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EFFECTS OF LIPOSOMAL FORM OF PHOSPHATIDYLCHOLINE ON OXIDATIVE-NITROSATIVE STRESS IN RENAL TISSUES OF RATS IN BURN DISEASE

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Abstract

This experiment carried out on 77 white Wistar rats aimed at studying the effects of liposomal form of phosphatidylcholine (lipin) on oxidative-nitrosative stress in renal tissues in burn disease (BD). This condition was modelled by immersing the epilated skin surface of the hind limb of the experimental animals into hot water (t + 70 – 75°C) under light ether anaesthesia for 7 s that led to the development of III A-B degree burns. Lipin was administered intraperitoneally daily in a dose of 50 mg / kg immediately after BD modelling.

The research has demonstrated BD modelling is accompanied by the development of oxidative-nitrosative stress in renal tissues as evidenced by a significant increase in the superoxide anion radical production by various sources (endoplasmic reticulum and NO synthase, mitochondrial respiratory chain, leukocyte NADPH oxidase); by the growth in the activity of inducible isozyme of NO-synthase (in the stage of burn shock in 2.83 times, $p<0.001$) with a decline in the constitutive isoforms activity, by the rise in the concentration of peroxynitrite (in the stage of burn shock in 3.18 times, in the stage of septicotoxemia in 2.56 times).

The administration of lipin significantly limits the development of oxidative-nitrosative stress in renal tissues in the dynamics of experimental BD, as evidenced by a probable decrease in superoxide anion radical, NO-synthase activity due to its inducible isoform (in the stage of burn shock – by 35.9%; in the stage of toxemia – by 43.1%, $p<0.001$), by redressing the imbalance between inducible and constitutive isozymes of NO-synthase, by reducing the concentration of peroxynitrite (in the stage of burn shock – by 34.3%, in the stage of late toxemia – by 44.3%; in the stage of septicotoxemia – by 44.3%, $p<0.001$).

Key words: burn disease; oxidative-nitrosative stress; free radical processes; kidney; liposomal form of phosphatidylcholine (lipin).

Introduction

Burns are among the commonest types of trauma throughout the world. The WHO estimates that 11 million burn injuries of all types occur annually worldwide, 180,000 of which are fatal [1]. Data from the National Centre for Injury Prevention and Control in the USA show that approximately 2 million fires are reported each year which result in 1.2 million people with burn injuries. Moderate to severe burn injuries requiring hospitalization account for approximately 100,000 of these cases, and about 5,000 patients die each year from burn-related complications [2].

Thermal damage is known to trigger a systemic cascade of metabolic, functional and structural changes described as burn disease (BD) that adversely affects the internal organs and contributes to the development of multiple organ failure [3, 4]. Acute kidney injury (AKI) may develop as one of the consequences of severe burns [5]. On average, in 15% of cases, the complex of functional changes in the kidneys in burn disease (BD) meets the AKI criteria [6, 7].

Kidneys contain large amounts of unsaturated fatty acids, which make them particularly vulnerable to oxidative stress regardless of the type of injury [8]. The role of free radicals in burn-induced AKI has been shown in the burn modelling experiment on rats [9].

There are certain expectations for the pathogenetic therapy of renal pathology during BD that come with the use of lipin, a liposomal form of natural lyophilized phosphatidylcholine [10]. Lipin, administered at the period of burn shock and early toxemia, showed the positive effect on the function of external respiration [10], but has not been studied yet as a nephroprotective agent in BD.

The aim of this study is to investigate the effects of liposomal form of phosphatidylcholine (lipin) on oxidative-nitrosative stress in renal tissues of rats in BD.

Materials and methods

The study was conducted on 77 mature rats of the Wistar line. BD was modelled by immersing the epilated skin surface of the hind limb of the experimental animals into hot water (t +70–75 °C) under light ether anaesthesia for 7 s that led to the development of III A-B degree burns. Lipin was administered intraperitoneally daily in a dose of 50 mg / kg immediately after BD modelling.

The research was conducted in compliance with the standards of the Convention on Bioethics of the Council of Europe 'European convention for the protection of vertebrate animals used for experimental and other scientific purposes' (Strasbourg, 18.III.1986). The experimental animals were euthanized under ether narcosis on the 1st, 7th, 14th, 21st, and 28th day that was consistent with the stages of burn shock, toxemia, and septicemia [11].

The production of superoxide anion radical ($\cdot O_2^-$) was evaluated by a test with nitro blue tetrazolium using spectrophotometry of the kidney homogenate by the following inductors: NADH was used to evaluate O_2^- production by the mitochondrial electron transport chain (ETC), NADPH was used to evaluate O_2^- production by endoplasmic reticulum and NO-synthase (NOS), and *S. typhi* lipopolysaccharide (LPS) was used to assess O_2^- production by NADPH oxidase of white blood cells [12].

The total activity of NOS was determined by the difference in the concentration of nitrite ions before and after the incubation of homogenate into the medium containing L-arginine (NOS substrate) and NADPH. The concentration of nitrite ions was assessed by the formation of diazo-compounds in the reaction with sulfanilic acid, and then we carried out the reaction with N-(1-Naphthyl)ethylenediamine, resulting in the production of red colour derivatives [13]. To determine the activity of constitutive NO-synthases (cNOS), we added 0.1 ml of 1% aminoguanidine hydrochloride solution ("Sigma-Aldrich") [14]. The activity of inducible NO-synthase (iNOS) was evaluated by subtracting the cNOS activity from the total NOS activity. The concentration of peroxynitrite in the homogenates was evaluated spectrophotometrically by wavelength of 355 nm [13].

The findings obtained were statistically processed. To verify the normality distribution, the Shapiro-Wilk test was applied. If they corresponded to the normal distribution, then the Student's t-test was used to compare independent samples. When the results ranges were not subject to normal distribution, statistical processing was performed using a nonparametric method, the Mann-Whitney test. Statistical calculations were performed using the Microsoft Exel (2007) for Windows Professional.

Results and discussion

Table 1 demonstrates the results of the $\cdot O_2^-$ production in the renal tissues of the rats in the dynamics of experimental BD course. In a day since the onset of modeled BD, the production rate of this radical by microsomal ETC increased 2.28-fold compared with the control.

Table 1 - The production of superoxide anion radical in the renal tissues of the rats under modelled burn disease (numerator) and under its correction with liposomal form of phosphatidylcholine (denominator) (M ± m)

Group of experimental animals	Inductors of superoxide anion radical production, nmol / g • s		
	NADPH	NADH	LPS
Control	17.32±0.96	19.32±1.20	0.79±0.08*
BD, 1st day	39.60±0.68* 27.47±1.54*,**	42.76±1.02* 30.43±2.15*,**	1.94±0.06* 1.29±0.19*,**
BD, 7th day	36.00±1.60* 22.7±1.42*,**	40.55±1.98* 25.49±1.73*,**	1.65±0.12* 1.10±0.10*,**
BD, 14th day	34.42±0.82* 22.38±1.18*,**	37.31±1.03* 25.48±1.64*,**	1.63±0.09* 1.08±0.10*,**
BD, 21st day	32.79±1.30* 21.04±1.31*,**	36.52±1.87* 23.64±1.93**	1.55±0.10* 1.04±0.12**
BD, 28th day	29.02±2.44* 18.55±1.68**	31.60±3.51* 20.98±2.69**	1.37±0.19* 0.91±0.14

Note: * – $p<0.05$, when compared to the control; ** – $p<0.05$, compared with a particular stage of burn disease with no correction.

On the 7th day of BD modelling, this indicator exceeded that in the control animals in 2.08 times, on the 14th day it was higher in 1.99 times, on the 21st day – in 1.89 times, and on the 28th day – in 1.68 times. It points out a significant and long-term role of NADPH-dependent ETC (endoplasmic reticulum and NOS) in the $\cdot O_2^-$ production with its maximum in the phases of burn shock and toxemia.

The $\cdot O_2^-$ production by mitochondrial ETC (by adding NADH as an inducer) in a day since BD modelling increased in 2.21 times compared with the control. On the 7th day, this value exceeded the values of findings in the control animals by 2.09 times, on the 14th day – by 1.93 times, on the 21st day – by 1.89 times, and on the 28th day – by 1.64 times that confirms the role of NADH-dependent mitochondrial respiratory chain as the main source of reactive oxygen species in nephrocytes [12].

Then this figure was found out also to be significantly higher than the result in the control animals: on the 7th day of BD it was higher in 2.08 times, on the 14th day – in 2.06 times, on the 21st day – in 1.96 times. On the 28th day, the rate of $\cdot O_2^-$ production by phagocytes in the kidney tissues increased in 1.73 times.

Lipin administration since the onset of experimental BD did not considerably change the rate of $\cdot O_2^-$ production by NADPH-dependent ETC (microsomes and NOS) for 3 weeks, and remained elevated. However, the value of this indicator was probably inferior to the results in the relevant groups of rats, which did not receive lipin correction: in a day after BD modelling this was found out lower by 30.6%, on the 7th day – by 37.0%, on the 14th day – by 35.0%, on the 21st day – by 35.8%, and on the 28th day – by 36.1%. This indicates the capability of lipin to correct functioning of NADPH-dependent ETC (endoplasmic reticulum and NOS) and to restrict the $\cdot O_2^-$ production, having normalized its rate by the 28th day of the experimental BD.

When using lipin since the onset of experimental BD, the rate of $\cdot O_2^-$ productions by NADH-dependent ETC (mitochondria) remained at an elevated level for 2 weeks. The value of this indicator was significantly inferior to the values of the relevant groups without BD correction: in a day after BD modelling it was lower by 28.8%, on the 7th day – by 37.1%, on the 14th day – by 31.7%, on the 21st day – by 35.3%, and on the 28th day – by 33.6%.

That is, the liposomal form of phosphatidylcholine demonstrated the capability to produce a positive effect on the functioning of the mitochondrial respiratory chain in the experimental BD course, limiting the possibility of 1-electron reduction of molecular oxygen with the $\cdot O_2^-$ formation, and normalizing its production by the 21st day of the experiment.

The lipin administration did not affect the rate of $\cdot O_2^-$ production by leukocyte NADPH-oxidase and it remained increased for 2 weeks. The value of this indicator was significantly lower than that of the relevant groups, which did not receive BD correction: in a day after BD modelling it was found out lowered by 33.5%, on the 7th day – by 33.3%, on the 14th – by 33.7%, on the 21st day – by 32.9%. On day 28, the rate of $\cdot O_2^-$ production by phagocyte NADPH oxidase did not differ significantly from those in the animals, which did not received lipin during modelled BD.

We suggest lipin can restrict the $\cdot O_2^-$ production not only by intracellular ETC, but also by leukocyte NADPH-oxidase, which is found on the plasma membrane of leukocytes, and can normalize this process by the 21st day of the experiment.

The role of nitric oxide system in acute renal pathology is controversial and sometimes difficult to predict, because NO has both negative and nephroprotective effects [15, 16]. There have been reports on this molecule as a mediator of systemic inflammatory response caused by skin burns, as well as on the changes in the activity of constitutive and inducible isoforms of NO-synthase that attend functional and metabolic disorders of various organs in BD [17]. However, the effect of thermal trauma on the NO-ergic system of the kidneys has not been studied sufficiently.

Table 2 presents the data on the activity of NOS isoforms in the renal tissues of the rats under modelled BD. After one day since BD modelling, the total activity of NOS in the kidney tissues of the rats grew up in 2.34 times, apparently due to its inducible isoform, whose activity increased in 3.22 times compared to the data of the control group. At the same time, the cNOS activity significantly went down, in 1.66 times, that is indicative of endothelial dysfunction in the kidneys at the stage of burn shock.

Table 2 - Indicators of nitrosative stress in the kidney tissues of rats under modelled burn disease (numerator) and under its correction by liposomal form of phosphatidylcholine (denominator) (M ± m)

Group of experimental animals	NO-synthase activity, μmol / min •·g· of protein			Concentration of peroxynitrite- ions, μmol / g
	Total	cNOS	iNOS	
Control	1.94±0.24	0.59±0.04	1.35±0.33	0.98±0.04
BD, 1st day	4.54±0.14* 3.15±0.36*,**	0.20±0.03* 0.37±0.04*,**	4.34±0.12* 2.78±0.36*,**	3.12±0.02* 2.05±0.06*,**
BD, 7th day	4.05±0.33* 2.59±0.27**	0.22±0.05* 0.41±0.07*,**	3.83±0.36* 2.18±0.30**	2.73±0.04* 1.64±0.05*,**
BD, 14th day	3.92±0.18* 2.51±0.25**	0.28±0.04* 0.48±0.04**	3.64±0.21* 2.03±0.35**	2.71±0.03* 1.51±0.06*,**
BD, 21st day	3.71±0.23* 2.43±0.28**	0.29±0.03* 0.46±0.04*,**	3.42±0.25* 1.97±0.38**	2.51±0.03* 1.47±0.06*,**
BD, 28th day	3.26±0.55* 2.12±0.33	0.33±0.03* 0.48±0.06**	2.93±0.70 1.64±0.31	2.10±0.09* 1.17±0.04*,**

Note: * – $p<0.05$, when compared to the control; ** – $p<0.05$, when compared to a particular stage of burn disease with no correction.

Subsequently, the total NOS activity in renal tissues remained higher compared to the control. In the period of toxemia, on the 7th day of modelled BD, this figure exceeded the value of the control group in 2.08 times and on the 14th day in 2.02 times. In the stage of

septicemia, on the 21st day of BD modelling, the total NOS activity was in 1.91 times higher, and on the 28th day – in 1.68 times ($p < 0.05$) higher than in the control animals.

The iNOS activity in the renal tissues on the 21st day of the BD course was above the values in the control group. On the 7th day of modelled BD, this figure was 2.83 time higher than the result in the control group, on the 14th day – 2.69 time higher. In the stage of septicemia, on the 21st day of the BD modelling, iNOS activity was 2.53 times higher than the control data. However, on the 28th day following the thermal injury, no significant differences compared to the control group were detected.

The cNOS activity in the renal tissues throughout the observation period was probably lower than the value of the control group. On the 7th day of modelled BD, this value was lower than the result in the control group in 1.63 times, on the 14th day – in 1.53 times, on the 21st day – in 1.51 times, and on the 28th day – in 1.44 times.

Simultaneous nephrocyte production of high NO concentrations by the iNOS and $\cdot O_2^-$ by various sources, including the mitochondrial respiratory chain, cNOS, leukocyte NADPH-oxidase, creates preconditions for the production of highly toxic reactive nitrogen species peroxynitrite [18, 19].

Starting from the phase of burn shock, the concentration of peroxynitrites in the renal tissues probably exceeded the control result: in 24 hours it was higher in 3.18 times, on the 7th day – in 2.78 times, on the 14th day – in 2.76 times, on the 21st day – in 2.56 times, and on the 28th day – in 2.14 times.

The lipin administration since the onset of experimental BD resulted in the normalization of the total NOS activity in 2 weeks.

The values of this indicator were probably inferior to that in the relevant comparison groups: in a day since BD modelling they lowered by 30.6%, on the 7th day – by 36.0%, on the 14th day – by 36.0%, on 21st day – by 34.5%. On day 28, the total NOS activity did not differ significantly from that obtained in rats, which did not receive lipin.

Similar dynamics was found out when we assessed iNOS activity in the renal tissues. The values of iNOS activity were also significantly inferior to the results of the relevant comparison groups: in a day since BD modelling they were lower by 35.9%, on the 7th day – by 43.1%, on the 14th day – by 44.2%, on 21st day – by 42.4%. On the 28th day, iNOS activity did not significantly differ from the findings obtained in the rats, which did not receive lipin after BD modelling.

However, the cNOS activity in renal tissues with under the lipin administration since the onset of experimental BD throughout the study significantly exceeded the values in the relevant comparison groups: in a day following the onset it was higher by 85.0%, on the 7th day – by 86.4%, on the 14th day – by 71.4%, on the 21st day – by 58.6%, on the 28th day – by 45.5%.

The content of peroxynitrites in the renal tissues in the rats, which received lipin since the onset of the experimental BD throughout the study was significantly inferior to the values of the relevant comparison groups: in a day it was lowered by 34.3%, on the 7th day – by 44.3%, on the 21st day – by 41.4%, and on the 28th – by 44.3%.

Lipin shows antihypoxic, antioxidant, membrane-protective, anti-inflammatory, detoxifying and organoprotective effects, as well as the ability to improve regional hemodynamics in different organs [10]. The use of phospholipids in the form of liposomes can improve the oxygen supply to the tissues, reduce the degree of tissue hypoxia and concomitant metabolic acidosis, and diminish hypoxic damage to the kidneys and other organs.

Conclusions

1. The research has demonstrated the burn disease modelling, starting from the phase of burn shock, is accompanied by the development of oxidative-nitrosative stress in renal tissues as evidenced by a significant increase in superoxide anion radical production by various sources (endoplasmic reticulum and NO synthase, mitochondrial respiratory chain, leukocyte NADPH-oxidase), increased activity of inducible isoenzyme of NO-synthase with the decrease in the activity of its constitutive isoforms , and rise in the concentration of peroxynitrite.

2. The administration of liposomal form of phosphatidylcholine significantly limits the development of oxidative-nitrosative stress in renal tissues in the dynamics of experimental burn disease, as evidenced by a significant decrease in superoxide anion radical, NO-synthase activity due to its inducible isoform, by redressing the imbalance between iNOS and cNOS, by lowering the peroxynitrite concentration.

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