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## Section Pharmacy

### REPAIR PROPERTIES OF THE NEW COMBINED CREAM DERMALIPOIN ON THE MODEL OF EXTENDED SCHEDULED WOUNDS IN RATS

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#### Abstract

The **aim** of the work was to study the reparative properties of the new combined cream on the model of stencil wounds in rats.

**Materials and methods.** Reparative properties of a new combined preparation in the form of a cream for the treatment of inflammatory and microbial skin diseases conditionally named Dermalipoin, which included:  $\alpha$ -lipoic acid, urea, olive oil, tea tree oil, PEG-400, was investigated on the model of extruded stencil wounds in rats. The effectiveness of treatment was assessed using planimetric indicators, which included determining: the area of wounds, the period of epithelialization, the number of animals with wounds that healed at different times and the coefficient of the rate of wound healing in comparison with the "Rescuer Forte" balm. The severity of the inflammatory reaction was drawn from changes in peripheral blood

parameters: blood clotting time, hemoglobin content, the number of erythrocytes and leukocytes, leukocyte formula. To assess the effect of the cream on reparative processes, the activity of enzymes-markers of cytolysis ALAT and ASAT in the blood serum of experimental animals was determined. To assess the intensity of destruction of tissue proteins in the blood serum of experimental animals, the urea content was determined.

**Results.** On the model of stencil wounds in rats, it was found that when applying the new cream Dermalipoin, rapid wound healing occurred: the coefficient of the rate of wound healing was 3,31 versus 2,79 and 1,45 in animals from the groups of the reference drug and control pathology, respectively ( $p \leq 0,05$ ). The study of the dynamics of biochemical parameters showed that the use of Dermalipoin cream was accompanied by a significant (compared to the control pathology) decrease in the activity of ALAT and ASAT, as well as a decrease in the content of urea in the blood serum of experimental animals on the ninth day of the experiment, which indicates a significant decrease in the severity of cytotoxic processes. Changes in hematological parameters indicate a decrease in the severity of the inflammatory process when using Dermalipoin cream and Rescuer Forte balm: at the end of the experiment, there was a significant decrease in the total number of leukocytes and an increase in hemoglobin levels.

**Conclusions.** It was found that on the model of squeezed out stencil wounds in rats, Dermalipoin cream has a pronounced wound-healing effect and, in terms of the severity of the effect, is not inferior to the comparison drug, the Rescuer Forte balm. Therefore, further study of the new cream as a potential wound-healing and anti-burn drug is promising.

**Key words:** wounds;  $\alpha$ -lipoic acid; urea; olive oil; tea tree oil; repair.

## РЕПАРАТИВНІ ВЛАСТИВОСТІ НОВОГО КОМБІНОВАНОГО КРЕМУ ДЕРМАЛІПОІН НА МОДЕЛІ ВИДАВЛЕНИХ ТРАФАРЕТНИХ РАН У ЩУРІВ

Н. М. Кононенко, А. М. Шейхалі

**Метою роботи** було вивчення репаративних властивостей нового комбінованого крему на моделі трафаретних ран у щурів.

**Матеріали та методи.** На моделі видавлених трафаретних ран у щурів досліджено репаративні властивості нового комбінованого препарату у формі крему для лікування запальних і мікробних захворювань шкіри умовно названий

«Дермаліпоін», до складу якого увійшли:  $\alpha$ -ліпоева кислота, сечовина, олівкова олія, олія чайного дерева, ПЕГ-400. Ефективність лікування оцінювали за допомогою планіметричних показників, що передбачали визначення: площі ран, періоду епітелізації, кількості тварин з ранами, що зарубцювалися в різні терміни та коефіцієнту швидкості загоєння ран у порівнянні з бальзамом «Спасатель форте». Про виразність запальної реакції робили висновок за змінами показників периферійної крові: часу згортання крові, вмісту гемоглобіну, кількості еритроцитів та лейкоцитів, лейкоцитарної формули. Для оцінки впливу крему на репаративні процеси проводили визначення активності ферментів-маркерів цитолізу – АлАТ і АсАТ у сироватці крові піддослідних тварин. Для оцінки інтенсивності деструкції тканинних білків у сироватці крові піддослідних тварин визначали вміст сечовини.

**Результати.** На моделі трафаретних ран у щурів встановлено, що при застосуванні нового крему Дермаліпоін відбувалося швидке загоєння ран: коефіцієнт швидкості загоєння ран становив 3,31, проти 2,79 та 1,45 у тварин з груп препарату порівняння та контрольної патології відповідно ( $p \leq 0,05$ ). Дослідження динаміки біохімічних показників показало, що застосування крему Дермаліпоін супроводжувалося достовірним (порівняно з контрольною патологією) зменшенням активності АлАТ та АсАТ, а також зниженням вмісту сечовини у сироватці крові дослідних тварин на дев'яту добу експерименту, що свідчить про суттєве зменшення виразності цитодеструктивних процесів. Зміни гематологічних показників свідчать про зменшення виразності запального процесу при застосуванні крему Дермаліпоін та бальзаму Спасатель форте: наприкінці експерименту спостерігалось достовірне зменшення загальної кількості лейкоцитів та підвищення рівня гемоглобіну.

**Висновки.** встановлено, що на моделі видавлених трафаретних ран у щурів крем «Дермаліпоін» виявляє виразну ранозагоювальну дію та не поступається за виразністю ефекту препарату порівняння бальзаму Спасатель форте. Тому перспективним є подальше вивчення нового крему як потенційного ранозагоювального та протиопікового лікарського засобу.

**Ключові слова:** рани;  $\alpha$ -ліпоева кислота; сечовина; олівкова олія; олія чайного дерева; репарація.

## Introduction

According to the WHO, in the structure of the mortality rate of the population is the third accounted for by injuries, burns, poisoning, etc. More than 100 thousand cases of wound and burn injuries are registered in Ukraine every year.

Modern requirements for local wound-healing and anti-burn drugs include: the possibility of using the drug in various phases of the wound process, the absence of toxic, allergenic and local irritating effects, a wide range of antibacterial action, high antimicrobial and anti-inflammatory activity. In addition, topical wound healing preparations should not cause disturbances in gas exchange in the skin [1].

When using wound-healing drugs in surgical practice, a number of problems can be distinguished: an increase in the resistance of causative agents of wound infection to existing drugs, especially staphylococci, which dominate among the association of microorganisms; insufficient effectiveness of many drugs associated with a monocomponent composition; the absence of drugs that have moderate osmotic activity and can be used in both phases I and II of the wound process [2].

Despite a significant number of drugs for the treatment of wound healing on the Ukrainian pharmaceutical market, today the problem of pharmacological correction of wounds and their complications remains relevant and unresolved. The main source of skin and soft tissue infection is microorganisms that contaminate and colonize the skin surface. *S. aureus* and *S. pyogenes*, capable of penetrating the epidermis in the presence of damage, undoubtedly play a leading role in the etiology of purulent skin infections. Moreover, *S. aureus* is the most common pathogen; infections caused by *S. pyogenes*, as well as a mixed infection with the participation of both microorganisms, are not so common.

$\alpha$ -Lipoic acid is a powerful antioxidant and effective anti-inflammatory agent; when applied topically, it has keratolytic, keratoplastic, bacteriostatic and fungistatic effects [3]. Olive oil has analgesic and anti-inflammatory effects, promotes healing of wounds, ulcers and burns. Tea tree oil is an adaptogen. It has a strong bactericidal, anti-inflammatory, anti-edematous and reparative effect. It has been successfully used in the fight against such pathogenic microorganisms as: *Staphylococcus* (*aureus*, *epidermidis*, *pneumoniae*, *faecalis pyogenes*), *Propionibacterium acnes*, *Betahaemolytic streptococcus*, *Citrobacter* spp., *Shigella sonnei*, *Proteus mirabilis*, *Legionella* spp., *Pseudomonas aeruginosa*, *Candida albicans* etc. PEG-400 is a solvent with a pronounced osmotic activity, which determines its widespread use in the production of creams for the treatment of infected wounds, where it provides an osmotic and dehydration effect, which in turn accelerates the wound healing period.

That is why the National University of Pharmacy has developed a new combined preparation in the form of a cream for the treatment of inflammatory and microbial skin diseases, conventionally named Dermalipoin, which includes:  $\alpha$ -lipoic acid, urea, olive oil, tea tree oil, PEG-400.

**Aim.** To study the reparative properties of the combined cream on the model of stencil wounds in rats.

### **Materials and methods**

The experiments were carried out on 32 nonlinear sexually mature white male rats weighing 200-220 g. Animals, under barbamil anesthesia, on a previously depilated and treated with 5% alcohol solution of iodine on the skin on the back bilaterally from the spine, inflicted a wound using a metal scarifier-punch with a diameter of 0,8 cm [4].

The animals were divided into five groups: intact control; control pathology; animals treated with the cream and animals that were treated with the reference drug - the "Rescuer-Forte" balm.

The test cream and the reference drug were applied to the wound surface one day after wounding at a dose of 0,1 ml/cm<sup>2</sup>.

The conclusion about the effect of the new cream and the reference drug on the course of the wound process was made, first of all, according to the rate of decrease in the area of wounds. The area of the wounds was measured every four days from the moment of reproduction of the pathology using a transparent stencil.

The coefficient of the rate of wound healing was calculated by the formula:  $V = (S_{\max} - S_{\text{exp}}) / S_{\text{exp}}$ , where  $S_{\max}$  is the maximum area of the wound, mm<sup>2</sup>;  $S_{\text{exp}}$  - wound area on the day of measurement, mm<sup>2</sup>;

In addition, the number of animals (%) with healed wounds was also determined.

The conclusion about the severity of the inflammatory reaction was made on the basis of changes in peripheral blood parameters: blood coagulation time, hemoglobin content, the number of erythrocytes and leukocytes, leukocyte formula. Peripheral blood parameters were determined at the beginning of the experiment (initial data), on the 5th and 13th days. To assess the effect of the cream on reparative processes, the activity of enzymes markers of cytolysis ALT and AST in the blood serum of animals was determined. Violation of the integrity of cell membranes and the destruction of cellular structures leads to the release of enzymes into the blood that have intracellular localization, as a result of which their activity in blood serum increases significantly.

To determine the activity of transaminases, a unified dinitrophenylhydrazine method was used. This method was proposed to determine the activity of ALT and AST in kits from Lachema (Czech Republic).

To assess the intensity of destruction of tissue proteins in the blood serum of animals, the content of urea was determined.

The content of urea was determined by the color reaction with diacetyl monooxime [5]. The calculation of the concentration of urea was made according to the formula:  $C = (E_d/E_{st}) \times 16.65$ , where  $C$  is the content of urea in the blood serum (mmol/l);  $E_d$  is the extinction of the test solution;  $E_{st}$  is the extinction of a standard solution; 16,65 - concentration of urea in a standard sample (mmol/l).

Biochemical parameters were recorded three times: on the 5th, 9th and 13th days of the experiment.

All interventions and euthanasia of animals were carried out in accordance with the "General moral principles of experiments on animals" adopted by the First National Congress on Bioethics (Kiev, 2001) and the Law of Ukraine "On the Protection of Animals from Cruelty" № 3477-IV (02.21.2006). Statistical processing of the obtained results was carried out on a personal computer Pentium III using the Statistica 6 program, using the Student's t-test for comparing independent samples at a probability level of  $p \leq 0.05$  [5].

## **Results**

The results of the experimental studies are presented in table 1-3. On the second day of the experiment, all experimental animals developed dry wounds. The inflammatory response was not clear: there was slight swelling and redness of the wound edges. The experimental data (table 2) show an increase in the activity of ALAT and ASAT (markers of cytolysis), as well as an increase in the content of urea in the blood serum on the fifth day of the experiment in animals of all experimental groups.

The nature of changes in hematological characteristics that occurred in animals of the control pathology group reflects the development of the inflammatory process. When determining the hematological parameters, a significant increase in the total number of leukocytes was observed compared to intact control, as well as a decrease in the level of hemoglobin, the number of erythrocytes and a decrease in the time of blood coagulation on the 5th and 13th days. Changes in the leukocyte formula were also recorded, which were manifested by neutropenia on the 5th day and an increase in the number of monocytes on the 5th and 13th days of the experiment (table 2).

Table 1

**Study of the reparative effect of Dermalipoin cream on a model of stencil wounds in rats  
(n = 8)**

Experimental conditions		Research terms, days				
		2	5	9	13	17
Control pathology	S, cm <sup>2</sup>	1,03 ± 0,02	0,91 ± 0,01	0,42 ± 0,02	0,17 ± 0,04	0,1 ± 0,01
	V, conv. units		0,13	1,45	5,05	9,3
	%	–	–	–	16,7	33,3
Dermalipoin cream	S, cm <sup>2</sup>	1,12 ± 0,01	0,80 ± 0,05	0,26 ± 0,03*	0,07 ± 0,01*	–
	V, conv. units		0,4	3,31	15,0	–
	%	–	–	–	66,6	100
Balsam "Rescuer forte"	S, cm <sup>2</sup>	1,10 ± 0,05	0,84 ± 0,06	0,29 ± 0,03*	0,08 ± 0,01*	–
	V, conv. units		0,31	2,79	12,75	–
	%				66,6	100

**Notes:**

S - is the average area of the wound;

V - coefficient of the rate of wound healing, conv. units;

% - the number of animals with healed wounds;

\* - the discrepancy is significant in relation to the control pathology (p < 0,05).

Table 2

**Influence of Dermalipoin cream on the dynamics of some biochemical blood parameters  
in rats (model of squeezed stencil wounds, n = 8)**

Experimental conditions	Indicators		
	ALAT, mmol/g•l	ASAT, mmol/g•l	Urea, mmol/l
Intact control	0,61 ± 0,06	0,68 ± 0,06	5,80 ± 0,56
5th day of research			
Control pathology	1,04 ± 0,10*	1,08 ± 0,15*	9,67 ± 1,02*
Dermalipoin cream	0,92 ± 0,07*	0,93 ± 0,13*	8,21 ± 0,35*/**
Balsam "Rescuer forte"	0,93 ± 0,11*	0,95 ± 0,11*	8,10 ± 0,51*/**
9th day of research			
Control pathology	0,88 ± 0,09*	0,98 ± 0,09*	9,28 ± 0,86*
Dermalipoin cream	0,73 ± 0,10*/**	0,80 ± 0,05*/**	7,48 ± 0,54*/**
Balsam "Rescuer forte"	0,75 ± 0,09*/**	0,82 ± 0,05*/**	7,43 ± 0,65*/**
13 research day			
Control pathology	0,83 ± 0,11*	0,77 ± 0,08	7,82 ± 0,56*
Dermalipoin cream	0,67 ± 0,08	0,75 ± 0,06	6,33 ± 0,69**
Balsam "Rescuer forte"	0,65 ± 0,09	0,75 ± 0,10	6,62 ± 0,57**

**Notes:**

\* - the discrepancy is significant relative to the intact control (p < 0,05);

\*\* - the discrepancy is significant in relation to the control pathology (p < 0,05).

**Effect of Dermalipoin cream on the dynamics of some hematological parameters in rats  
(models of squeezed stencil wounds, n = 8)**

Indicators	Study timing	Control pathology	Dermalipoin cream	Balsam "Rescuer forte"
Clotting time, sec	initial data	200,33 ± 7,45	194,3 ± 36,68	196,17 ± 5,15
	5 days	170,17 ± 12,09*	202,67 ± 5,50	200,83 ± 8,28
	13 days	125,83 ± 11,44*	164,33 ± 11,43*/**	161,83 ± 7,76*/**
Hemoglobin, g/l	initial data	130,4 ± 8,71	127,4 ± 7,30	132,0 ± 5,43
	5 days	106,6 ± 3,44	108,4 ± 5,27	108,8 ± 4,32
	13 days	109,4 ± 2,19	118,2 ± 5,81**	118,8 ± 8,35**
Erythrocytes, x10 <sup>12</sup> /l	initial data	4,23 ± 0,17	4,21 ± 0,23	4,35 ± 0,34
	5 days	3,73 ± 0,36	3,72 ± 0,37	3,59 ± 0,33
	13 days	3,95 ± 0,25	4,01 ± 0,41	4,04 ± 0,22
Leukocytes, x10 <sup>9</sup> /l	initial data	7,48 ± 0,51	7,70 ± 0,93	7,38 ± 0,88
	5 days	16,73 ± 0,94	17,04 ± 1,19	16,40 ± 1,06
	13 days	13,40 ± 1,58	10,24 ± 1,16**	9,47 ± 1,15**
Some indicators of the leukocyte formula, %				
Lymphocytes	initial data	61,17 ± 2,48	60,17 ± 3,06	59,83 ± 2,04
	5 days	64,00 ± 3,16	63,50 ± 2,59	61,83 ± 3,13
	13 days	62,83 ± 2,79	61,83 ± 3,49	61,00 ± 2,00
Monocytes	initial data	5,00 ± 0,63	5,33 ± 0,51	5,67 ± 0,82
	5 days	8,83 ± 0,75*	9,33 ± 0,52*	9,50 ± 0,55*
	13 days	6,67 ± 1,50*	8,33 ± 0,82*	8,17 ± 1,17*
Stab neutrophils	initial data	1,83 ± 0,41	2,00 ± 0,63	1,83 ± 0,41
	5 days	0,83 ± 0,17*	1,00 ± 0,10*	1,17 ± 0,17
	13 days	2,33 ± 0,21	2,50 ± 0,22	2,17 ± 0,17
Segmented neutrophils	initial data	31,3 ± 33,33	33,00 ± 2,00	30,33 ± 2,73
	5 days	23,00 ± 2,53*	22,83 ± 2,48*	24,67 ± 2,42*
	13 days	30,00 ± 3,16	29,83 ± 3,19	28,50 ± 2,17

**Notes:**

\* - the discrepancy is reliable relative to the initial data (p <0,05);

\*\* - the discrepancy is significant in relation to the control pathology (p <0,05).

The healing of wounds in the animals of the control pathology group was slower than in the animals of the experimental groups treated with the test cream and the reference drug.

On the ninth day of the experiment, the coefficient of the rate of wound healing in the experimental groups increases significantly. The average area of wounds in animals treated with the investigated cream and balsam "Rescuer Forte" was statistically significantly less than the values of this indicator in animals of the control pathology group; the coefficient of the rate of wound healing was 3,31 and 2,79, respectively, versus 1,45 in animals from the control pathology group (2,28 and 1,92 times higher, respectively).

On the 13th day, the coefficient of the rate of wound healing in animals treated with



the investigated cream and “Rescuer Forte” balm were, respectively, 2,97 and 2,52 times higher than in animals of the control pathology group.

Complete healing of wounds in animals treated with Dermalipoin cream and Rescuer Forte balm was observed between the 13th and 17th days, while in animals of the control pathology group, complete wound healing occurred between the 17th and 19th days.

The study of the dynamics of biochemical parameters showed that the use of the cream "Dermalipoin" and the balsam "Rescuer Forte" was accompanied by a significant (compared with the control pathology) decrease in the activity of ALAT and ASAT, as well as a decrease in the content of urea in the blood serum of experimental animals on the ninth day of the experiment, which indicates about a significant decrease in the severity of cytotoxic processes. On the 13th day of the experiment, there was a complete normalization of the activity of marker enzymes and the level of urea in the blood serum of experimental animals.

Changes in hematological parameters indicate a decrease in the severity of the inflammatory process when using Dermalipoin cream and Rescuer Forte balm: at the end of the experiment, there was a significant decrease in the total number of leukocytes and an increase in hemoglobin levels, but no significant changes in leukocyte counts were recorded.

Thus, it was found that on the model of squeezed out stencil wounds in rats, Dermalipoin cream has a distinct wound-healing effect and is not inferior in the severity of the effect to the comparison drug, the Rescuer Forte balm.

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