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The Effect of Dinitrosyl Iron Complexes in Free Radical Oxidation in Organs of Rats during Hypoxia Caused by Thermal Injury

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Abstract: The aim of this work was to investigate the effect of deposited form of nitric oxide, dinitrosyl iron complexes (DNICs) on the processes of lipid peroxidation and the status of antioxidant system in the liver, kidney, heart and lung of rats with combined thermal injury. The experiment was conducted on 45white rats of Wistar line. Combined thermal trauma (contact burn on area of 20% of the body surface and termoinhalation impact) was applied under anesthesia. Animals with burn daily was injected intraperitoneally 10% solution of DNICs. The intensity of lipid peroxidation and total antioxidant activity, the specific activity of catalase, superoxide dismutase, concentration of malonic dialdehyde (MDA) were determined in homogenates of organs at days 3 and days 10 after burn. It was established that DNICs have pro - and antioxidant properties. The results showed that the introduction of dinitrosyl iron complexes to the rats with combined thermal trauma had a normalizing effect on the processes of lipid peroxidation in the lungs and heart. The deposited form of nitric oxide in the form of DNICs had caused a decrease of MDA level and intensity of lipid peroxidation in the liver on the day 10 after injury. A reduction of lipid peroxidation in the kidney in thermal injury under the influence of DNICs was

revealed. The specific activity of superoxide dismutase and catalase on the day 10 after injury increased in the lung, heart and liver of rats under the influence of the deposited form of NO.

Keywords: lipid peroxidation; antioxidants; burn; dinitrosyl iron complexes

INTRODUCTION

The thermal trauma has a high prevalence. The thermal injury is one of the central places in the general structure of traumatism[1]. Modern principles of complex treatment of burns are constantly being improved however the mortality rate for burns is quite high in connection with the development of multiple organ failure[2]. Activation of free radical processes in thermal injury leads to hypoxia and oxidative stress, which is one of the universal mechanisms of tissue damage[3]. In this regard, the study of free radical oxidation in various organs and tissues in case of burns and search for possible ways of its correction are of interest.

Nitric oxide (NO) can be used for the treatment of burn disease. NO is a universal regulator of various physiological processes in the organism of animals and humans. Nitric oxide functions in the cardiovascular, secretory, reproductive, nervous and excretory systems[4]. Product-NO donors (nitroglycerin and its analogues) are often used in clinical practice as drugs. They are rapidly metabolized with release of NO. However, prolonged use of nitroglycerin leads to tolerance to a certain dose. A forced increase in dose can cause a NO hyper production. In this regard, currently, the properties and possibilities of application other NO donors are actively studied[5].

Some of the most promising NO sources are dinitrosyl-iron complexes (DNICs), in particular DNICs containing thiol ligands, such as cysteine, glutathione. NO does not have the disadvantages of organic nitrates and potentially is acceptable for biomedical applications[6-8]. DNICs protect NO from superoxide anions, provide intracellular and intercellular transport of nitrogen oxide and deposit of NO[9]. DNICs are formed in the body of endogenous[10]. DNICs act as regulators of diverse physiological processes, inhibit thrombosis, have an antioxidant effect, accelerate the healing of skin wounds and reduce the necrotic zone after experimental myocardial infarction[11]. DNICs are low-toxic and have prolonged effect[10,12]. However, the body's response to exogenous injection of DNICs to rats with combined thermal injury (CTI) is unclear.

The aim of this work was the study of influence of dinitrosyl iron complexes on the lipid peroxidation (LPO) and antioxidant system in the organs of rats with combined thermal injury.

MATERIALS AND METHODS

The experiment was carried out on 45 white male rats of Wistar line weighing among 200 – 250g. Animals were derived from the farm «Stolbovaja» (Moscow). All animals were kept in standard vivarium conditions in cages with free access to food and water[13]. Conditions of working with animals corresponded with the rules of the European Convention ET/S 129, 1986 and the directives 86/609 ESC. After quarantine (days 14) the following groups of animals were formed: 1. Intact group: healthy animals (n=15); 2. Control group: animals with combined thermal injury which daily were injected intraperitoneally with 1 ml of saline (n=15); 3. Experimental group: animals with CTI which daily were intraperitoneally injected with 10% solution of DNICs (1 ml; 0,3 mmol/l) (n=15).

DNICs with glutathione were obtained by the method of A. F. Vanin[7] by mixing 300 mM $NaNO_2$, 200 mM restored glutathione and a solution of $FeSO_4$. DNIC concentration was determined spectrophotometrically on the spectrophotometer Power Wave XS (Bio-Tek, USA) in the wavelength range 410-700 nm.

CTI including contact burns on 20% of the body surface and inhalation the impact by hot air and combustion products within 20-30 seconds in the conditions of inhalation chamber was done under anesthesia (Zoletil 100, VIRBAC, France, 60 mg/kg + XylaVET, Pharmamagist Ltd., Hungary, 6 mg/kg)[14]. Animals were taken out of the experiment on days 3 and 10 by decapitation with previous transection of carotid artery under anesthesia (Zoletil (60 mg/kg) + XylaVET (6 mg/kg)).

After decapitation of the rats the abdominal cavity was opened. Liver, kidneys, heart and lungs quickly were removed and were washed from the blood in chilled saline using a thick needle and syringe for 10 ml. The washed organs were immediately placed in Petri dish standing on ice and were crushed by scissors. In a glass Potter homogenizer with a Teflon pestle 10% homogenate of tissue was prepared on the basis of the medium containing 0,25 M sucrose, 1mM EDTA, 0,01 M Tris–HCl – buffer (pH=7,5). The tissue was homogenized for 30-40 seconds. The homogenate was centrifuged 10 min at 1000g in a centrifuge Multifuge 1 SR (t=0 + 2C°) to remove intact cells and nuclei. Loose sediment was discarded, the supernatant was used for the study[15].

Prooxidant and antioxidant status was assessed in the homogenates of liver, heart, kidneys and lungs. The activity of free radical oxidation (FRO) was studied using the method of induced biochemiluminescence[16] on the device BHL-06 (Medozons, Nizhny Novgorod, Russia). The following parameters of biochemiluminescence were evaluated: tg 2α – characterizes the speed of reduction of FRO processes in the plasma and evidences about total antioxidant activity (TAA); S – light sum of chemiluminescence for 30 sec. – reflects the potential ability of a biological object to FRO. The concentration of malonic dialdehyde (MDA) was determined by M. Mihara et al.[17]. For the evaluation of catalase activity we used the spectrophotometric method based on determination of rate of decomposition of hydrogen peroxide by catalase from the sample[18]. The activity of superoxide dismutase (SOD) was determined by inhibition of formation of product of adrenaline oxidation[19]. Protein concentration was calculated by the modified method of Lowry[20]. Statistical data processing was performed by the software (Statistica 6.0 (StatSoft Inc., USA)). The significance of differences between groups was assessed using Student's t-test. The differences were considered statistically significant at $p<0,05$.

RESULTS AND DISCUSSION

The failure of the respiratory system is most frequent component occurring in burn disease[21]. The study showed that the light sum of chemiluminescence was increased by 68,5% (p=0,009) on days 3 after CTI in the lung homogenate compared to healthy animals. The S indicator increased by 11,5% (p=0,021) for days 10 after CTI (**figure 1**). The introduction of DNICs to rats with thermal injury caused a statistically significant decrease of free radical oxidation in the lungs by 45% (p=0,011) at days 3 after injury compared with control. This contributes to the normalization of FRO. There was a tendency to decrease the FRO under the influence of DNICs in the lungs for days 10 after injury by 12% (p=0,064) compared with rats of the control group (**figure 1**).

Activation of the FRO when CTI was accompanied by a slight increase of secondary product of lipid peroxidation (MDA) in the lung at days 3 after injury compared to control group rats (**figure 2**). On days 10 after CTI a concentration of MDA decreased by 29,1% (p=0,007) compared to healthy rats.

This is probably due to the increase in the activity of enzymes involved in the utilization of highly toxic aldehydes, in particular, aldehyde dehydrogenase[22].

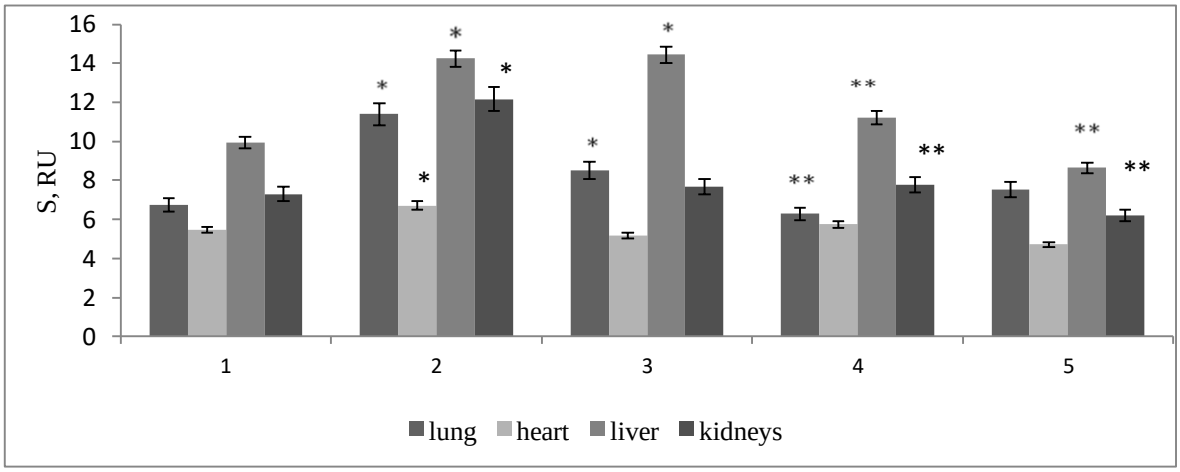

Figure 1: The light sum of chemiluminescence in rat organs after the CTI and after the introduction of dinitrosyl iron complexes. Notes: 1. Intact healthy animals (n=15); 2. Control 1: animals with CTI days 3 (n=8); 3. Control 2: animals with CTI days 10 (n=7); 4. Experiment 1: animals with CTI which daily were injected with DNICs days 3 (n=8); 5. Experiment 2: animals with CTI which daily were injected with DNICs days 10 (n=8); * - differences were statistically significant compared with the healthy animals ($p<0,05$); ** - differences were statistically significant compared with the control ($p<0,05$).

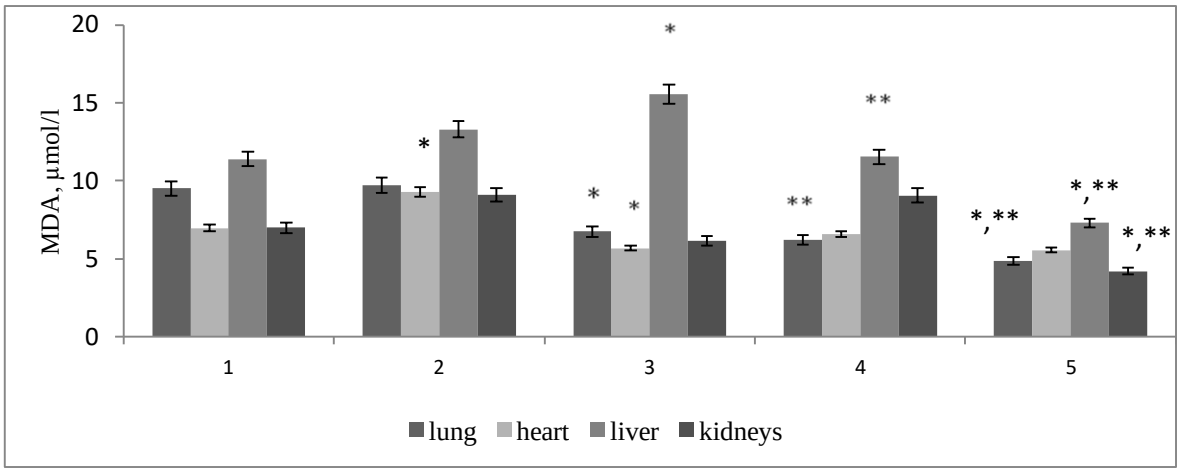

Figure 2: The concentration of malonic dialdehyde in rat organs after the CTI and after the introduction of dinitrosyl iron complexes. Notes: 1. Intact healthy animals (n=15); 2. Control 1: animals with CTI days 3 (n=8); 3. Control 2: animals with CTI days 10 (n=7); 4. Experiment 1: animals with CTI which daily were injected with DNICs days 3 (n=8); 5. Experiment 2: animals with CTI which daily were injected with DNICs days 10 (n=8); * - differences were statistically significant compared with the healthy animals ($p<0,05$); ** - differences were statistically significant compared with the control ($p<0,05$).

Malonic dialdehyde concentration decreased by 36% (p=0,008) and 28% (p=0,021) respectively after days 3 and days 10 after injury after exposure to DNICs in the lungs compared to the control group of rats. It is known that prooxidant-antioxidant balance is disturbed, free-radical reactions are intensified and oxidative stress develops in case of burns. This leads to hyperproduction of active forms of oxygen. Various cells and cellular structures are the target of active forms of oxygen[23,24]. It was shown that DNICs had a normalizing effect on the processes of lipoperoxidation in the lungs during thermal injury.

Statistically significant differences in the total antioxidant activity between rats of the control group and healthy animals were not found in the lungs according to induced biochemiluminescence (**figure 3**). However, the introduction of DNICs in CTI caused an increase in TAA for days 3 after the trauma by 28% (p=0,076) compared with the control.

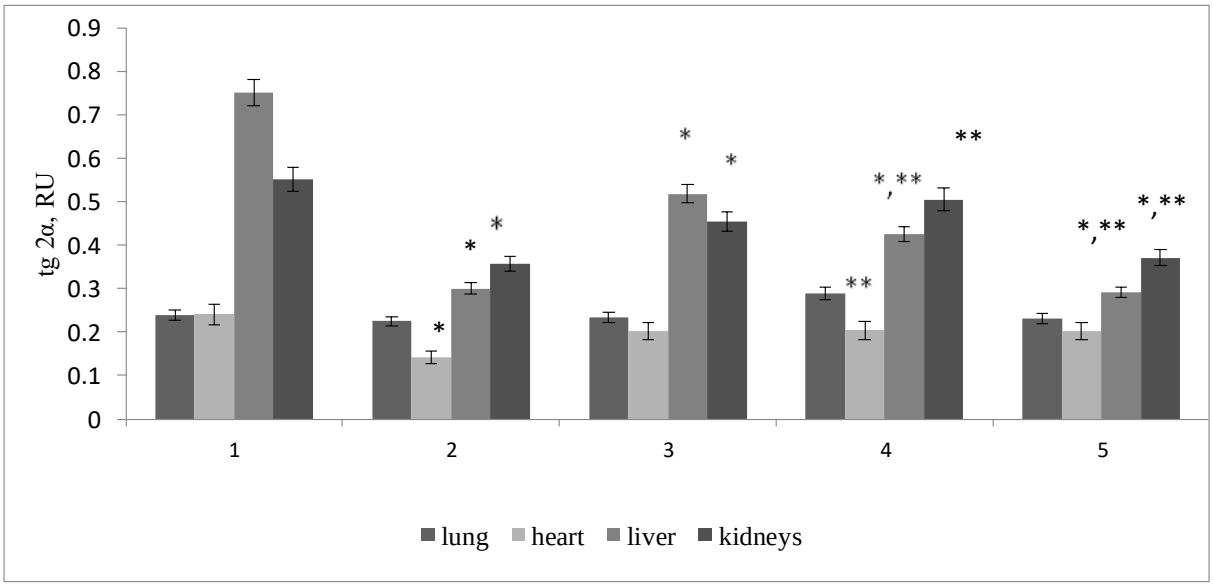

Figure 3: The tg2α indicator in rat organs after the CTI and after the introduction of dinitrosyl iron complexes. Notes: 1. Intact healthy animals (n=15); 2. Control 1: animals with CTI days 3 (n=8); 3. Control 2: animals with CTI days 10 (n=7); 4. Experiment 1: animals with CTI which daily were injected with DNICs days 3 (n=8); 5. Experiment 2: animals with CTI which daily were injected with DNICs days 10 (n=8); * - differences were statistically significant compared with the healthy animals (p<0,05); ** - differences were statistically significant compared with the control (p<0,05).

The reactions catalyzed by superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6) are important for the support of the prooxidant-antioxidant balance and the intracellular reduction potential in cells. The study of the enzymatic link of antioxidant protection in the lungs showed a decrease of the SOD activity in CTI transforming superoxide radicals into hydrogen peroxide. The specific activity of SOD decreased in 1,2 times (p=0,034) for days 3 and in 1,8 times (p=0,004) for days 10 after CTI in comparison with indicator of healthy animals (**Table 1**). The catalase activity decreased in the lungs by 4,5 times (p=0,001) for days 3 and by 1,2 times (p=0,009) for days 10 after CTI in comparison with intact rats (**Table 2**).

Table 1: Specific activity of superoxide dismutase (RU/mg) in rat organs after the CTI and after the introduction of dinitrosyl iron complexes

groups	lung	heart	liver	kidneys
1	1586,70±64,14	1083,51±76,26	758,23±30,60	652,9±39,92
2	1324,59±53,62*	1380,07±78,52*	962,39±27,31 *	734,51±37,27
3	897,01±43,27*	742,99±23,82 *	476,10±35,34 *	697,49±29,76
4	599,53±31,97*/**	963,94± 37,91 **	410,84±9,47 */**	601,91±26,81 **
5	998,23±65,92*	781,26±44,14 */**	579,76±13,18*	583,64±41,05

Notes: 1. Intact healthy animals (n=15); 2. Control 1: animals with CTI days 3 (n=8); 3. Control 2: animals with CTI days 10 (n=7); 4. Experiment 1: animals with CTI which daily were injected with DNICs days 3 (n=8); 5. Experiment 2: animals with CTI which daily were injected with DNICs days 10 (n=8); * - differences were statistically significant compared with the healthy animals (p<0,05); ** - differences were statistically significant compared with the control (p<0,05).

Table 2: Specific activity of catalase (RU/min×mg) in rat organs after the CTI and after the introduction of dinitrosyl iron complexes

groups	lung	heart	liver	kidneys
1	27,60±2,42	10,61±0,15	18,77±2,39	40,57±6,21
2	6,35±0,51 *	10,90±0,24	6,21±0,69 *	11,18±0,53 *
3	22,56±0,89 *	6,71±0,39 *	15,38±1,41	15,74±1,07 *
4	17,08±1,20*/**	7,99±0,37*/**	7,81±0,74*	9,34±0,36*/**
5	8,43±0,27*/**	13,11±0,41*/**	26,18±1,78*/**	8,19±0,41*/**

Notes: 1. Intact healthy animals (n=15); 2. Control 1: animals with CTI days 3 (n=8); 3. Control 2: animals with CTI days 10 (n=7); 4. Experiment 1: animals with CTI which daily were injected with DNICs days 3 (n=8); 5. Experiment 2: animals with CTI which daily were injected with DNICs days 10 (n=8); * - differences were statistically significant compared with the healthy animals (p<0,05); ** - differences were statistically significant compared with the control (p<0,05).

It has been showen that the activity of SOD and catalase in the lungs was statistically significantly lower than the indicator of healthy animals at injection of DNICs to rats with thermal burn. However, there was an increase in catalase activity for days 3 after injury at injection of DNICs in 2,8 times (p=0,003) compared with the control. There was a tendency to increase the specific activity of SOD by 11% (p=0,087) for days 10 after CTI (**Table 1**) in the lungs under the influence of the deposited form of nitric oxide in comparison with the control group of rats. This contributes to the reduction of highly reactive superoxide influencing on the formation of S-nitrosohemoglobin and stimulating the release of NO from S-nitrosoalbumin[3,25].

An increase in the intensity of induced chemiluminescence was found for days 3 after the burn by 27,8% (p=0,015) compared to healthy animals when studying the processes of lipoperoxidation in the heart of rats with CTI (**figure 1**).

It is known according to the literature that the elevated production of active forms of oxygen initiates the lipid peroxidation of biological membranes[24]. The study showed an increase in concentration of MDA in the heart by 33,3% (p=0,024) for days 3 after injury compared to intact rats. This indicated the activation of lipid peroxidation. The decrease in the process of induced chemiluminescence and the decrease in MDA by 20% (p=0,027) for days 10 after CTI compared with healthy animals was revealed. DNICs when CTI normalized the lipid peroxidation and MDA level in heart (**figure 2**). There was a decrease of tg2α in heart when days 10 after CTI and a decrease of tg2α by 41,1% (p=0,005) for days 3 after injury compared to healthy animals (**figure 3**). The total antioxidant activity in the heart at days 3 after the CTI increased by 43,70% (p=0,001) under the influence of deposited form of NO compared to control.

Compensatory increase in specific activity of SOD by 1,3 times (p=0,031) in comparison with intact rats was revealed for days 3 after thermal exposure. This indicates the mobilization of protective mechanisms[24]. The activity of SOD and catalase decreased in 1,5 times (p=0,023) and 1,6 times (p=0,030) respectively on the days 10 after CTI in the homogenate of heart compared with healthy animals (**Table 1, 2**). This can lead to the accumulation of hydrogen peroxide and superoxide radicals. By days 10 after CTI the specific activity of catalase increased by 2 (p=0,003) and 1,2 times (p=0,041) in comparison with control and healthy rats.

Features of hepatic insufficiency are well studied in burn disease. They are manifested in violations of pigment metabolism, protein- forming, synthesizing and detoxification functions, coagulopathies[21]. As a result, enzymes synthesized in the liver and bilirubin enter the blood in excess[23].

The study of induced chemiluminescence processes in the liver showed an increase in the lipid peroxidation by days 3 and 10 after injury by 43,4% (p=0,012) and 45,3% (p=0,006) respectively compared with healthy animals (**figure 1**). The results indicate about an intensification of peroxide processes in the liver during burns. DNICs caused a decrease of FRO in liver by 27% (p=0,023) on days 3 and by 40,1% (p=0,017) on days 10 after CTI compared to control (**figure 1**).

MDA level increased by 21% in the liver (p=0.087) by days 3 and MDA concentration increased by 36,6% (p=0,031) by days 10 after injury compared to healthy animals (**figure 2**). Thus, the elevated level of MDA may be an additional marker of acute parenchymal liver damage. Injection of DNICs to rats with thermal injury led to a decrease of MDA in the liver by 13% (p=0,042) for days 3 and by 53% (p=0,003) for days 10 after injury compared to control. The MDA level on days 10 after CTI under the influence of DNICs was lower by 35,9% (p=0,005) compared to intact animals. Thus, DNICs contribute to the reduction and reduction of the intensification of peroxidation processes in the liver in rats with CTI.

TAA in the liver decreased by days 3 and 10 after injury by 60% (p=0,017) and 30% (p=0,022) respectively compared to healthy rats (**figure 3**). The observed increase in peroxide processes on the background of antioxidant system depression indicates the development of oxidative stress in the liver of rats with CTI. Oxidative stress is probably the fact of active mobilization of all systems, and the plastic system did not have time to join in the adaptation process. DNICs caused the increase in tg 2α at days 3 after injury by 39% (p=0,031) compared with the control.

SOD activity in the liver compensatory increased by 27% (p=0,012) at days 3 after burn and decreased by 1,6 times (p=0,005) on days 10 after CTI compared to intact animals. There was a tendency to increase the activity of SOD under the influence of DNICs by 22% (p=0,065) on the days 10 after CTI compared to control. The activity of catalase decreased in the liver in 3,2 times (p=0,001) in days 3 after CTI compared to healthy animals. DNICs increased the catalase activity in the liver at days 10 after injury in 1,7 times (p=0,011) compared with the control animals and in 1,4 times (p=0,022) compared with healthy rats.

Burn injury is a severe form of pathology, which is accompanied by the formation of systemic inflammatory response syndrome. It adversely affects the state of internal organs and participates in the development of multi-organ insufficiency[21]. The light sum and the level of MDA in the kidneys increased by 3 days after the burn by 67% (p=0,014) and 21% (p=0,034), respectively, compared with intact rats (**figure 1, 2**). According to the literature data, the processes of LPO increase, the formation of active forms of oxygen increases, the antioxidant system is quenched under the influence of chemical or physical factors on an organism[24].

DNICs helped to reduce the processes of lipid peroxidation in the kidneys for days 3 after CTI compared with control animals by 34% (p=0,027) (**figure 1**). The MDA and LPO decreased by 33% (p=0,021) and 15% (p=0,039), respectively, at days 10 after an injury under the influence of DNICs.

Total antioxidant activity in rat kidney homogenate decreased on days 3 after CTI – by 35% (p=0,021), on days 10 – by 17,7% (p=0,035) relative to healthy animals. These data indicate the inhibition of the antioxidant system of protection in thermal injury (**figure 3**). TAA increased on days 3 by 41,3% (p=0,026) relative to control with the introduction of DNICs, but on days 10 TAA was lower than healthy and control rats by 32,5% (p=0,009) and 18,1% (p=0,021) respectively.

The decrease of catalase activity in kidneys for days 3 by 3,6 times (p=0,007), for days 10 after injury by 2,5 times (p=0,009) in comparison with healthy animals was revealed. Introduction of DNICs to rats contributed to a statistically significant reduction in catalase activity during CTI and compared with intact animals and compared to control (**Table 2**). There were not statistically significant changes in the activity of SOD in the kidneys during CTI (**Table 1**).

Thus, studies have shown that the thermal injury causes intensification of free radical oxidation on the background of a decrease in antioxidant reserves in all investigated organs. The amplification of peroxidation processes, accumulation of MDA, the oppression of the antioxidant system were identified in the liver, kidney, heart and lung of rats with CTI. A decrease in the catalytic properties of catalase in all organs during CTI was noted. The decrease of specific activity of SOD for days 10 after injury in liver, heart and lungs was revealed. The most expressed increase in LPO and decrease in TAA were noted in the liver. A factor reinforcing oxidative stress is the acidification of the medium caused by the activation of glycolysis in tissues deprived of oxygen during hypoxia. This leads to the accumulation of lactic acid in the tissues in conditions of circulatory disorders. Acidification of the medium leads to the release of iron ions, inducing free radical oxidation.

CONCLUSION

It was installed that DNICs have pro - and antioxidant properties. DNICs have a normalizing influence on the processes of lipid peroxidation in lung and heart of rats with CTI. There was a statistically significant decrease in MDA and LPO for days 10 after injury under the influence of DNICs in the liver. A reduction of lipid peroxidation in the kidneys during CTI under the influence of DNICs has been shown. Specific activity of SOD and catalase increased by days 10 after injury in the lungs, heart and liver of rats after exposure to deposited form of nitric oxide. Because DNICs are donors of nitric oxide, it can be assumed that NO acts in the investigated organs as an antioxidant grabbing alkoxyl and alkylperoxyl radicals. As a result a chain reactions of peroxidation are terminated[3,12], nitro-derivatives of lipids are formed and adduct (alkylperoxynitrite), similar to peroxynitrite, is formed[9]. In addition, one of the mechanisms of antioxidant action of NO is binding of free iron ions in the composition of nitrosyl complexes. Free radical oxidation reactions catalyzed by redox-active iron ions are inhibited[3]. Thus, nitric oxide can protect biological molecules from oxidative modification and can affect the hemoproteins (catalase).

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