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Evaluation Of The Effectiveness Of Wound Healing Using Collagen Matrices In A Thermal Burn Model

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Abstract: Every year, dozens of new topical products for wound treatment are developed and improved worldwide. Due to the similarity of the general stages of the wound process in humans and laboratory animals, these products undergo initial testing in experimental animal models. The wound-healing effect was studied in a thermal burn model in Wistar rats divided into four groups: 1 – control group (natural wound healing); 2 – intact animals (normal, without burns); 3 – experimental group (collagen treatment); 4 – experimental group 2, comparison group ("Levomekol"). Wound healing was assessed by planimetry on days 1, 3, 5, 7, 9, and 13, as well as by histological analysis of skin tissues on days 6, 13, and 20. Results. Thermal skin injury resulted in a grade IIIA burn accompanied by the development of dry coagulation necrosis. The use of collagen matrices led to restoration of the total leukocyte count and a reduction in the burn wound area. Histomorphometric studies confirmed the dynamics of skin tissue regeneration after thermal injury. The effectiveness of collagen matrices was compared with the pharmacopoeial drug Levomekol. A higher wound-healing effect was observed with the use of collagen. Conclusion. Using a thermal burn model, a pronounced wound-healing effect of collagen matrices was demonstrated, as evidenced by restoration of leukocyte levels, reduction of the burn wound area, and recovery of the skin histostructure.

Keywords: Collagen matrices, thermal burn, skin, wound-healing effect, morphology.

Introduction: Burns are among the most common types of skin injuries. Every year in the Russian Federation, 420,000–450,000 victims seek medical care for burns. Moreover, about 70% of them can be treated on an outpatient basis, as they sustain superficial burns of limited area [1–3]. Methods, means, and tactics for the treatment of burn patients are continuously being improved, and their optimal selection remains a pressing issue in modern combustiology [4].

In recent years, research has focused on obtaining collagen-based preparations and evaluating the possibilities of their use in the production of medicinal products and cosmetics [5–7]. Such preparations serve as bioplastic materials and as matrices for the formation of the body's own connective tissue [8–10]. The main advantages of collagen products include biodegradability, biocompatibility, low antigenicity, the ability to form complexes with drugs, and the capacity to stimulate regeneration [11–13].

All newly developed products undergo preliminary testing in laboratory animals due to the similarity of the general stages of the wound process in humans and animals. Modeling skin wounds in laboratory animals makes it possible not only to study the course of the pathological process, but also to substantiate the mechanism of action of the studied preparation based on its positive effects [14].

METHODS

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

Experimental studies were conducted on 32 white Wistar rats of both sexes, weighing 180–200 g. The animals were divided into four groups of eight each:

Control group – animals after burn injury with "natural wound healing";

Experimental group 1 – animals after burn injury

treated with collagen;

Experimental group 2 (comparison group) – animals after burn injury treated with Levomekol ointment (Nizhpharm, Russia);

Intact group – intact animals (normal, without burns).

The studied agents were applied daily, once a day, in a dose of 0.5 g to the wound surface starting from the day after the burn and until complete healing. During the observation period, skin defects were left open.

The thermal burn model in experimental animals was created according to the method of B.A. Paramonova et al. [15] under ether anesthesia. One day before burn modeling, hair was clipped from a visible skin area (4 × 4 cm) on the lateral surface of the animal. 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 to two-thirds of its height with hot water (100 °C), which was then applied to the skin surface for 10 seconds. After the burn, the rats were placed in individual cages for observation.

Experiments on animals were carried out in the vivarium of the Moscow Research Laboratory of the Tashkent Medical Academy. All manipulations with animals were performed in accordance with international ethical standards, the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes. Housing and care of animals were carried out in accordance with GOST standards.

All animals received a uniform (standard) diet with free access to food and water. The light regime in the vivarium was maintained by alternating light and dark periods every 12 hours (“day/night”). Air temperature was maintained at 20–25 °C, and relative humidity at 60–70%.

Wound healing was assessed based on planimetry results. Wound contours were traced using a transparent stencil applied to the wound, and the wound area was calculated. The average wound area was evaluated over time on days 1, 3, 5, 7, 9, and 13 of the experiment. In addition, the appearance of wounds, the presence and nature of the scab, and the time to complete healing were recorded. During the experiment, the scab was not mechanically removed until it was spontaneously rejected.

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

Blood samples from animals were collected into tubes containing heparin (50 U/mL), and the following parameters were determined: total antioxidant activity (in blood serum), total leukocyte and erythrocyte counts, catalase enzyme activity (in blood), and malondialdehyde content (in blood serum).

The total leukocyte count in blood was determined in a Goryaev chamber after diluting the sample with 3% acetic acid stained with methylene blue. The total erythrocyte count in blood was also determined by counting after diluting the sample with physiological saline [19].

The obtained study results are presented as median (Me) and upper and lower quartiles (Q1–Q3). Statistical significance of differences was assessed using the nonparametric Mann–Whitney test. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

On day 1 after induction of a thermal burn, a pronounced local inflammatory reaction was observed in animals of both the control and experimental groups. Burns of a round shape with a bright red wound bed were formed. Marked hyperemia (with a hyperemic zone up to 0.7 cm wide) and swelling of the skin tissues at the border with the wound surface were recorded.

The study preparations, collagen and Levomekol, were applied on the day following the burn to a softened burn scab with small, sparse blisters, without signs of suppuration. Skin surface sensitivity was preserved.

By day 3 of the experiment, a burn scab had formed on the wound surface in the animals; however, in the control group rats (without treatment) it was dense, and tighter adherence to the wound surface was noted. On days 6–7 of the experiment, partial rejection of the burn scab was observed in rats of the experimental groups (with treatment). Visual observation revealed intensive formation of young connective tissue at the bottom of the burn wound. On days 13–15 after burn injury, epithelialization of wounds was observed in rats of the experimental groups (with treatment), which was almost completely completed by day 21. In control group rats, reparative processes proceeded more slowly and were completed only by days 23–25.

It should be noted that in Experimental Group 1 (animals treated with collagen after burn injury), compared with other experimental groups, earlier onset of scab rejection, more pronounced formation of young connective tissue, more intensive

epithelialization of the wound surface, as well as a greater reduction in the severity of inflammation and edema were observed.

It is known that burn injury is characterized by leukocytosis (an increase in the number of leukocytes) [19]. The effect of collagen matrices KM1 and KM2 on this parameter is presented in Table 1.

As can be seen from the data (Table 1), in the control group of animals an increase in leukocyte levels was observed on days 6 and 13 of observation, amounting to 20.6% and 61.0%, respectively, compared with intact (normal) rats. Treatment of post-burn wounds in animals with the studied agents contributed to normalization of the investigated parameter, and this effect was most pronounced in Experimental Group 1 (treatment with a collagen matrix).

Table 1

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

Group Control	Treatment Burn	Leukocyte count, $\times 10^9$/L	
		Day 6 of the experiment	Day 13 of the experiment
Experimental Group 1	Burn + collagen	6,88(6,17–7,59)*n	9,13 (8,82–9,75)*n
Experimental Group 2	Burn + Levomekol	6,36 (6,15–6,58)*2,3	5,20 (4,93–5,45)*k, 2, 3
Intact animals	Normal (no burn)	5,58 (5,33–5,83)*k, 1, 2	6,84 (6,67–7,01)*n, k, 1
Group	Treatment	5,46 (4,76–6,16)	5,67 (4,97–6,37

Note. * – deviation, respectively: n – normal, k – control; 1, 2, 3 – statistically significant relative to groups 1, 2, and 3 ($p \leq 0.05$).

The use of the studied drugs helped prevent the development of general leukocytosis, contributing to a higher dynamic of wound healing. The effect of KM1 and KM2 collagen matrices on the wound area is presented in Table 2.

As can be seen from the data in Table 2, from day 3 of the experiment, the burn injury sites in animals of groups 1 and 2 differed statistically significantly from the results of the control group. The data obtained during the study demonstrate the wound-healing effect of the collagen matrices, which can be compared to the effect of the pharmacopoeial Levomekol preparation. The collagen matrix exhibited the most pronounced reparative effect (experiment 1). It should be noted that in animals treated with collagen and Levomekol, the final healing of the burn wounds occurred on days 19–21, whereas in rats of the control group it occurred on days 23–25.

Table 2

Dynamics of changes in the wound area after thermal burns and treatment with the studied agents, Me (1–Q3)

Group Control	Treatment Burn	Leukocyte count, $\times 10^9$/L					
		1-kun	3-kun	5-kun	7-kun	9-kun	13-kun
Control group	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)
Experimental Group 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
Experimental Group 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

Histological and morphometric analysis during thermal burns and subsequent treatment with the studied agents confirmed the dynamics of skin tissue regeneration. In the experimental groups of animals, wound healing was characterized by a smooth course of the process and prevention of necrosis spreading into the deeper layers of the skin. In experimental group 1, which was treated with the collagen matrix, granulation tissues matured uniformly, there were no purulent-necrotic complications during the healing process, and the structure of regenerated tissue closely resembled the normal skin of rats (intact animals).

Thus, based on the above data, collagen matrices demonstrated a pronounced reparative effect in a thermal burn model comparable to the effect of the pharmacopoeial Levomekol preparation.

CONCLUSION

This study investigated the wound-healing effect of collagen matrices. The use of the studied matrices in a thermal burn model restored the total leukocyte count in the blood of experimental animals and reduced the burn wound area. Their anti-inflammatory and reparative effects were also confirmed by histomorphological studies. Compared to the control group, more intensive healing was observed with collagen matrices (compared to natural healing without treatment), which was manifested by a smaller leukocyte-necrotic crust thickness, as well as accelerated epithelialization and complete closure of skin defects. Examination of the epidermal and dermal layers revealed, by day 20 of the experiment, the absence of pathological processes. The efficacy of the matrices was compared with that of the pharmacopoeial Levomekol preparation. At the same time, a more pronounced wound-healing effect was observed when using the collagen matrix.

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