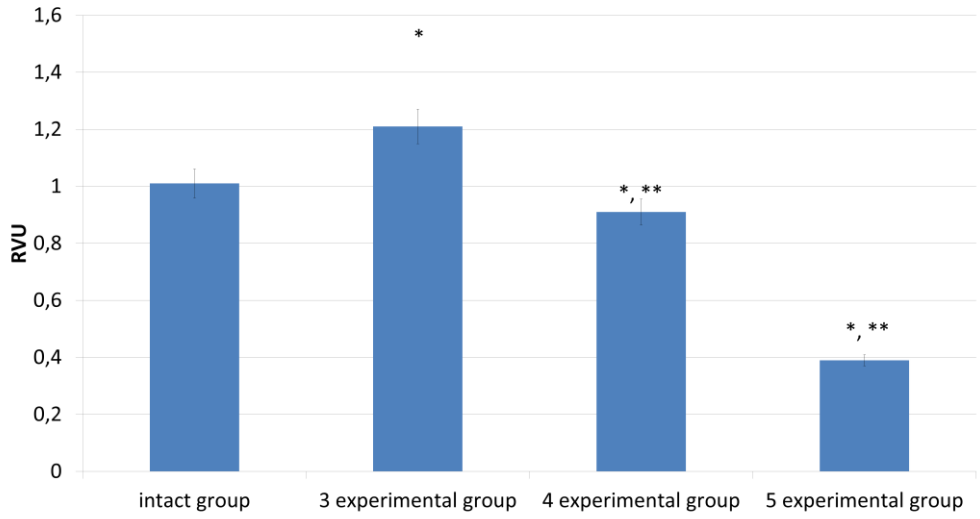

Fig. 6. The index of the microcirculation bypass in rats with CTT under the influence of DNIC.
Note: * – differences are statistically significant compared to intact group ($p < 0.05$); ** – differences are statistically significant compared to rats of 3 experimental group ($p < 0.05$)

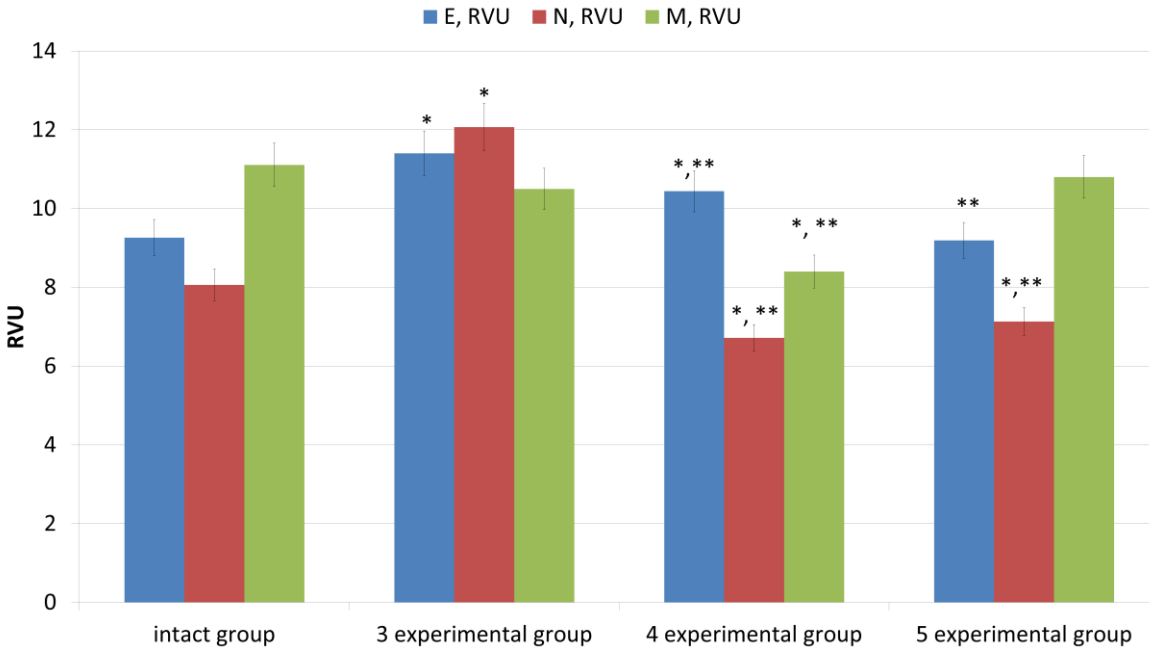

Fig. 7. Active factors of microcirculation regulation (E – endothelial fluctuations of microcirculation, N – neurogenic fluctuations of microcirculation, M – myogenic fluctuations of microcirculation) in rats with CTT under the influence of DNIC
Note: * – differences are statistically significant compared to intact group ($p < 0.05$); ** – differences are statistically significant compared to rats of 3 experimental group ($p < 0.05$)

CTT by the 10th day led to an increase in the respiratory component by 69% relative to the intact values (Fig. 8). In the group with intraperitoneal administration of saline solution to animals with a burn on the 10th day, respiratory fluctuations increased by 116% and 28% of the indicator of the intact and 3 experimental groups, respectively. The CTT treatment regimen with the use of DNIC in similar terms contributed to a high (by 48%) level of the respire-

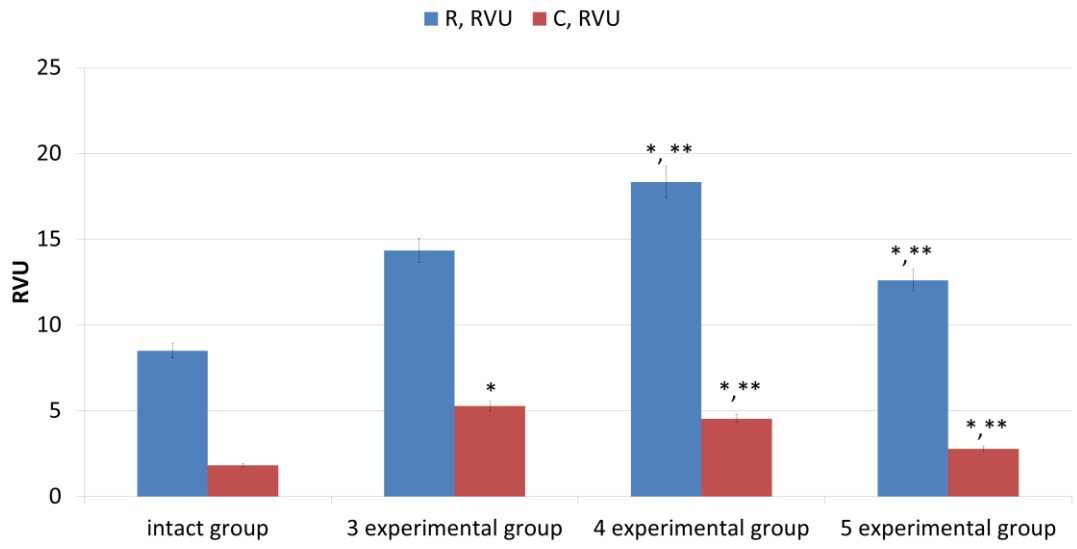

Fig. 8. Passive factors (R – respiratory fluctuations of microcirculation, C – cardiac fluctuations of the microcirculation) of local regulation of microcirculation in rats with CTT under the influence of DNIC
Note: * – differences are statistically significant compared to intact group ($p < 0.05$); ** – differences are statistically significant compared to rats of 3 experimental group ($p < 0.05$)

ory component relative to intact data, and at the same time there was a decrease of 12% and 31% from the parameters of the 3 and 4 experimental groups.

In the group with CTT without treatment, the cardiac component increased almost 3 times from the intact level (Fig. 8).

The use of saline solution in the 4 experimental group with CTT contributed to a decrease in the fluctuations of the cardiac spectrum by 14% relative to the values of the 3 experimental group, but the indicator remained high (by 150%) relative to the intact data.

When adding DNIC to the CTT therapy regimen on the 10th day, cardiac fluctuations decreased by 47% and 39% compared to the 3 and 4 experimental groups, along with this, the indicator in the group with DNIC was higher than the intact values by 54%.

Discussion

It is scientifically proven that NO is one of the main regulators of renal hemodynamics and glomerular filtration, an inhibitor of sodium transport (Lewandowska *et al.*, 2010). NO participates in the formation of S-nitrosothiols, affects Fe/S-groups in the catalytic centers of proteins (Shumaev *et al.*, 2007; 2008; 2021). DNIC, according to the literature data, practically do not change the gas composition of the blood and the indicators of the acid-base state of the body (Selivanov *et al.*, 2012).

When exposed to DNIC, it was noted that changes in regulatory factors in groups of healthy animals are multidirectional. Thus, 1 experimental group with saline solution is close to intact levels in terms of indicators. The endothelial component in the 2 experimental group of animals with DNIC was increased. This may be due to the effect of exogenous NO on the endothelium of microvessels. The neurogenic component in the 2 experimental group with DNIC was lower than the intact values. This indicates a decrease in the influence of the sympathetic nervous system. The growth of the myogenic component in healthy animals under the influence of DNIC was revealed, which indicates the onset of vasodilation processes. The use of DNIC contributed to a decrease in the respiratory amplitude. This is probably due to the improvement of blood outflow processes.

According to the literature, in case of a burn, structural and functional changes of liver cells are noted, depending on the severity of the injury, which are aggravated by circulatory disorders, hypoxia, which, in the absence of compen-

sation mechanisms, can lead to damage to the function of the organ (Marusyanov *et al.*, 1995).

A direct relationship between the content of stable products of the metabolism of NO and markers of the activity of the inflammatory process (C-reactive protein and circulating immune complexes), due to the activation of inducible NO-synthase (iNOS), is proved. The literature shows that after the action of the immune stimulus, iNOS is localized in the glomerular cells and a small amount of it is located in the tubules. NO is of great importance in the regulation of renal blood flow, excretory function of the kidneys, tubulo-glomerular balance. NO is constantly synthesized in endothelial and smooth muscle cells of renal vessels, epithelial and mesangial tubular cells, as well as as a result of the interaction of NO with the renin-angiotensin system and other bioregulators of the functions of this organ (Markov, 2000). Angiotensin-converting enzyme is a physiological regulator of angiotensin II and bradykinin. Angiotensin II is a stimulator of the formation of free radicals, in particular, superoxide anions that inactivate nitric oxide, promote the formation of peroxynitrite and reduce the effectiveness of NO-mediated vascular dilation (Belous *et al.*, 2011).

Overexcitation of the central nervous system during a burn leads to increased vascular permeability and plasma loss. As a result, hypoproteinemia develops, the cellular and mineral composition of the blood changes, the volume of circulating blood decreases, hemoconcentration decreases, the shaped elements of the blood are destroyed. The excitation of the sympathetic-adrenal system, the release of catecholamines and corticosteroids play a leading role in vasoconstriction in burns. Corticosteroids potentiate the action of pressor substances, catecholamines cause and maintain generalized spasm of arterial vessels, which is a characteristic early sign of microcirculation disorders. There are stagnant phenomena in the postcapillary venular vessels and the intensity of blood flow in the nutritive link of the capillary bed decreases. The termination of active vasomotion in the part of the capillary bed in which the resistance to blood flow is higher leads to a decrease in volumetric blood flow, and, as a result, to the appearance of signs of stasis, as well as the predominance of anaerobic metabolism in the tissues themselves. The loss of vasomotion leads to shunting of the blood flow. Most of the blood entering the microcirculatory bed moves along a smaller part of the capillaries. The subtle mechanisms that regulate transcapillary mass transfer and volumetric processes in tissues suffer. In case of thermal trauma, the amplitude of the regulatory components increases in comparison with healthy animals. However, there is a decrease in active and passive regulatory factors in relation to animals with a burn without treatment in the group with DNIC. This fact may indicate the processes of restoring endothelial function, reducing sympathetic hyperregulation, reducing congestion in the venous microvascular bed, optimizing the processes of filling the arteries and arterioles of the microcirculatory link.

A decrease in the vascular tone of arterioles (removal of spasm) due to a decrease in neurogenic activity increases the heart rate in the microcirculatory bed due to an increase in the flow of arterial blood, which brings a pulse wave (Krupatkin & Sidorov, 2013). The HbNO complex is a stable compound and, therefore, cannot control the supply of oxygen to tissues, unlike the nitrosated form of hemoglobin, in which NO interacts with the cysteine amino acid residue contained in the b-subunit of the globin part of the molecule (bCys93 for human Hb) (Doctor *et al.*, 2005).

NO-Hb undergoes conformational changes that lead to the release of NO from the Hb-bCys93 complex and its diffusion into the surrounding tissues Against the background of a decrease in the O2 voltage and an increase in the concentration of hydrogen ions in the blood. This has a local vasodilating effect on the smooth muscles of the vessels (Gross & Lane, 1999).

In the microcirculation system, NO prevents the adhesion of white blood cells on the surface of microvessels, aggregation of blood plates, inhibits the release of biologically active substances from platelets that can damage the smallest vessels (Gladwin *et al.*, 2004).

It was revealed that the index of the microcirculation of the border region in the 3 and 4 experimental groups with CTT was lower than the intact values. However, in the 5 experimental group with DNIC, an intensification of microhemodynamics is observed, possibly due to the predominance of nutritive blood flow (IMB < 1). In case of thermal trauma, the amplitude of the regulatory components increases in comparison with healthy animals. However, there is a decrease in active and passive regulatory factors in the group with DNIC in relation to animals with CTT without treatment. This fact may indicate the processes of restoring endothelial function, reducing sympathetic hyperregulation, reducing congestion in the venous microvascular bed, optimizing the processes of filling the arteries and arterioles of the microcirculatory link.

The possibility of the existence of the described mechanism of action of the deposited form of NO in the mammalian body is also conf-irmed by the studies of A.F. Vanin, who showed that DNIC have a hypotensive effect, realized due to the release of NO by these compounds (Vlasova *et al.*, 2003).

Consequently, there is a decrease in the neurogenic and respiratory components, an increase in perfusion and the myogenic component of the microcirculatory bed with a course application of 0.3 micromol/l DNIC in rats under normal conditions. The work reveals the physiological and biochemical mechanisms of disorders in CTT, including the growth of endothelial, neurogenic and respiratory components of microcirculation, microhemodynamics of the border area of the burn, IMB. The role of 0.3 micromol/l of DNIC in the normalization of microcirculation in CTT was established.

Thus, DNIC have an activating effect on the state of microcirculation, in conditions of relative norm. For the first time, using an integrated research approach, it was found that the use of DNIC in experimental CTT normalizes the state of microcirculation, increasing the adaptive potential of the body.

References

BELOUS YU.A., DROZDOVA G.A., KOMAREVCEVA I.A., MUSTYACA V.F., ORLOVA E.A., KOMAREVCEVA E.V. & FILIPPOVA O.I. (2011): Renin-angiotensin system in the regulation of apoptosis in the kidneys: the role of nitric oxide. *Medicinskij vestnik Severnogo Kavkaza* **3**, 57–59.

DOCTOR A., PLATT R., SHERAM M. L., EISCHEID A., MCMAHON T., MAXEY TH., DOHEROY J., AXELROD M., KLINE J., GURKA M., GOW A. & GASTON B. (2005): Hemoglobin conformation couples erythrocyte S-nitrosothiol content to O_2 gradients. *Proc. Natl. Acad. Sci. USA* **102**(16), 5709–5714.

GLADWIN M.T., CRAWFORD J.H. & PATEL R.P. (2004): The biochemistry of nitric oxide, nitrite and hemoglobin: role in blood flow regulation. *Free radical biology and medicine* **36**(6), 707–717.

GLANC S. (1998): *Mediko-biologicheskaya statistika*. Moscow: Praktika, 459 pp.

GOL'DZON M.A. & DOLGIH V.T. (2011): The effect of severe thermal trauma on the contractility and metabolism of the heart. *Obshchaya reanimatologiya* **7**(1), 11–14.

GROSS S.S. & LANE P. (1999): Physiological reactions of nitric oxide and hemoglobin: A radical rethink. *Proc. Natl. Acad. Sci. USA* **96**(18), 9967–9969.

HERBERT J., GOODYER I.M., GROSSMAN A.B., HASTINGS M.H., DE CLOET E.R., LIGHTMAN S.L., LUPIEN S.J., ROOZENDAAL B. & SECK J.R. (2006): Do corticosteroids damage the brain? *Neuroendocrinology* **18**, 393–411.

KARKISHCHENKO N.N. & GRACHEVA S.V. (2010): *Rukovodstvo po laboratornym zhivotnym i al'ternativnym modelyam v biomedicinskih issledovaniyah*. Moscow: Profil' – 2C, 358 pp.

KRUPATKIN A.I. & SIDOROV V.V. (2013): *Funkcional'naya diagnostika sostoyaniya mikrocirkulyatorno-tkanevyh sistem: Kolebaniya, informaciya, nelinejnost'. Rukovodstvo dlya vrachej*. Moscow: Knizhnyj dom «LIBROKOM», 496 pp.

KUZNECOVA V.L. & SOLOVEVA A.G. (2015): Nitric oxide: properties, biological role, mechanisms of action. *Sovremennye problemy nauki i obrazovaniya* **4**, URL: http://science-education.ru/ru/article/view?id=21037.

LEKMANOV A.U., AZOVSKIJ D.K. & PILYUTIK S.F. (2018): Analysis of survival in children with severe thermal trauma delivered in the first 72 hours after injury. *Vestnik anesteziologii i reanimatologii* **15**(5), 30–38.

LEWANDOWSKA H., BRZÓSKA K., MECZYŃSKA-WIELGOSZ S., RUMIANEK K., WÓJCIUK G. & KRUSZEWSKI M. (2010): Dinitrosyl iron complexes--structure and biological functions. *Postepy Biochem*. **56**(3), 298–304.

MARKOV H.M. (2000): The role of nitric oxide in the pathogenesis of childhood diseases. *Rossijskij vestnik perinatologii i pediatrii* **4**, 43–47.

MARTUSEVICH A.K., SOLOVEVA A.G., PERETYAGIN S.P., KARELIN V.I. & SELEMIR V.D. (2014): The effect of the NO-containing gas flow on some parameters of the energy metabolism of red blood cells. *Byulleten' eksperimental'noj biologii i mediciny* **158**(7), 40–42.

MARUSYANOV V.E., MIHAJLOVICH V.A. & DOMANSKAYA I.A. (1995): Characteristics of the stages of endogenous intoxication. *Efferentnaya terapiya* **1**(2), 26–30.

OSTROVSKIJ, N.V., BABKIN V.B. & BELYANINA I.B. (2006): *Neotlozhnaya pomoshch' pri termicheskoj travme*. Saratov: Izdatel'stvo Saratovskogo medicinskogo universiteta, 35 pp.

SELIVANOV E.A., REMIZOVA M.I., GERBUT K.A., BURGOVA E.N. & VANIN A.F. (2012): The effect of the dinitrosyl complex of iron with glutathione on the course of hemorrhagic shock during its infusion therapy. *Medicinskij akademicheskij zhurnal* **12**(2), 84–89.

SHULAEVA N.M., KUSPIC E.V., SHCHUKOVSKIJ V.V. & OSTROVSKIJ N.V. (2013): Intensive therapy of endogenous intoxication syndrome in victims of severe thermal burns. *ZHurnal im. N.V. Sklifosovskogo. Neotlozhnaya medicinskaya pomoshch'* **3**, 27–32.

SHUMAEV K.B., GUBKIN A.A., GUBKINA S.A., GUDKOV L.L., LAKOMKIN V.L., TOPUNOV A.F., VANIN A.F. & RUUGE E.K. (2007): Interaction of albumin-bound dinitrosyl complexes of iron and reactive oxygen species. *Biofizika* **52**(3), 534–538.

SHUMAEV K.B., KOSMACHEVSKAYA O.V., GRACHEV D.I., TIMOSHIN A.A., TOPUNOV A.F., LANKIN V.Z. & RUUGE E.K. (2021): Possible mechanism of antioxidant action of dinitrosyl iron complexes. *Biomedicinskaya himiya* **67**(2), 162–168.

SHUMAEV K.B., GUBKIN A.A., SEREZHENKOV V.A., LOBYSHEVA I.I., KOSMACHEVSKAYA O.V., RUUGE E.K., LANKIN V.Z., TOPUNOV A.F. & VANIN A.F. (2008): Interaction of reactive oxygen and nitrogen species with albumin − and hemoglobin bound dinitrosyl iron complexes. *Nitric Oxide* **18**, 37–46.

VANIN A.F. (2015): *Dinitrozil'nye kompleksy zheleza s tiolsoderzhashchimi lignadami: fizikohimiya, biologiya, medicina.* Moscow – Izhevsk: Izdatel'stvo «Institut komp'yuternyh issledovanij», 220 pp.

VLASOVA M.A., VANIN A.F., MYULLER B., SMIRIN B.V., MALYSHEV I.YU. & MANUHINA E.B. (2003): Identification and characterization of different pools of nitric oxide depots in the vessel wall. *Byulleten' eksperimental'noj biologii i mediciny* **136**(9), 260–264.

VOROB'EV A.V., PERETYAGIN S.P., RAZMAHOV A.M., MARTUSEVICH A.K., VAZINA I.R., KVICINSKAYA N.A., LUZAN A.S. & STRUCHKOV A.A. (2010): Sposob modelirovaniya kombinirovannoj ozhogovoj travmy. *Patent RF* 2408081 dated 27.12.2010. *Byulleten'* **36** (RU).