

одночасного ураження токсикантами є статевонезрілі шури. Найвищий вміст С-реактивного протеїну зареєстровано у сироватці крові шурів старечого віку, у яких він підвищився в 3,3 раза. Карболайн виявився ефективним в останні терміни дослідження. Після ураження спостерігався зсув балансу між цитокинами в сторону прозапальних процесів, на що вказувало підвищення вмісту прозапального та зниження вмісту протизапального інтерлейкіну. У статевозрілих шурів ентеросорбент призвів до вірогідного відновлення дисбалансу цитокинів у сироватці крові після ураження.

Ключові слова: натрію нітрит, тютюновий дим, метгемоглобін, карбоксигемоглобін, цитокини, С-реактивний протеїн

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одновременному поражению токсикантами были неполовозрелые крысы. В сыворотке крови пораженных старых крыс отмечено наиболее высокое содержание С-реактивного протеина (в 3,3 раза выше нормы). Карболайн оказал эффективное влияние на этот показатель в конце исследования. В сыворотке крови пораженных крыс всех возрастных групп наблюдался сдвиг цитокинового баланса в сторону провоспалительных процессов (повышение содержания интерлейкина-6 и снижение интерлейкина-4). Наиболее эффективное влияние оказал энтеросорбент на цитокиновый баланс у половозрелых крыс.

Ключевые слова: нитритно-табачная интоксикация, мет- и карбоксигемоглобин, цитокины, С-реактивный протеин

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BIOCHEMICAL FEATURES OF POSTOPERATIVE SKIN WOUNDS HEALING AGAINST THE BACKGROUND OF DIABETES MELLITUS IN RATS WITH DIFFERENT WAYS OF WOUND CLOSURE

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The study is devoted to identifying the features of free radical processes changes in skin homogenate of the rats with diabetes mellitus, after using different methods of surgical wounds closing. Analysis of the results suggests that the use of skin glue significantly reduces the intensity of the free radical oxidation course in the cells of scarred skin tissues in animals with simulated diabetes mellitus compared to the application of nodal sutures to the wound under similar conditions.

Key words: free radical oxidation, oxidative stress, abnormal scar.

The work is a fragment of the research project "Systemic and organ disorders due to emergency factors effect on the body, mechanisms of their development and pathogenetic correction", state registration No. 016U003390.

Diabetes mellitus (DM) is considered to be one of the most important non-communicable diseases worldwide and is also one of the global health challenges of the 21st century [3]. It ranks fourth among the major causes of death in developed countries [15].

In 2017, 1,270,929 cases of diabetes were registered in Ukraine. Today, almost 50% of diabetes cases are undiagnosed. Development of late complications leads to a significant reduction in quality of life, reducing its duration by 10-30%, disability, increased mortality of patients by 2-3 times and significant budget expenditures for their treatment. The number of patients with diabetes mellitus is increasing by 5-7% annually. Such indices indicate a non-infectious epidemic of diabetes.

Wound healing is a coordinated process that goes through certain stages with different types of cells and their waste products participating, which regulate the healing process. It is proved that in patients with DM there is the wound healing process disorder, but not all features of this process in diabetes mellitus are completely understood.

At the same time, the quality and structure of the material, the chemical composition of the suture material effect the reaction of tissues to their introduction, as well as the final result of the operation. Free radical oxidation (FRO) is an important biochemical conversion process of lipids, proteins, nucleic acids and other compounds under the action of free radicals, and peroxide oxidation of lipids (LPO) and proteins is one of the further consequences [2, 13]. At all stages of the FRO, due to the interaction of free radicals and biological macromolecules, numerous intermediate products are formed. Such peroxidation compounds, in their excess, are characterized by pronounced cytotoxic activity. As a result, the processes of energy production in the cell are suppressed, the synthesis of proteins and nucleic acids is disrupted, which in its turn leads to the formation of pathological scar tissue.

The purpose of the study to identify the features of free radical processes changes in the skin homogenate of rats in different ways of surgical wounds closing, in the conditions of diabetes mellitus.

Materials and methods. An experiment was performed on 60 adult male rats weighing 240-280 g. The rats were simulated diabetes mellitus by intraperitoneal administration of streptozotocin

("Sigma", USA) at the dose of 65 mg/kg body weight. 15 minutes before administration of streptozotocin the animals were intraperitoneally administered nicotinamide at the dose of 230 mg/kg body weight. All animals were obese, which was caused by keeping the animals on a high-fat diet for 4 weeks. By determining the concentration of glucose in the blood with ContourNext (USA) glucometer, the development of type 2 DM was confirmed. Under the thiopental anesthesia (40 mg/kg body weight), all experimental animals underwent straight incisions 2 cm long in the anterior-lateral region of the abdomen. In experimental group I (30 rats) surgical sutures "Vikril5 / 0" were used to close the postoperative wound. In experimental group II (30 rats) "Dermabond" skin glue (Ethicon, USA) was applied. Animals were sacrificed on the 3rd, 7th, 28th days after surgery by overdose of thiopental anesthesia (90 mg/kg body weight of rats).

The scarred skin pieces homogenate and the homogenate supernatant were under study.

To determine the degree of free radical oxidation processes activation, the content of reactive oxygen species (ROS) [5], lipid hydroperoxides (LHP) [5], diene conjugates (DC) and triene conjugates (TC) [1] and indices of blood plasma proteins oxidative modification were determined (OMP₃₇₀ and OMP₄₃₀) [10].

In order to study the antioxidant defense system, the activity of superoxide dismutase (SOD) in the supernatant was determined by the method of Chevari S. et al. [12]. Catalase activity was determined by the method of Korolyuk M.A. [6], sulfhydryl groups (SH groups) – according to Ellman G.L. [14].

Statistical processing of the obtained data was carried out by standard methods of variation statistics using a package of statistical software. The results are given as (M±m), where M is the mean value of the index, m is the standard error. The significance of discrepancies between the studied indices was determined using the Student's t-test.

Results of the study and their discussion. Our study showed that the activity of free-radical oxidation (FRO) increased during the wound process against the background of diabetes mellitus. However, in the skin homogenates of the animals treated with "Dermabond", all values were significantly lower than in the group of animals treated with interrupted sutures during all study periods. Thus, the number of ROS was greater in animals of group I, compared to those of group II, on the 3rd, 7th and 28th days of the experiment. They also showed a gradual decrease from the beginning of the study to the last time interval, the gradient of decrease being more significant in group II (table 1). The content of diene and triene conjugates in the skin homogenate of group I rats exceeded that of animals in group II on the 3rd day. In group I, the content of DC was (10.77±0.23) relative units (RU)/kg, TC - (9.72±0.41) RU/kg, in II - (7.66±0.37) RU/kg and (7.53±0.37) RU/kg, respectively. On days 7 and 28, a linear decrease in the content of primary LPO products was observed in both experimental groups I and II. Lipid hydroperoxides (LHP) content was also higher in group I rats, compared those in group II, on the 3rd day of the experiment and significantly decreased on the 7th and 28th days in the both groups of animals.

Table 1

Indices of free radical oxidation in the skin homogenate of rats with simulated diabetes mellitus using suture material and skin glue in the dynamics

Index		ROS, %	DC, RU/kg	TC, RU/kg	LHP, RU/h
Intact animals		19.20±0.37	4.05±0.08	4.09±0.18	5.05±0.06
Day 3	Group I	90.76±1.26	10.77±0.23	11.49±0.55	9.72±0.41*
	Group II	74.67±1.06*	7.66±0.37*	9.71±0.19*	7.53±0.37*
Day 7	Group I	88.27±2.08	9.88±0.56	9.29±0.39	7.20±0.38*
	Group II	43.93±1.31*	6.82±0.29*	7.84±0.20*	6.24±0.35*
Day 28	Group I	50.1±3.21	5.65±0.26	8.76±0.26	5.13±0.21
	Group II	29.07±0.95*	5.46±0.16	6.51±0.26*	4.93±0.23*

Notes. Henceforward in the tables: * - the difference is significant between experimental groups I and II within one day

The content of OMP₃₇₀ and OMP₄₃₀ products in the skin homogenate of group I animals was by 2.3 and 1.5 times higher, respectively, similar to skin of those in group II on the 3rd day of the experiment. A slight decrease in these indices occurred in both experimental groups on day 7. On day 28, the content of OMP₃₇₀ products in the skin homogenate of group I animals was by 3.6 times higher than the same index in the skin of group II animals (table 2).

Analysis of all indices permits to state that in the skin of animals treated with glue, the intensity of FRO processes is much lower compared to the imposition of interrupted sutures. Given the strengthening of the LPO processes, which cause the phenomena of primary and secondary alteration when using suture material to close wounds in rats with diabetes mellitus, the beginning of reparative mechanisms in them is delayed.

Indices of proteins oxidative modification in skin homogenate against the background of diabetes mellitus using suture material of skin glue in the dynamics

Group/Index, %		OMP ₃₇₀	OMP ₄₃₀
Intact animals		1.70±0.06	0.33±0.46
Day 3	Group I	7.25±0.20	2.79±0.10
	Group II	3.12±0.10*	1.84±0.09*
Day 7	Group I	6.78±0.17	2.10±0.11
	Group II	2.67±0.11*	1.42±0.09*
Day 28	Group I	5.83±0.26	1.69±0.06
	Group II	1.62±0.04*	1.02±0.06

At the early stages of scar formation (on the 3rd day), we observed an increase in the activity of the antioxidant defense system in the two groups presented. In the groups of animals to which interrupted sutures were applied, the activity of superoxide dismutase (SOD) and catalase was much higher than in the skin of the animals to which the glue was applied, and amounted to (199.81±7.59) RU and (175.02±8.31) kat/kg, respectively. Such data indicated the inevitable start of free radical oxidation due to the traumatic factor against the background of diabetes mellitus. A significant decrease in all parameters was determined in the skin of experimental animals of the both groups on the 7th day of the experiment. However, this difference in the change of SOD activity data was higher in group II animals than in the group treated with the skin glue, where the index decreased by 1.4 times, while in group I the this index decreased by 1.15 times (table 3).

Table 3

Indices of the antioxidant defense system in the skin homogenate under the conditions of diabetes mellitus using suture material and skin glue

Index		SOD, RU	Catalase, kat/kg	SH-groups, mmol/L
Intact animals		71.45±2.28	35.05±2.91	37.50±2.02
Day 3	Group I	199.81±7.59	175.02±8.31	90.44±2.00
	Group II	118.57±3.51*	96.05±2.43*	68.50±1.42*
Day 7	Group I	173.19±7.05	146.72±7.19	71.50±2.77
	Group II	84.72±1.81*	74.28±1.83*	62.90±1.27*
Day 28	Group I	140.51±5.93	111.12±5.13	64.18±2.25
	Group II	83.18±2.29*	73.02±1.40*	62.27±1.33*

Similarly, the reduced glutathione indices were lower in rats of group II, compared to those in group I, on the 3rd and the 7th days from the beginning of the experiment.

Thus, in the animals in the wound process against the background of diabetes mellitus, the indices of FRO activity are much higher, compared to the control rats. The obtained data are confirmed by the results of other authors in the study of the oxidative-antioxidant system status of the liver in alloxandiabetic rats under the conditions of melatonin administration and in the study of the vanadium and chromium citrates effect on the antioxidant system in the blood of experimental rats [4, 9, 11]. The activity of superoxide dismutase and catalase, which are important components of the antioxidant system, in the skin homogenate of rats with diabetes mellitus is significantly reduced, compared to healthy animals. Similar activity of these indices was found in the study of the antioxidant defense system in rats under the action of carrageenan against the background of streptozotocin diabetes, as well as in the study of lipid peroxidation system and antioxidant defense enzymes in rats of different ages in experimental diabetes mellitus [7, 8, 11].

Conclusion

The use of skin glue significantly reduces the FRO intensity in the cells of scar tissue in animals with simulated diabetes mellitus compared to the imposition of interrupted sutures on the wound: in skin homogenates of animals, which were applied skin glue "Dermabond", all indices are significantly lower than in the group of animals which were imposed interrupted sutures during all terms of the study. In particular, in the long term of the study (the 28th day) the content of hydro lipid peroxides in experimental group I was 8.76±0.26 RU/kg, which is by 1.3 times more than in the animal group II - 6.51±0.26 RU/kg.

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Реферат

БІОХІМІЧНІ ОСОБЛИВОСТІ ЗАГОСННЯ ПІСЛЯОПЕРАЦІЙНИХ РАН ШКІРИ НА ФОНІ ЦУКРОВОГО ДІАБЕТУ У ЩУРІВ ПРИ РІЗНИХ СПОСОБАХ ЗАКРИТТЯ РАН

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Дослідження присвячено вивченню особливостей змін вільнорадикальних процесів у гомогенаті шкіри щурів при різних способах закриття операційних ран, за умов цукрового діабету. Аналіз отриманих результатів дає змогу стверджувати, що застосування шкірного клею достовірно знижує інтенсивність перебігу ВРО у клітинах рубцевозмінених тканин шкіри тварин зі змодельованим цукровим діабетом порівняно із накладанням на рану вузлових швів за аналогічних умов.

Ключові слова: вільнорадикальне окиснення, оксидативний стрес, патологічний рубець.

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БИОХИМИЧЕСКИЕ ОСОБЕННОСТИ ЗАЖИВЛЕНИЕ ПОСЛЕОПЕРАЦИОННЫХ РАН КОЖИ НА ФОНЕ САХАРНОГО ДИАБЕТА У КРЫС ПРИ РАЗЛИЧНЫХ СПОСОБАХ ЗАКРЫТИЯ РАН

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Исследование посвящено изучению особенностей изменений свободнорадикальных процессов в гомогенате кожи крыс при различных способах закрытия операционных ран, при сахарном диабете. Анализ полученных результатов позволяет утверждать, что применение кожного клея достоверно снижает интенсивность течения свободно радикальных процессов в клетках рубцово-измененных тканей кожи животных с смоделированным сахарным диабетом по сравнению с наложением на рану узловых швов при аналогичных условиях.

Ключевые слова: свободно радикальное окисление, оксидативный стресс, патологический рубец.

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THE ROLE OF ARGININE/CITRULLINE CYCLE DISORDERS IN THE PATHOGENESIS OF DOXORUBICIN-INDUCED LIVER INJURY ASSOCIATED WITH NONALCOHOLIC STEATOHEPATITIS IN RATS

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The article presents the results of an experimental study aimed to investigate the features of arginine / citrulline cycle disorders associated with doxorubicin-induced liver injury in rats, concomitant with NASH. The study involved adult fertile rats (n=30; male rats=15 (50%); female rats=15 (50%)) weighing 160-220 g. The rats were assigned into 3 groups: Group I (n=10) involved rats with NASH, administered with intraperitoneally 5 mg/kg doxorubicin for 3 days; Group II (n=10) involved rats without NASH, administered with intraperitoneally doxorubicin simultaneously; Group III (n=10) – control group. It has been shown that administration of doxorubicin in rats with NASH leads to disorders of arginine/citrulline cycle, which are characterized by inhibition of arginase activity and activation of citrulline synthesis.

Key words: arginine, citrulline, arginase, doxorubicin, hepatotoxic reactions, nonalcoholic steatohepatitis.

The work is a fragment of the research project “Development of methods for prevention and treatment of the drug-induced internal organs damages”, state registration No. 0115U001087.

Doxorubicin, an anthracycline antibiotic, has been extensively used in oncology and oncohematology for over 30 years. Currently, it is considered one of the most effective antitumor drugs with pharmacological feature to accumulate in malignant cells. However, the use of doxorubicin is accompanied by a high risk of side effects due to its high toxicity [3, 8, 10, 13].