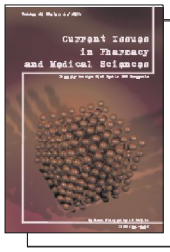

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Oxidizing modification of proteins in case of a burn injury in rats against the topical treatment with new wound healing preparations

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ABSTRACT

A significant increase in the degree of the oxidizing modification of proteins in rats after the reproduction of the third-degree burns was established. In the course of the treatment, positive changes in aldehydephenylhydrazones and ketophenylhydrazones indices and their restoration to the level of the intact control were received, indicating the inactivation of free radical and peroxide compounds under the influence of wound healing preparations, as opposed to the animals that did not receive treatment. The further study of the influence of new wound healing drugs on the state of the antioxidant system and lipid peroxidation in order to establish their mechanisms of reparative action is promising.

Keywords: a burn injury, oxidizing modification of proteins, wound healing preparations

INTRODUCTION

Burn injuries are a type of traumatic pathology that is characterized by a complex set of polysystemic shifts. The body's response to a burn injury corresponds to the mechanism of the stress response with the development of the adaptation syndrome, resulting in disruption of the normal flow of oxidizing processes [6]. Oxidizing modification of proteins (OMP) is one of the early and most reliable indicators of tissue damage in case of a free radical pathology. These are proteins but not lipids or nucleic acids that are effective traps of the generated reactive oxygen species (ROS) and their oxidizing modification is regarded as one of early and reliable markers of the oxidizing stress [1, 9]. The oxidates of proteins in oxidizing damages in tissues appear earlier and are more stable compared to the products of lipid peroxidation, in particular plasma proteins subjected to oxidizing degradation having quite long half-life. [1] OMP causes changes in physical and chemical properties of the protein molecule: the fragmentation, aggregation and susceptibility to pro- teolysis [2, 8]. As a result, either the formation of products with a high functional activity or either inactivation of zymophores, or modification of protein molecules, that can have a toxic effect and aggravate the pathologic process, occur.

The presence of ROS in the wound defect leads to denaturation of the synthesized protein and inactivation of fibroblasts that impede wound healing [9]. The study of the biochemical aspects of the development of a burn injury is necessary to generally understand the mechanisms of the healing process and develop a strategy for its treatment and development of new wound healing medicines that affect the majority of pathologic links of the wound process. In most studies, devoted to the mechanisms of development of a burn wound, the content of lipid peroxidation products is analyzed, the concentration of oxidized proteins is virtually paid no attention [6,7].

The aim of this study is to examine the state of oxidizing modification of proteins in the treatment with new wound healing preparations at the model of the third A-degree burn injury in rats.

MATERIALS AND METHODS

A model of a burn injury was reproduced on 168 mature male albino rats weighing 200–240 g. In animals, under general anesthesia using thiopental (40 mg/kg) on the depilated skin area on the back, retreating from the spine for 1.5 cm, a burn was simulated with an instrument

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with a fixed temperature scale and electric soldering iron, at the end of which a round metal plate with a diameter of 2.5 cm is mounted. Exposure time of the contact plate heated to 200°C was 10 seconds [8]. The histological examination of the damaged skin confirmed that under the above conditions the third A-degree burn was formed, according to the clinical classification of burns [4]. The rats were withdrawn from the experiment using thiopental anesthesia at different phases of wound process: on the 2nd, on the 9th and on the 18th days of the experiment. Simultaneously, the material was taken from the rats of intact groups.

To evaluate the intensity of peroxidation process for proteins the method of determining the oxidizing modification of proteins by the level of carbonyl derivatives formed after reaction with ROS with histidine, arginine, lysine, and proline residues was used [2, 3]. The principle of E.E. Dubinina's method is based on the reaction of interaction of oxidized amino acid residues of proteins with 2,4-dinitrophenylhydrazones, which are recorded spectrophotometrically. The concentration of the formed oxidized reaction products, aldehyde- (APH) and ketophenylhydrazones (KPH) is proportional to the optical density at 270, 363, 370 nm as measured by a spectrophotometer SF-46 (Russia). The content of carbonyl derivatives of oxidized proteins was expressed in units of optical density, referred to 1 mg of protein (U/mg of protein) [1].

Experimental animals in series I of the experiment were divided into 5 groups: I – an intact control (IC), II – a positive control (PC), III – an experimental group, in which the animals were treated with "Bioflorin" ointment, IV – an experimental group of animals received the preparation "Prolidoxyd" ointment, and group of animals V received a comparator preparation – "Algofin" ointment (company "Krasnaya zvezda", Ukraine). In series II of the experiment the animals were also divided into 5 groups: I – IC, II – PC, III – an experimental group, which was treated with "Dexpanthenol with ceramides" cream, IV – an experimental group of animals received the preparation "Ceramides" cream and in group V the animals were treated with the comparator preparation – "Bepanthen" cream ("Bayer" company, Germany). Comparator preparation were selected as analogues according to the dosage form, in part according to the composition of the active ingredients or their origin, pharmacological effects and indications for use. The preparations were applied on the surface of burns in rats with a sterile spatula once a day in an arbitrary therapeutic dose of 20 mg/cm^2. The treatment was given until the complete healing of burns.

All interventions and euthanasia of animals were carried out in compliance with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1986). The results obtained were treated with the program Statistica 6.0 based on one-way ANOVA using the Kruskal-Wallis and Newman-Keuls criteria at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

After the reproduction of the third-degree burn injury in rats a thick crust of a dark-brown colour with a clearly bounded zone of necrosis and apparent inflammatory changes in the surrounding tissues were formed. On the 2nd day of the experiment (the peak of pathology), which corresponds to the stage of a shock in burn disease, the content of OMP products has increased dramatically in animals. Definitely, in comparison with the intact, the levels of APH (by 1.8 times) and KPH (by 2.1 times and 2.5 times), recorded at a wavelength of 363 and 370 nm (Table 1 and 2), increased. The data obtained show an increase in the intensity of OMP process in the acute period of thermal injury and confirm the destruction of the membranes of tissues, as well as the predominance of catabolic processes over anabolic ones.

Table 1. The content of products of oxidizing modification of proteins (U/mg of protein) in the blood serum of rats on a model of the third-degree burn, $x \pm S_x$ (the 1st series of the experiment)

Experimental groups	Indices of oxidizing modification of proteins		
	Aldehyde- phenylhydrazones	Ketophenylhydrazones	
	λ=270 nm	λ=363 nm	λ=370 nm
Intact control (n=6)	0.072±0.003	0.097±0.004	0.126±0.003
2nd day (n=6)			
Positive control	0.133±0.003*	0.209±0.11*	0.303±0.013*
9th day (n=6)			
Positive control	0.104±0.004*	0.133±0.006*	0.267±0.009*
"Prolidoxyd" ointment	0.086±0.004*	0.113±0.002*/**	0.228±0.007*/**
"Bioflorin" ointment	0.064±0.004**/***	0.120±0.003*	0.175±0.005*/**/***
"Algofin" ointment	0.078±0.002**	0.121±0.003*	0.208±0.005*/**
18th day (n=6)			
Positive control	0.074±0.004	0.083±0.004	0.182±0.004*
"Prolidoxyd" ointment	0.070±0.004	0.099±0.004	0.135±0.004**
"Bioflorin" ointment	0.060±0.003**	0.081±0.007	0.127±0.003**
"Algofin" ointment	0.068±0.004	0.082±0,005	0.137±0.004**

* the differences are valid relative to the intact control group, $p<0.05$;

** the differences are valid relative to the positive control group, $p<0.05$;

*** the differences are valid relative to the comparator preparation that is "Algofin" ointment, $p<0.05$;

n=6 – the number of animals in each experimental group.

Table 2. The content of products of oxidizing modification of proteins (U/mg of protein) in the blood serum of rats on a model of the third-degree burn, $x \pm S_x$ (the II[nd] series of the experiment)

Experimental groups	Indices of oxidizing modification of proteins		
	Aldehyde- phenylhydrazones	Ketophenylhydrazones	
	λ=270 nm	λ=363 nm	λ=370 nm
Intact control (n=6)	0.075±0.003	0.100±0.004	0.128±0.003
2nd day (n=6)			
Positive control	0.134±0.003*	0.208±0.012*	0.315±0.014*
9th day (n=6)			
Positive control	0.107±0,004*	0.135±0.006*	0.279±0.008*
"Ceramides" cream	0.083±0.005**	0.089±0.003**/ ***	0.174±0.004*/ **/ ***
"Dexpanthenol with ceramides" cream	0.074±0.004**/ ***	0.103±0.005** / ***	0.150±0.006*/ **/ ***
"Bepanthen" cream	0.110±0.004*	0.125±0.004*	0.217±0.007*/ **
18th day (n=6)			
Positive control	0.074±0.005	0.088±0.003	0.184±0.004*
"Ceramides" cream	0.057±0.004**/ ***	0.070±0.003 **/ ***	0.128±0.003**
"Dexpanthenol with ceramides" cream	0.066±0.003***	0.093±0.003***	0.122±0.004**/ ***
"Bepanthen" cream	0.082±0.003	0,105±0.004**	0.128±0.006**

* the differences are valid relative to the intact control group, $p<0.05$;

** the differences are valid relative to the positive control group, $p<0.05$;

*** the differences are valid relative to the comparator preparation that is "Bepanthen" cream, $p<0.05$

Further observation of the dynamics of the level of OMP products showed that in all groups there was a significant reduction in the concentration of carbonyl groups and the approximation of these indices to the values of intact animals.

In the PC group of animals on the 9th and 18th days of the experiment the level of APH was significantly reduced by 1.3 and 1.8 times, and the level of KPH was reduced by 1.4 and 2.4 times (λ = 363 nm) and by 1.1 and 1.4 times (λ = 370 nm) compared with the peak of pathology. It should be noted that the level of KPH on the 18th day (λ = 370 nm) slightly decreased and significantly exceeded the level of IC, indicating the further flow of the pathological process in this group.

Treatment of animals with the study preparations (series I of the experiment) contributed significantly to the significant decrease in OMP performance, in relation to the PC group, already on the 9th day of treatment. Under the influence of "Prolidoxyd" ointment the level of KPH significantly decreased, and under the influence of "Bioflorin" and "Algofin" ointments the levels of APH and KPH decreased (at λ = 363 nm). On the 18th day of treatment, all indices recovered to the level of IC under the influence of preparations. Comparison of the effectiveness of the study preparations showed that according to the influence on OMP process "Prolidoxyd" ointment was not worse than the comparator preparation – "Algofin" ointment, and on the 9th day "Bioflorin" ointment exceeded the activity of the latter, as in its application APH and KPH indices (λ = 370 nm) were significantly different from "Algofin" ointment.

In series II of the experiment on the 9th and 18th day of the treatment of animals with "Ceramides" cream, the level of APH was significantly lower by 1.2 and 1.3 times, and the level of KPH at λ = 363 nm was lower by 1.5 and 1.3 times, and at ë = 370 nm it was lower by 1.6 and 1.4 times as compared with the PC group. When applying "Dexpanthenol with ceramides" cream the level of APH was significantly lower by 1.5 and 1.1 times, and the level of KPH was significantly lower on the 9th day by 1.3 times (λ = 363 nm), and on the 9th and 18th days was lower by 1.9 and 1.6 times (λ = 370 nm) in comparison with the PC group. In case of treatment with "Bepanthen" cream on the study terms the concentration of APH and KPH (λ = 363 nm) was at the level of PC and only the KPH index (λ = 370 nm) for both terms was significantly reduced by 1.3 and 1.4 times compared with the PC group. Consequently, the effectiveness of treatment with "Ceramides" and "Dexpanthenol with ceramides" creams exceeded the effectiveness of the comparator preparation that is "Bepanthen" cream.

Thus, the results obtained have showed that in the development of a burn injury the balance between formation and neutralization of free radicals is expressly disrupted. New wound healing preparations have showed an inhibitory effect on the OMP process and by the intensity of the therapeutic action exceeded the effectiveness of the reference preparations. The probable mechanism of wound healing action of preparations is associated with inhibition of processes of OMP formation due to the stabilization of biological membranes.

CONCLUSIONS

The conducted study of processes of oxidizing modification of proteins has showed that after modeling of burns in animals their significant activation is observed and confirmed by a significant increase in aldehydephenylhydrazones and ketophenylhydrazones indices as compared with the intact control. The use of new wound healing preparations in the treatment of burns significantly affects the reduction of aldehydephenylhydrazones and ketophenylhydrazones concentration in the blood serum of rats on the 9th day and the normalization of these indices to the level of the intact control on the

18th day, in contrast to the positive control. By normalization of oxidizing modification of proteins processes under the influence of study preparations, their effectiveness is not worse than that of comparator preparations.

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