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ANALYSIS OF ANTIOXIDANT SYSTEM OF TEST ANIMALS IN THE DYNAMICS OF THE WOUND PROCESS AT LOCAL APPLICATION OF HYDROGEL DRESSINGS

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Introduction

It is known that during development of inflammation, a significant number of multicomponent reactions occur in the body, the cascade of which is triggered by the action of an altering factor. One of these important physiological processes is the uncontrolled formation of free radicals. As a result of damage to membrane walls and membranes, free radical oxidation is activated, which is controlled by the antioxidant system. The development of the inflammatory process is always accompanied by the activation of lipid peroxidation (LPO) caused by tissue hypoxia. This, in turn, catalyses changes in the physiological parameters of the antioxidant system [2, 5, 7, 8, 11].

Free radical oxidation products can have an anti-inflammatory effect, increase the permeability of biological membranes (including lysosomal membranes), and facilitate the release of inflammatory mediators. Catalase is an enzyme of the oxyreductase class involved in the detoxification of the non-radical reactive oxygen species H_2O_2 . The enzyme is localized mainly in cell peroxisomes, and the large molecular weight of the enzyme prevents its penetration through the cell membrane [4, 6].

Taking into account that the introduction of antioxidants of both natural and artificial origin prevents the depletion of its reserves, the dynamics of the anti-protective balance and its correlation under the influence of topical application of hydrogel dressings saturated with silver ions and an antioxidant preparation have been studied [1, 10, 12, 13].

Purpose of study

Analysis of the dynamics of antioxidant balance of wounds in experiment with the use of hydrogel dressings saturated with silver ions and an antioxidant drug for the local treatment of inflammatory processes.

Materials, design and methods of study

During the performance of this study, generally accepted international norms and rules for working with experimental animals were followed, in accordance with the "General Ethical Principles of Animal Experiments", approved by the III National Congress in Kyiv in 2007, and the Declaration of Helsinki: "Rules for the use of experimental animals in experimental research" 1964–2000.

The experimental study was carried based on the vivarium of the Danylo Halytsky Lviv National Medical University. All animals were kept in accordance with the Sanitary Regulations for the arrangement, equipment and maintenance of experimental biological clinics (vivarium), where they received a standard diet.

The experimental study was conducted on 80 sexually mature male Wistar rats with weight from 150 to 200 grams.

Healing of an experimental infected wound in experimental animals using hydrogel dressings saturated with medicinal substances have been studied. For this purpose, white sexually mature male Wistar rats were depilated on the back in the inter-lobar region one day before surgery. Modelling of the infected wound was performed under sterile conditions under general anaesthesia using diethyl ether. On the depilated area, after aseptic treatment, a skin area with subcutaneous fatty tissue measuring 2×2 cm to the superficial fascia was excised.

After that, a small bacillus saturated with a culture of *Staphylococcus aureus* was introduced into the wound. The wound was left open. On the third day, rats developed a full-fledged purulent wound with all signs of inflammation [14, 15].

Disposition of experimental groups of animals in the study of the features of the course of purulent wound process was as follows:

- 1) intact animal (10 male rats);
- 2) control group - the wound was washed with 3% H_2O_2 solution and a sterile ointment dressing with Levomycil was applied to the animals of this group on the third day after its modelling (10 male rats);
- 3) experimental group 1 - on the third day after wound modelling, the wound was washed with H_2O_2 solution and a hydrogel dressing saturated with silver ions was applied (20 male rats);
- 4) experimental group 2 - on the third day after modelling the wound with H_2O_2 solution, the animals were treated with a hydrogel dressing saturated with the antioxidant drug Quercetin (20 male rats);
- 5) experimental group 3 - animals of this group, on the third day after modelling the infected wound, were treated with H_2O_2 solution and a hydrogel dressing saturated with silver ions and the antioxidant drug Quercetin was applied (20 male rats).

All hydrogels were fixed to the wounds on the animals' backs with a gauze bandage to prevent their displacement and licking by the animals.

The effectiveness of the proposed local therapy was assessed using biochemical methods. In the analysis of laboratory parameters, the indicators of intact animals that did not have an infected wound modelled were considered as normal. Animals were withdrawn from the experiment on days 3, 7, 10, 14. Blood was taken from the cervical vessels for biochemical studies.

Method for determination of catalase activity

Determination of catalase activity is based on the ability of hydrogen peroxide to form a stable colour complex with molybdenum salts. The intensity of the colour was measured on the SF-26 at a wavelength of $\lambda=410$ nm against the control sample, which was supplemented with water instead of hydrogen peroxide. The reaction was started by adding 0.1 ml of blood serum to 2 ml of 0.03% hydrogen peroxide solution. The blank sample was added with 0.1 ml of distilled water. Reaction was stopped after 10 min by adding 1 ml of 4% ammonium molybdate. The colour intensity was measured at a light beam length of 410 nm against a control sample to which 2 ml of distilled water was added.

Activity was determined by formula:

$$A = \frac{(E_x - E_0)}{V \cdot t \cdot K},$$

where

A - catalase activity, mmol H_2O_2 /ml-s; E_x - extinction of the control sample, in which the experimental tissue is replaced with water, units; E_0 - extinction of the experimental sample, units; K - molar extinction coefficient of hydrogen peroxide, which was $22.2 \times 10^3 \text{ mmol}^{-1} \text{ cm}^{-1}$; V - sample volume, ml; t - incubation time, s.

A unit of catalase activity is the amount of enzyme involved in the conversion of 1 mcg of hydrogen peroxide per 1 second under the specified conditions [3].

Study results and their discussion

Maintaining the prooxidant-antioxidant balance is an important mechanism for an adequate response to the inflammatory process. The antioxidant system is a multifactorial regulatory complex of active compounds and components for the control of free radical oxidation. One of the antioxidant enzymes is catalase, which is involved in the detoxification of non-radical active hydrogen peroxide to two stable water molecules and an oxygen molecule. The dynamics of antioxidant activity is shown in Table 1.

Table 1
Catalase activity in the blood plasma of experimental animals, ($M \pm m$, $\mu\text{Cat/l}$)

Study group	Term of study, day			
	3d	7th	10th	14th
Intact animals (n=10)	0,33 \pm 0,05			
Control group (n=10)	0,38 \pm 0,11	0,41 \pm 0,08	0,35 \pm 0,08	0,33 \pm 0,04
Experimental group 1 (n=20)	0,39 \pm 0,04	0,43 \pm 0,06	0,35 \pm 0,03	0,32 \pm 0,06
Experimental group 2 (n=20)	0,54 \pm 0,14	0,52 \pm 0,07*	0,44 \pm 0,05	0,36 \pm 0,04
Experimental group 3 (n=20)	0,55 \pm 0,15	0,51 \pm 0,03*	0,42 \pm 0,02	0,38 \pm 0,06

Note: * there is a significant difference ($p < 0.05$) between the levels of catalase in the blood plasma of rats compared to intact animals;

There was no significant difference ($p > 0.05$) between the levels of catalase in the blood plasma of rats compared to the control group.

Catalase activity on the *third day* of the experiment had insignificant differences between different groups of animals, which depended on the factor that influenced the healing process. In all experimental animals with modelled purulent wounds, an increase in catalase activity was observed in response to an increase in the formation of peroxidation products. In animals of the control group, the content of catalase in the blood plasma was $0.38 \pm 0.11 \mu\text{Cat/l}$, which is 15.2% ($p > 0.05$) higher than in intact animals - $0.33 \pm 0.05 \mu\text{Cat/l}$. If to compare the indicators in the experimental groups with the animals treated with traditional topical treatment, it should be noted that the catalase content in the first experimental group was statistically insignificant

different from the control - 0.39 ± 0.04 and $0.38 \pm 0.11 \mu\text{Cat/l}$, respectively ($p > 0.05$). Such data can be explained by the absence of antioxidant drugs in the treatment regimen of animals of both groups. Significantly higher rates of catalase activity growth were observed with the use of hydrogel dressings saturated with an antioxidant drug alone or this drug together with silver. In experimental group 2 ($0.54 \pm 0.14 \mu\text{Cat/l}$), an increase in CA was noted by 42.1% from the level of the control group and by 44.7% more in experimental group 3 ($0.55 \pm 0.15 \mu\text{Cat/l}$). The data obtained in the experimental groups in which the animals were administered topical administration of the antioxidant drug demonstrates a difference in catalase activity and a slight difference with the data in the control group and the first experimental group.

The data obtained on the *seventh day* of the

study had fundamental differences from the previous study. Subsequently, there was a tendency to a slight increase in catalase activity in the control group ($0.41 \pm 0.08 \mu\text{Cat/l}$), relative to the value on the third day and relative to intact animals ($0.33 \pm 0.05 \mu\text{Cat/l}$). The results obtained were not statistically different from each other, as well as on the third day of the experiment. The content of catalase on day 7 of the experiment in the first experimental group was $0.43 \pm 0.06 \mu\text{Cat/l}$, which was not statistically different from animals treated with Levomikol ointment. In rats treated with hydrogel dressings saturated with an antioxidant preparation, an increase in antioxidant activity was further observed, which was almost the same and statistically significantly different from the intact group ($p < 0.05$). In the second experimental group, the catalase activity was $0.52 \pm 0.07 \mu\text{Cat/l}$, in the third experimental group - $0.51 \pm 0.03 \mu\text{Cat/l}$.

On the 10th day, there was a tendency to decrease catalase activity in all groups of animals. A decrease was observed in the control group of animals ($0.35 \pm 0.08 \mu\text{Cat/l}$), in which an unreliable statistical difference with intact animals by 6.1% was noted. The index of the antioxidant system in animals treated with hydrogel dressings saturated with silver ions did not statistically differ from that of the control group - $0.35 \pm 0.03 \mu\text{Cat/l}$, which indicates the same therapeutic value of these active substances on the antioxidant intensity in wound healing. The results were somewhat higher in the second ($0.44 \pm 0.05 \mu\text{Cat/l}$) and third experimental groups ($0.42 \pm 0.02 \mu\text{Cat/l}$) - by 25.7% and 20% compared to the control group. However, when compared with the values in these groups on day 7 of the experiment, a significant decrease in catalase activity on day 10 should be noted.

At the final stage of the study on the 14th day, the analysis of catalase content showed that in all animals with a modelled inflammatory process, the antioxidant activity did not differ significantly from each other and, importantly, from the intact animals ($0.33 \pm 0.05 \mu\text{Cat/l}$). The indicators of the control ($0.33 \pm 0.04 \mu\text{Cat/l}$) and experimental groups were as follows: the first experimental group - 0.32 ± 0.06 , the second experimental group - 0.36 ± 0.04 , the third experimental group - $0.38 \pm 0.06 \mu\text{Cat/l}$. The antioxidant protection was supplemented by the local release of an antioxidant drug from hydrogel dressings, which contributed to a decrease in the intensity of free radical oxidation processes. That is, in almost all groups of animals with purulent-inflammatory wounds, the catalase activity levels approached the level of intact animals on day 14.

Conclusions

Analyzing the obtained results, it can be concluded that in all experimental animals with simulated purulent wounds, an increase in catalase activity was observed in response to an increase in the formation of peroxidation products. The highest indicators of the antioxidant system were observed in animals that were treated with hydrogel ban-

dages saturated with the antioxidant drug "Quercetin" in the scheme of local treatment of purulent wounds. Leveling of the catalase index in all experimental groups was observed on the 14th day of the experiment.

The results in the second and third experimental groups indicate that the use of hydrogel dressings saturated with an antioxidant drug, which is released into the wound surface for a long time, promotes the activation of antioxidant protection and suppresses the processes of free radical formation for local treatment.

Prospects of research

The results of the study can be used for further clinical study of the effectiveness of the use of hydrogel dressings saturated with silver ions and an antioxidant drug for the local treatment of odontogenic abscesses and phlegmon.

Authors' contribution

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Conflict of interest

The authors declare no conflict of interest.

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**Стаття надійшла
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Summary

Introduction. The development of the inflammatory process is always accompanied by the activation of lipid peroxidation, which is caused by tissue hypoxia. This, in turn, catalyzes a change in the physiological parameters of the antioxidant system.

The aim of the study is to analyze the dynamics of the antioxidant balance of wounds in an experiment using hydrogels saturated with silver ions and an antioxidant drug for the local treatment of inflammatory processes.

Object and research methods. The experimental study was carried out on 80 Wistar rats weighing 150 to 200 grams. Animals were removed from the experiment. for 3, 7, 10, 14 days. Catalase activity in the dynamics of the wound process was determined.

Research results and their discussion. The content of catalase on the 7th day in the third experimental group is 0.51 ± 0.03 $\mu\text{Kat/l}$. On the 10th day, the indicator of the antioxidant system in the second (0.44 ± 0.05 $\mu\text{Kat/l}$) and third experimental groups was 0.42 ± 0.02 $\mu\text{Kat/l}$. At the final stage of the study on the 14th day, the analysis of catalase content showed that in all animals with a simulated inflammatory process, the indicator of antioxidant activity had no significant differences between themselves and, importantly, with the indicators of intact animals (0.33 ± 0.01 $\mu\text{Kat/l}$). The indicators of the control (0.33 ± 0.04 $\mu\text{Kat/l}$) and experimental groups were as follows: the first experimental group – 0.32 ± 0.06 , the second experimental group – 0.36 ± 0.04 , the third experimental group – 0.38 ± 0.06 $\mu\text{Kat/l}$. Antioxidant protection was supplemented by local release of a drug with antioxidant action from the hydrogels, which contributed to a reduction in the intensity of free radical oxidation processes. That is, in almost all groups of animals, which were simulated purulent-inflammatory wounds, indicators of catalase activity approached the level of intact animals already on the 14th day.

Conclusions. Application for local treatment of hydrogel bandages saturated with an antioxidant drug, which is released into the wound surface for a long time, promotes the activation of antioxidant protection and suppresses the processes of free radical formation.

Key words: inflammatory processes, antioxidant system, catalase, hydrogel bandages.

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АНАЛІЗ АНТИОКСИДАНТНОЇ СИСТЕМИ ПІДДОСЛІДНИХ ТВАРИН У ДИНАМІЦІ РАНОВОГО ПРОЦЕСУ ЗА МІСЦЕВОГО ЗАСТОСУВАННЯ ГІДРОГЕЛЕВИХ ПОВ'ЯЗОК

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Резюме

Вступ. Розвиток запального процесу завжди супроводжується активацією перекисного окиснення ліпідів (ПОЛ), що зумовлено тканинною гіпоксією. Це своєю чергою каталізує зміну фізіологічних показників антиоксидантної системи.

Мета дослідження. Аналіз динаміки антиоксидантного балансу ран в експерименті при застосуванні гідрогелевих пов'язок, насичених іонами срібла й антиоксидантним препаратом для місцевого лікування запальних процесів.

Об'єкт і методи дослідження. Експериментальне дослідження було проведено на 80 статевозрілих щурах-самцях лінії Вістар масою від 150 до 200 грам. Виводили тварин з експерименту на 3, 7, 10, 14 добу. Визначали каталазну активність у динаміці ранового процесу.

Результати досліджень та їх обговорення. Уміст каталази на 7-му добу в третій дослідній групі – $0,51 \pm 0,03$ мкКат/л. На 10-ту добу показник антиоксидантної системи в другій ($0,44 \pm 0,05$ мкКат/л) і третій дослідній групах – $0,42 \pm 0,02$ мкКат/л. На завершальному етапі дослідження на 14-ту добу аналіз умісту каталази показав, що в усіх тварин із модельованим запальним процесом показник антиоксидантної активності не мав суттєвих розбіжностей між собою і, що важливо, із показниками інтактних тварин ($0,33 \pm 0,01$ мкКат/л). Показники контрольної ($0,33 \pm 0,04$ мкКат/л) і дослідних групи були такі: перша дослідна група – $0,32 \pm 0,06$, друга дослідна група – $0,36 \pm 0,04$, третя дослідна група – $0,38 \pm 0,06$ мкКат/л. Антиоксидантний захист доповнювався виділенням місцево з гідрогелевих пов'язок препарату антиоксидантної дії, що сприяло зниженню інтенсивності процесів вільнорадикального окиснення. Отже, майже в усіх групах тварин, яким було змодельовано гнійно-запальні рани, показники каталазної активності наближалися до рівня інтактних тварин уже на 14-ту добу.

Висновки. Застосування для місцевого лікування гідрогелевих пов'язок, насичених антиоксидантним препаратом, який пролонговано виділяється в ранову поверхню, сприяє активації антиоксидантного захисту і пригнічує процеси вільнорадикального утворення.

Ключові слова: запальні процеси, антиоксидантна система, каталаза, гідрогелеві пов'язки.