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Evaluation of wound healing efficiency using collagen matrices on a thermal burn model

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Abstract Every year dozens of new local wound treatment agents are developed and improved in the world, which undergo preliminary testing on laboratory animals due to the presence of common phases of the wound healing process with humans. Wound healing effect was studied on a thermal burn model on Wistar rats in 4 groups: 1st - control (natural wound healing); 2nd - intact animals (normal, without burn). 3rd - experimental (collagen treatment); 4th - experimental 2 , comparison group (" Levomekol "); Wound healing was assessed based on the results of planimetry on the 1st, 3rd, 5th, 7th, 9th and 13th days and histological analysis of skin tissue on the 6th, 13th and 20th days.

Results. Thermal skin damage resulted in a IIIA degree burn, accompanied by the development of dry coagulation necrosis. The use of collagen matrices restored the total leukocyte count and reduced the area of the burn wound. Histomorphometric studies confirmed the dynamics of skin tissue recovery after the burn. The effectiveness of the matrices was comparable to the pharmacopoeial drug " Levomekol ". A higher wound-healing effect was noted with the use of collagen.

Conclusion. The wound-healing effect of collagen matrices was established on the thermal burn model, as evidenced by the results of restoration of the number of leukocytes, reduction of the burn wound area and restoration of the skin histostructure .

Key words: collagen matrices, thermal burn, skin, wound healing effect, morphology,

Introduction Burns are one of the most common skin lesions, and annually in the Russian Federation 420-450 thousand victims seek medical care. Moreover, 70% of them can be treated on an outpatient basis, since they have minor superficial burns [1-3]. Methods, means and tactics of treating patients with burns are constantly being improved, and their choice remains a pressing problem of modern combustiology [4]. Research in recent years is aimed at obtaining collagen-based drugs and assessing the possibility of their use in the production of drugs and cosmetics [5-7]. Such drugs serve as a bioplastic material and a matrix for the formation of native connective tissue [8-10]. The main advantages of collagen products are biodegradability, biocompatibility , weak antigenicity , the ability to form complexes with drugs and stimulate regeneration [11-13]. All developed products undergo preliminary testing on laboratory animals due to the presence of common phases of the wound process with humans. Modeling skin wounds in laboratory animals allows not only to study the course of the pathological process, but also to reasonably assume the mechanism of action with a positive effect of the studied agent [14].

Materials and methods of the study The collagen matrix was white, had a gel-like consistency with a sour-milk odor; pH = 4.42; the average molecular weight of the molecules was 333 kDa . Since the obtained collagen matrices had thick gel-like consistencies, they were easily used in the experiment as ointments.

Wistar rats of both sexes, weighing 180–200 g. The animals were divided into 4 groups of 8 animals each: 1st — control (animals with a burn and “natural wound healing”); 2nd — experimental 1 (animals with a burn were treated with collagen); 3rd — experimental 2, a comparison group in which Levomekol ointment (Nizhpharm , Russia) was used to treat the animals’ wounds after a burn; 4th — a group of intact animals (normal, without a burn). The studied agents were applied to the wound area daily, once a day, 0.5 g at a time, the day after the burn and until complete healing. Skin defects remained open throughout the entire observation period.

The thermal burn model was reproduced on experimental animals using the method of B.A. Paramonov et al . [15] under ether anesthesia. The day before the burn modeling, the visible cutaneous part of the hair on the animal’s lateral surface was trimmed with scissors (4 × 4 cm). To reproduce the burn model, a glass test tube with a diameter of 22 mm and a length of 20–25 cm was used, filled 2/3 full with hot water (100 °C), with subsequent contact with the skin area for 10 s. After the burn, the rats were placed in individual cages for observation.

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Animal experiments were carried out in the vivarium of the Tashkent Medical Academy's Scientific Research Laboratory. All manipulations with animals were carried out in accordance with international moral and ethical standards, according to the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986) and in accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals. The maintenance and care of animals was carried out in accordance with GOST.

All animals were on the same (standard) diet with free access to food and water. The light regime in the vivarium was provided by changing the lighting "day/night" every 12 hours. The air temperature was 20-25 °C, the relative humidity was 60-70%.

Wound healing was assessed based on the results of planimetry, using a transparent template that was placed on the wound, wound contours were transferred and their area was calculated. The average wound area was assessed dynamically - on the 1st, 3rd, 5th, 7th, 9th and 13th days of the experiment. The appearance of the wounds, the presence and nature of the scab were also assessed, and the time of complete healing was noted. During the experiment, the scab was not mechanically removed until it was rejected by itself.

On the 6th, 13th and 20th day after the burn, the rats were gradually removed from the experiment using chloroform vapors. Blood was taken from the femoral artery, and after euthanasia, skin tissue samples were taken, fixed in a 10% formalin solution and, using generally accepted methods of histological analysis, the morphological state of the skin tissue during healing was assessed.

Blood was collected from animals in a test tube with heparin (50 U /ml) and the following parameters were determined: total antioxidant activity (in blood serum), total leukocyte and erythrocyte content, catalase enzyme activity (in blood) and malonic acid content dialdehyde (in blood serum).

The total content of leukocytes in the blood was determined by counting in a Goryaev chamber with a sample diluted with 3% acetic acid, stained with methylene blue. The total content of erythrocytes in the blood was also determined by counting with a sample diluted with a physiological solution [19].

The obtained results of the study are presented as median (Me), upper and lower quartiles (Q1–Q3).

The statistical significance of differences was assessed using the nonparametric Mann–Whitney test. Results were considered statistically significant when the significance level of differences reached $p \leq 0.05$.

Research results On the first day after the thermal burn, an intense local inflammatory reaction was observed in animals of the control and experimental groups. Round burns with a bright red wound bottom were formed. Pronounced hyperemia (hyperemia zone up to 0.7 cm wide) and swelling of skin tissues at the border with the wound surface were noted.

The studied preparations collagen and " Levomekol " were applied the next day after the burn to a soft burn crust with small, rare blisters, without suppuration. The skin surface retained its sensitivity.

By the 3rd day of the experiment, a burn scab had formed on the surface of the burn wound in the animals, but in the rats of the control group (without treatment) it was dense; its tighter adhesion to the wound surface was noted. On the 6th–7th day of the experiment, fragmentary rejection of the burn scab was observed in the rats of the experimental groups (with treatment). Visual observation revealed intensive formation of young connective tissue at the bottom of the burn wound. On the 13th–15th day from the moment of infliction of the burn injury, epithelialization of the wounds was observed in the rats of the experimental groups (with treatment), which was almost completely completed by the 21st day. In the rats of the control group, reparative processes were slower, with final completion by the 23rd–25th day. It should be noted that in experimental group 1 (animals were treated with collagen after a burn), the onset of scab rejection, the formation of young connective tissue, more pronounced epithelialization of the wound surface, as well as a decrease in the severity of inflammation and edema were observed at an earlier stage compared to other experimental groups .

It is known that burn injury is characterized by leukocytosis (an increase in the number of leukocytes) [19]. The effect of collagen matrices on this indicator is presented in Table 1.

As follows from the data (Table 1), in the control group of animals on the 6th and 13th days of observation, an increase in the level of leukocytes, which amounted to 20.6% and 61.0%, respectively, in relation to intact rats (the norm). Treatment of animal wounds after a burn with the studied agents contributed to the

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normalization of the studied indicator, and this process was most pronounced in experimental group 1 (with treatment with a collagen matrix).

Table 1

Leukocyte content in the blood of rats after thermal burn and treatment with the studied agents, Me (Q1–Q3)

Group	Means	Number of leukocytes, × 109/l	
		Day 6 of the experiment	Day 13 of the experiment
Control	Burn	6.88 (6.17–7.59)*n	9.13 (8.82–9.75)*n
Experienced 1	Burn + collagen	6.36 (6.15–6.58)*2, 3	5.20 (4.93–5.45)*k, 2, 3
Experienced 2	Burn + Levomekol	5.58 (5.33–5.83)*k, 1, 2	6.84 (6.67–7.01)*n, k, 1
Intact animals)	Norm	5.46 (4.76–6.16)	5.67 (4.97–6.37

Note. * – deviation is statistically significant in relation to respectively: n – norm, k – control, 1, 2, 3 – 1st, 2nd and 3rd groups ($p \leq 0.05$)

The suppression of the development of general leukocytosis when using the studied preparations contributed to higher dynamics of wound healing . The effect of collagen matrices on the area of wounds is presented in Table 2.

As follows from the data in Table 2, starting from the 3rd day of the experiment, the areas of burn wounds in animals of the 1st and 2nd groups statistically significantly differed from the results of the control group. The data obtained during the study indicate the wound-healing effect of collagen matrices, which is comparable to the effect of the pharmacopoeial drug "Levomekol". The most pronounced reparative effect was demonstrated by the collagen matrix (experimental 1). It should be noted that the final healing of wounds after a burn in animals treated with collagen and "Levomekol" occurred by the 19th-21st day, and in rats in the control group - by the 23rd-25th day .

Table 2

Dynamics of changes in the area of wounds after thermal burns and treatment with the studied agents, Me (Q1–Q3)

Group	Means	Number of leukocytes, × 109/l					
		Day 1	Day 3	Day 5	Day 7	Day 9	Day 13
Control	Burn	3.49 (3.32–3.63)	3.76 (3.60–3.92)	3.18 (2.97–3.39)	2.54 (2.44–2.64)	1.65 (1.47–1.83)	1.35 (1.16–1.54)
Experienced 1	Burn + collagen	3.49 (3.32–3.63)	3.06 (2.92–3.2)*k	2.94 (2.73–3.15)	1.91 (1.74–2.08)*k	1.4 (1.26–1.54)	0.96 (0.78–1.14)*k
Experienced 2	Burn + Levomekol	3.49 (3.32–3.63)	2.97 (2.73–3.21)*k	2.69 (2.55–2.83)*k	1.75 (1.64–1.86)*k	1.29 (1.16–1.42)*k	1.00 (0.88–1.12)*k

The analysis of histological and morphometric indices in thermal burns and subsequent treatment with the studied agents confirmed the dynamics of skin tissue restoration. Wound healing in the experimental groups of animals was characterized by an easier course of the process, stopping the spread of necrosis into the deep layers of the skin. For experimental group 1, in which a collagen matrix was used to treat thermal burns of experimental animals, a more uniform maturation of granulation tissue, the absence of purulent-necrotic

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complications in the healing process, the formation of a regenerate approaching in its structure to the normal skin of rats (intact animals) were characteristic.

Thus, from the above data it follows that collagen matrices had a pronounced reparative effect on the thermal burn model, comparable to the effect of the pharmacopoeial drug " Levomekol ".

Conclusion The study of the wound-healing effect of collagen matrices was conducted. The use of the studied matrices on the thermal burn model restored the total leukocyte content in the blood of experimental animals and reduced the area of the burn wound. The anti-inflammatory and reparative effect was also confirmed by histomorphological studies. When exposed to collagen matrices, more intense healing was observed than in the control group (with natural healing, without treatment), which was manifested by a smaller thickness of the leukocyte-necrotic scab, as well as accelerated epithelialization and complete closure of the skin defect. The results of studies of the histostructure of the epidermal and dermal layers of the skin showed restoration and the absence of pathological processes on the 20th day of the experiment. The effectiveness of the matrices was comparable to the effect of the pharmacopoeial drug "Levomekol". At the same time, a more pronounced wound-healing effect was noted when using the collagen matrix.

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