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Original article

MORPHOMETRIC CHANGES IN RAT PERIWOUND SKIN DURING HEALING OF EXCISIONAL WOUNDS AFTER EXPOSURE TO CHRONIC SOCIAL STRESS

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Background. Chronic stress is the most common systemic factor that negatively affects the body's overall resistance, including wound healing. While many aspects of skin recovery after a surgical wound are well defined, the involvement of cells in the periwound rarea emains unsufficiently studied.
Objective. To determine the morphological features of the periwound skin at different stages of the healing process after exposure to chronic social stress.
Methods. Chronic social stress was modeled through long-term psychoemotional exposure in Wistar laboratory rats of the experimental group. Animals in both control and experimental groups were wounded in the interscapular area by skin excision. The material of the periwounds was collected on the 1st, 3rd, 7th, 14th and 30th days of wound healing and processed according to standard histological methods.
Results. Exposure to chronic social stress led to thinning of the skin layers even before wounding. Visual wound healing was delayed. The main reparative processes by phases (inflammation, proliferation, and remodulation) also occurred with significant delays.
Conclusions. Aggressive-dominant social stress is a rather strong damaging factor for susceptible animals, leading to impaired physiological skin regeneration. This was evident in the thinning of skin layers observed in histological samples even before wound application, resulting from reduced proliferation and differentiation processes. The negative consequences of chronic social stress were further manifested in the healing of a surgical wound: the repair processes during the main phases, in particular inflammation and proliferation, were delayed, which ultimately led to the chronicity of reparative regeneration.

Keywords: wound healing; chronic social stress; periwound; epidermis; dermis; subcutaneous tissue.

Introduction

The skin is one of the most frequently injured organs in the body [1, 2]. After injury, the skin must restore homeostasis, structural integrity, and functional competence [3, 4]. Wound healing is a complex process involving the interaction of inflammatory cells, resident cells, components of the extracellular matrix, and soluble mediators [5, 6]. This process is typically divided into three consecutive, overlapping phases: vascular response, inflammation, proliferation, and remodeling [5–8].

Various factors can impair wound healing, with stress being one of the most common systemic contributors [9–13]. Stress affects individuals regardless of their position, social status, or material wealth. In the United States, which was among the first to recognize the problem of stress, statistics indicate that 90% of the population experiences severe stress. Of these, 60% report experiencing stress 1-2 times a week, while 30% experience it nearly every day. Similar data from other countries show that 50–60% of the population suffers from significant stress [14]. Moreover, the long-term stress induced by the COVID-19 pandemic has had a substantial impact on well-being, contributing to an increase in chronic illnesses, especially among individuals aged 35–44. The prevalence of chronic conditions in this group rose from 48% in 2019 to 58% in 2023. This age group also experienced the highest increase in mental health diagnoses – from 31% in 2019 to 45% in 2023. However, adults aged 18–34 continue to report the highest rates of mental illness, with 50% affected in 2023 [15]. In recent years, it has become increasingly evident that stress can significantly hinder healing: stressors of various magnitudes and durations have been shown to impair healing in both humans and animals [16–19].

Despite extensive research, many aspects of organ repair in mammals following injury remain poorly understood. The cutaneous wound is one of the most studied models for investigating reparative regeneration in adult mammals due to its ease of reproduction, the ability to precisely control wound depth and area, and the opportunity to visually monitor the healing process. Most studies have focused on the rat back scar model [20]. However, these studies often overlook the periwound area,

even though skin cells actively proliferate not only near the wound site but also in more distant regions. This proliferation is essential for restoring the lost mass of the organ while preserving its functionality [21].

Therefore, the objective of this study was to investigate the morphological characteristics of the periwound skin at different stages of the healing process following chronic social stress exposure.

Materials and Methods

The study was conducted on 50 sexually and socially mature male Wistar laboratory rats aged 12-13 months [22, 23], which were divided into two groups. The first group, the control group, consisted of 20 rats (4 rats for each wound healing period). Chronic social stress was induced in the second group, which was more susceptible to stress based on the results of the open field test. This group comprised 30 rats (6 rats for each wound healing period). Stress was modeled by three weeks of social isolation and prolonged psychoemotional exposure, achieved by placing four aggressive rats around each experimental animal [24, 25]. The presence of stress was confirmed through an open field test, performed before and after the chronic stress simulation.

Skin tissue excision was inflicted on the animals between the shoulder blades, creating a full-thickness wound. The full-thickness wound model was created as follows: after skin pretreatment under aseptic conditions and anesthesia, a 1–1.3 cm diameter excision of skin and subcutaneous tissue was made in the interscapular area of the rats' shaved backs [20]. Samples from the structural components of the wound bed and adjacent intact skin (up to 1 cm beyond the wound edge) were collected at 1, 3, 7, 14, and 30 days post-wounding. In this study, the periwound area (up to 5 mm beyond the wound edge) was also analyzed. According to modern wound healing periodization, days 1 and 3 correspond to the inflammation phase, days 7–14 to the proliferation phase, and day 30 to the remodeling phase.

Experimental animals were kept under standard sanitary conditions. After creating the incisional experimental wound, the animals were housed in separate cages with weekly bedding changes under aseptic conditions. No signs of contaminant bacterial infection were observed during the observation period in either group of animals. Throughout the study, they were housed in a vivarium maintained at a temperature of 20–25 °C, with humidity not exceeding 55%, on a natural light "day-night" cycle, individually in plastic cages, and fed a balanced diet.

The experimental procedures adhered to the "International Recommendations for Medical and Biological Research Using Animals" and the national "Joint Ethical Principles of Animal Experiments" (Ukraine, 2001), in accordance with Council Regulation 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on the protection of animals used for scientific purposes.

Skin samples were collected and fixed in a 10% neutral formalin solution in a vessel with darkened glass and stored at room temperature for 3 days before histological analysis. The skin was then processed according to standard histological techniques, placed in paraffin blocks, and serial sections (5 μm thick) were cut using a Thermo Scientific HM 325 microtome (Thermo Scientific, Massachusetts, USA). Sections were stained with hematoxylin and eosin. Photomicrographs were taken using a PrimoStar iLED microscope and an Axio Cam ERc5s camera (ZEISS, Germany). The stained images were analyzed using Quantitative Pathology and Bioimage Analysis (Qupath) software (v 0.4.4, Edinburgh, UK) to calculate the thickness of the skin layers.

Statistical analysis and presentation of the experimental results were performed using the IBM SPSS Statistics software, version 26 (IBM Corp., Armonk, NY, USA).

In each group of the obtained quantitative indicators, an analysis of the normality of the distribution was carried out using the one-sample Kolmogorov–Smirnov test. The Student's *t*-test was used to obtain statistical conclusions when comparing samples of variables. The difference was considered statistically significant at $p < 0.05$.

Generalized data from the study of morphological features of rat skin during wound healing were expressed as the arithmetic mean and its standard deviation (mean ± SD).

Results

Visual morphological features of healing of surgical wounds in control and experimental groups are presented in the Table.

In the control group, immediately after the defect was created, the wound area statistically significantly increased by 15.2% compared to the target area, the wound surface was covered with loose coagulated elements of the internal environment tissue, forming a preliminary wound matrix.

Table 1: Full-thickness wound area on the back of rats at different times after surgery in the control and after exposure to chronic social stress (mean ± SD)

Day of wound healing	Indicator			
	Control		Experiment	
	Absolute wound area, mm^2	Relative wound area, %	Absolute wound area, mm^2	Relative wound area, %
Before excision of the skin flap	94.99 ± 6.25	100.00 ± 6.59	95.01 ± 6.41	100.00 ± 6.97
Immediately after excision	109.43 ± 6.40*	115.20 ± 6.73*	109.55 ± 7.45*	115.30 ± 7.88*
Day 1	106.20 ± 7.87	111.80 ± 2.93	107.93 ± 8.72	113.59 ± 9.16
Day 3	97.14 ± 11.26	102.26 ± 11.88	104.32 ± 14.97	109.79 ± 15.77
Day 7	73.24 ± 9.19**	77.10 ± 9.68**	95.39 ± 11.82#	100.39 ± 12.42#
Day 14	16.62 ± 2.46***	17.49 ± 2.60***	50.64 ± 6.27**###	53.29 ± 6.61***###
Day 30	0.52 ± 0.44***	0.55 ± 0.47***	8.64 ± 1.56***###	9.09 ± 1.63***###

Notes. * – differences at different times after surgery within one group (control and experiment) are statistically significant ($p \le 0.05$); ** – differences at different times after surgery within one group (control and experiment) are statistically significant ($p \le 0.01$); *** – differences at different times after surgery within one group (control and experiment) are statistically significant, ($p \le 0.001$); # – differences between control and experiment at one time are statistically significant ($p \le 0.05$); ### – differences between control and experiment at one time are statistically significant ($p \le 0.001$).

The wound healing process occurred with the formation of a connective tissue scar by the 30th day. On the 1st and 3rd day, the wound matrix covering the wound became denser, while the tendency to decrease the area of the wound defect did not reach statistical differences with the initial values. On the 7th day, the wound area was 77.1% of the initial. By the 14th day, the wound area was 17.5% of the initial defect. On the 30th day, there were no signs of a wound, and a thin connective tissue scar was observed in its place.

In the group of animals that had undergone chronic social stress, similar to the control, immediately after the wound was applied, its area statistically significantly increased by 15.3% compared to the target area, but unlike the control, a delay in wound closure was observed already on the first and third days. Formation of a protective wound matrix from elements of internal tissues was inferior in quantity and density. On the 7th day after wounding, their area averaged 100.4% of the initial area, which demonstrated a significant lag behind the control at this observation period (77.1%, $p \le 0.01$). Signs of scab formation were absent, the primary matrix was observed. By the 14th day, the relative wound area was significant – 53.3%, which was three times lower than wound closure in the control (17.5%, $p \le 0.001$). By the 30th day of the experiment, the wound area remained at an average level of 9.1%.

The visual morphological features of the healing of surgical wounds in the control and experimental groups were adequate to their histological dynamics of reparative regeneration (Fig. 1).

The thickness of the epidermis was 14.82 ± 1.03 μm (Fig. 2), the boundaries of the epidermal layers were not clear (their thickness corresponded to the well-known ones (Fig. 3) [23, 24]), in all layers there were mainly 1-2 rows of keratinocytes. Epidermis and dermis, interacting with each other, formed weakly expressed epidermal cords and dermal papillae, blood vessels were localized mainly in the area of hair follicles (Fig. 1a). The dermis occupied the main part (thickness on the cross section – 439.51 ± 49.73 μm) of the skin, and its borders pass into the subcutaneous tissue. The thickness of the subcutaneous tissue was 263.37 ± 68.13 μm (Fig. 4). It contained a large number of adipocytes.

On the 1st day of wound healing, the thickness of the epidermis increased statistically significantly (25.36 ± 2.29 μm ($p \le 0.001$) (Fig. 2)) due to the reactive proliferation of basal layer cells (13.40 ± 1.48 μm ($p \le 0.001$) (Fig. 3a)) and tendencies to hyperplasia and hypertrophy (Fig. 1c) of the cells of the spinous layer (4.47 ± 0.56 μm) (Fig. 3b). Also, the thickness of the dermis increased statistically significantly ($p \le 0.01$), which was 598.67 ± 34.51 μm (Fig. 4a) due to increased vascular perfusion. Changes in the subcutaneous tissue were not statistically significant (Fig. 4b).

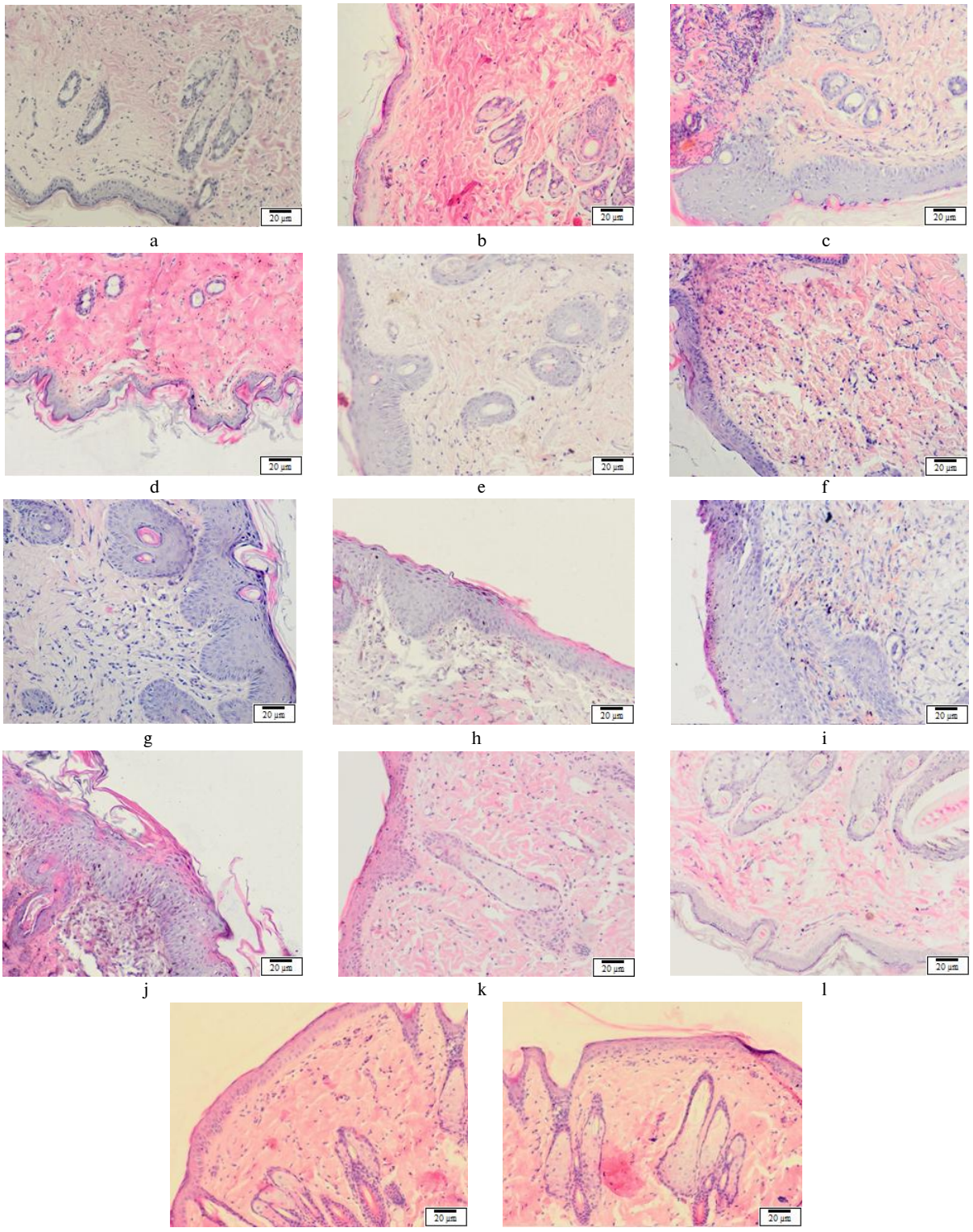

Figure 1: Microphotographs of a skin flap excised in periwound and wound area (i, j) in control and experimental groups of rats at different periods of healing: (a) day of wounding, control group; (b) day of wounding, experimental group; (c) day 1 of wound healing, control group; (d) day 1 of wound healing, experimental group; (e) day 3 of wound healing, control group; (f) day 3 of wound healing, experimental group; (g) day 7 of wound healing, control group; (h) day 7 of wound healing, experimental group; (i) day 7 of wound healing, control group – granulation tissue is formed; (j) day 7 of wound healing, experimental group – no granulation tissue, inflammatory infiltration of the dermis; (k) day 14 of wound healing, control group; (l) day 14 of wound healing, experimental group; (m) day 30 of wound healing, control group; (n) day 30 of wound healing, experimental group

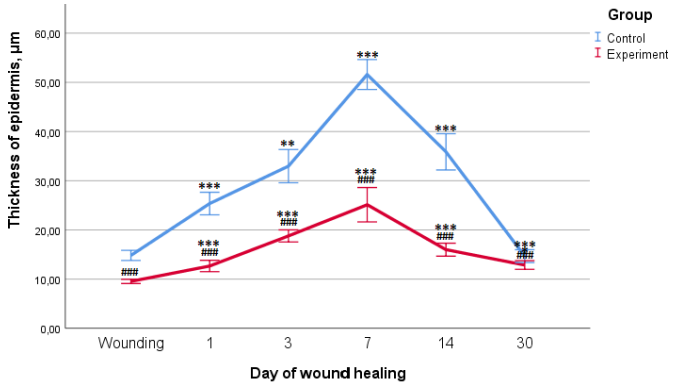

Figure 2: Dynamics of changes in the thickness of the periwound skin epidermis of rats during healing process in control and after exposure to chronic social stress. Mean values and their standard deviations are indicated. * – changes are statistically significant, compared to the previous period of wound healing ($p \leq 0.05$); ** – changes are statistically significant, compared to the previous period of wound healing ($p \leq 0.01$); *** – changes are statistically significant, compared to the previous period of wound healing ($p \leq 0.001$); *** – changes are statistically significant, compared to the previous period of wound healing ($p \leq 0.001$); ### – changes are statistically significant, compared to control ($p \leq 0.001$)

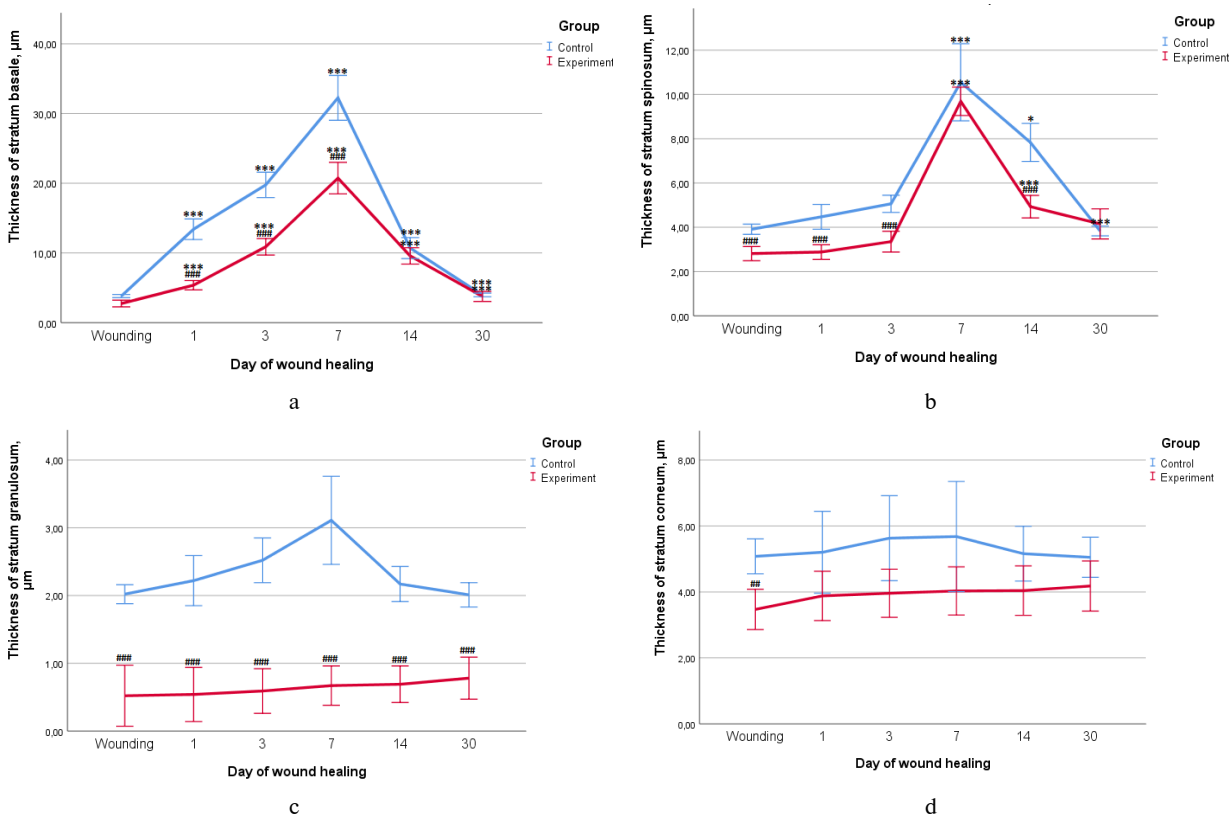

Figure 3: Dynamics of changes in the thickness of the periwound skin epidermal layers ((a) stratum basale, (b) stratum spinosum, (c) stratum granulosum, and (d) stratum corneum) of rats during healing process in control and after exposure to chronic social stress. Mean values and their standard deviations are indicated. * – changes are statistically significant, compared to the previous period of wound healing ($p \leq 0.05$); ** – changes are statistically significant, compared to the previous period of wound healing ($p \leq 0.01$); *** – changes are statistically significant, compared to the previous period of wound healing ($p \leq 0.001$); ## – changes are statistically significant, compared to control ($p \leq 0.01$); ### – changes are statistically significant, compared to control ($p \leq 0.001$)

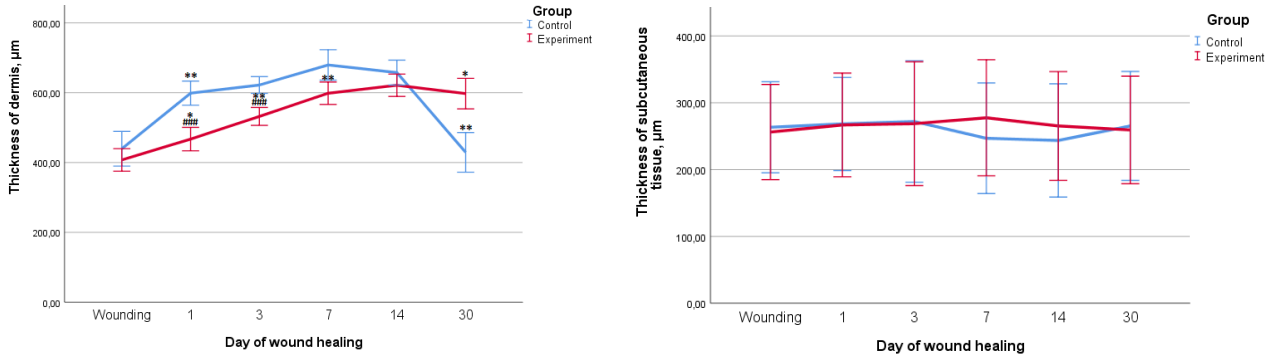

Figure 4: Dynamics of changes in the thickness of the periwound skin dermis (a) and subcutaneous tissue (b) of rats during healing process in control and after exposure to chronic social stress. Mean values and their standard deviations are indicated. * – changes are statistically significant, compared to the previous period of wound healing ($p \le 0.05$); ** – changes are statistically significant, compared to the previous period of wound healing ($p \le 0.01$); ### – changes are statistically significant, compared to control ($p \le 0.001$)

On the 3rd day, the thickness of the epidermis in periwound was 32.97 ± 3.38 µm (Fig. 2), which is statistically significantly more compared to the previous period of wound healing ($p \le 0.01$). The thickness of the basal layer was 19.76 ± 1.82 µm ($p \le 0.001$) (Fig. 3a). The thickness of the dermis at this healing time also tended to increase (Fig. 4a). Infiltration of the dermis by leukocytes and increased vascularization were observed (Fig. 1e). No significant changes in the subcutaneous tissue were found.

On the 7th day after the application of wounds, the formation of granulation tissue in the area of the wound surface and infiltration of this area by immunological cells was observed (Fig. 1i). The thickness of the epidermis of the marginal zone of the wound reached the maximum values – 51.59 ± 3.04 µm ($p \le 0.001$) (Fig. 2), mainly due to statistically significant ($p \le 0.001$) thickening of the basal layer and spinous layer ($p \le 0.001$) (Figs. 3a, 3b), which was accompanied by the phenomena of hyperplasia and hypertrophy of epidermal cells. The thickness of the dermis was in the upper limits of the species norm and amounted to 679.47 ± 43.63 µm, but did not have statistically significant changes compared to the previous period of observation (Fig. 4a). During the microscopic examination of samples stained with hematoxylin and eosin, an increase in collagen fibrils in periwound was observed (Fig. 1g). No changes were detected in the subcutaneous tissue.

By the 14th day, the thickness of the epithelium ($p \le 0.001$) (Fig. 2), in which 4-6 layers of cells were distinguished (Fig. 1k), and its basal ($p \le 0.01$) and spinous ($p \le 0.05$) layers decreased statistically significantly (Figs. 3a, 3b) in the periwound. The thickness of the dermis remained high, within the limits of the species norm, but no statistically significant changes were observed. The thickness of the subcutaneous tissue did not change statistically significantly (Fig. 4).

On the 30th day, the reparative regeneration of the wound process was completed with the return of histological indicators to the initial levels, which was visually emphasized on the slides (Fig. 1m).

The epidermis of animals that had undergone chronic social stress even before the wound was inflicted was significantly thinner (9.54 ± 0.43 µm ($p \le 0.001$) (Figs. 1b, 2)) compared to control, due to the thinning of the basal and spinous layers (2.74 ± 0.48 and 2.81 ± 0.32 µm ($p \le 0.001$), respectively). 3-5 layers of keratinocytes were determined. At the same time, the granular layer was especially thinned, up to its complete absence in short sections. Also, local parakeratosis was observed. In the dermis, there was a narrowing of the vessels of the superficial vascular plexus and the formation of a moderate leukocyte infiltration around them.

On the 1st day, proliferation of the epithelium was observed due to the cells of the basal layer (Figs. 1d, 3a), but it was 2 times less ($p \le 0.001$), compared to the control (Fig. 2). At the same time, the thickness of the dermis increased statistically significantly ($p \le 0.05$), however, its thickness was statistically significantly smaller ($p \le 0.001$) compared to the control. Changes in the subcutaneous tissue were not statistically significant (Fig. 4b).

On the 3rd day, the thickness of the epidermis in the marginal zone was 18.78 ± 1.23 µm, which is statistically significantly less than the control

($p \le 0.001$) and related to it, all layers of the epidermis (spinosum, granulosum, and corneal) were thinned (Fig. 3). The thickness of the basal layer was also almost 2 times smaller compared to the control (10.88 ± 1.19 μm ($p \le 0.001$)) (Fig. 3a). The thickness of the dermis at this time of healing was statistically significantly ($p \le 0.001$) less compared to the control (Fig. 4a). It was infiltrated with leukocytes and vascularized (Fig. 1f). No significant changes in the subcutaneous tissue were found.

On the 7th day, when the maximum values of the proliferative reaction should be observed, the thickness of the epidermis of the periwound was 25.12 ± 3.49 μm ($p \le 0.001$), which was 2 times less, compared to the control ($p \le 0.001$), due to the thinning of the basal and granular layers ($p \le 0.001$). As compensation for the reduced proliferative activity of the basal layer, the proliferative activity of the spinous layer increased and it almost reached the control level (9.69 ± 0.64 and 10.55 ± 1.74 μm, respectively, $p > 0.05$). However, further processes of differentiation of subsequent layers remained suppressed, so their thicknesses differed from the control. Granulations are not performed (Fig. 1j), however at this period granulation tissue was formed in the control group. A thickening of the dermis was observed, compared to the previous period of the study ($p \le 0.01$) due to the ongoing vascular-cellular inflammatory reaction of the dermis (Fig. 1h). Changes in the subcutaneous tissue were not statistically significant.

On the 14th day of the wound healing, a decrease in the thickness of the epidermis of the skin periwound was observed, both in comparison with the seventh day and with the control at the expense ($p \le 0.001$). The spinous and granular layers were thinned compared to the control ($p \le 0.001$). No significant changes were found in the dermis and subcutaneous tissue.

On the 30th day after wounding, a decrease in the thickness of almost all layers of the skin of the periwound was observed, but the return of indicators to the weekend was not observed. Thus, the thickness of the epidermis was 12.87 ± 0.88 μm, which is statistically significantly less than in the control and on the day of wounding in the experimental group ($p \le 0.001$). The thickness of the granular layer remained low, compared to the control ($p \le 0.001$). The thickness of the dermis decreased compared to the previous observation period ($p \le 0.05$), but was not significantly increased compared to the control. No changes were observed in the subcutaneous tissue.

Discussion

Physiological and reparative processes require a complex multi-level and multi-component system of regulation, the main one of which is carried out through the neuroimmunoendocrine system [26]. In this case, the adaptation of the mammalian organism in a constantly changing environment is carried out mainly through the activation of the hypothalamic-pituitary-adrenal axis and the subsequent secretion of glucocorticoids (GCs), mineralocorticoids, catecholamines, the functions of which in some cases overlap [27]. Thus, GCs ensure continuous optimal metabolism and morphogenetic integrity of the body, carried out through the homeostatic function of the immune system [28, 29]. Catecholamines regulate the optimal functioning of the cardiovascular system and the tone of the muscular system [29]. Mineralocorticoids, through the renin-angiotensin-aldosterone system, regulate the water-salt balance in the internal system of the body by optimizing its mineral composition and vascular tone together with the autonomic nervous system [30].

Current research has demonstrated that the systemic central neuroimmunoendocrine regulation of the body has regional representation in various organs. Specifically, skin cells are capable of secreting hormones of the central regulatory axis and play an active role in both physiological and reparative regeneration. In addition to their structural function, all skin cells are also involved in innate and adaptive immunity, forming a hierarchy of resident, recruited, and recirculating cells [31].

In the context of this broadly described homeostasis regulation, wound healing occurred in the skin of both control and experimental animal groups.

Wound healing is a complex, evolutionarily shaped process involving the interaction of molecular and cellular regulatory mechanisms, as mentioned above, which are implemented at both local and systemic levels. This process occurs in three overlapping phases: hemostasis/inflammation, proliferation/differentiation, and remodeling [5–7, 16, 21].

Comparative analysis of the dynamics of structural layer restoration in the skin revealed stereotypical cellular and morphogenetic responses in the corresponding phases of repair [22, 32]. However, the impact of various types of stress significantly influenced the efficiency of reparative processes. In the control group, these processes aligned with physiological parameters, whereas in the experimental group, although the direction of proliferative and differentiation responses was similar, the

amplitude of these changes and the temporal aspects of the key phases of the wound healing process were significantly delayed.

For animals in the control group, the excisional injury itself and the accompanying pain served as acute stress, whereas numerous studies have shown that acute stress, lasting from several minutes to a few hours, positively mobilizes all innate and adaptive defense mechanisms of restorative morphogenesis [31, 33–35].

Primarily, glucocorticoids like prednisone and catecholamines from the central regulatory axis, within physiological adaptive limits, trigger the redistribution of key circulating immune cells to the corresponding organs, including the skin. These include dendritic cells, macrophages, granulocytes, and T- and B-lymphocytes. Under the influence of these hormones, the local resident cell system of the regulatory axis in the skin is also activated, including keratinocytes, endothelial cells, fibroblasts, adipocytes, mast cells, and innate T- and B-lymphocytes. These cells synthesize analog mediators that help optimize the course of the wound healing process [31, 35–37].

In this context, in the control animals, a preliminary wound matrix quickly formed within the first hours of injury development, through the formation of complexes based on fibronectin, fibronectin with heparin, and collagen [29]. This matrix isolated the wound from the external environment, created conditions for wound edge contraction, and the opsonizing properties of fibronectin facilitated the optimal deployment of cellular and humoral components of the subsequent inflammatory response on the 1st and 3rd days of healing [30]. The optimal progression of this phase was evidenced by a significant increase in the proliferation of the basal epidermal layer and a notable thickening of the dermis as a result of the vascular-cellular reaction. The effective resolution of clearance issues and mobilization of the cellular diversity of skin tissues in the wound area, mainly through their recruitment from the bloodstream and deposition in the wound focus and periwound zone, contributed to the homeostatic course of the healing process and the transition to the next stage. This stage involved the proliferation and differentiation of the main histological cell lineages [38]. The main outcome of the proliferative reaction was the formation of granulation tissue on the 7th day, serving as a transitional tissue and a second temporary wound matrix [39]. This matrix developed as a continuation of the primary wound matrix formed within the first day of the wound process. This period was also characterized by high vascularization. As a result, by the 7th day, we observed maximal thickness of both the epidermis and dermis. By the 14th day, differentiation processes of tissue lineages predominated, leading to the transformation of the granulation matrix: complete epithelialization, and wound closure. These rehabilitative changes by the 30th day resulted in complete wound healing by secondary intention, with the formation of a thin connective tissue scar.

Successful wound healing in the control animals was also demonstrated by the temporal reduction in the relative wound area. The wound area, which initially increased after incision due to the elasticity of the surrounding tissues, rapidly decreased during the proliferative phase (by 17%) following the inflammatory period, approaching intact values. This finding is consistent with other studies [22, 32].

In the experimental group of animals, acute post-traumatic shock was superimposed on a pronounced chronic social stress (CSS), with the stressor factor being the dominant aggression from surrounding animals. This aggression was perceived by the sensitive animals as a life-threatening danger or the risk of injury, especially in the absence of the ability to isolate themselves from these animals. Chronic stress is defined as stress lasting weeks, months, or years [33]. As a result of three weeks of stress exposure, a pronounced deep stress response was observed, as recorded in the open field test. According to the literature, CSS increased hormonal tension in the central regulatory axis and its associated autonomic nervous system, as well as in its regional axis [10, 11, 31, 33, 40, 41]. Additionally, the increased levels of hormones disrupt the harmonious sequence of immune cell recruitment from the internal environment, including the skin, leading to a subsequent blockage of constant recirculation and renewal [31, 33].

Therefore, the effect of chronic social stress was already observed in skin samples prior to wound infliction: all layers of the epidermis and dermis were reduced in comparison with the control group. In our previous studies within the same experiment on mast cell dynamics, we found a significant increase in the number of mast cells and their degranulation activity, also in pre-wound samples from animals exposed to chronic social stress [42, 43]. Recent findings in the study of mast cell functions have provided evidence that they constitute a local, autonomous neuroimmune-endocrine system, the primary function of which is morphogenetic control of cellular and humoral components of the in-

ternal environment [16, 17, 44, 45]. Supporting this concept are data on the close synaptic connections between mast cells and nerve endings [44], as well as the tight receptor-ligand feedback mechanism. In light of this, it is reasonable to conclude that chronic social stress, through central regulatory pathways, disrupts their local tissue regulatory complexes. Therefore, the multiple increases in both the number and functional activity of mast cells in intact histological samples can be explained by the inhibition of their migration and deposition under the influence of glucocorticoids from the adrenal cortex, aimed at resolving alterative skin issues.

Our findings were confirmed by a recent report stating that chronic stress, by enhancing catecholamine synthesis activity, increases the mobilization of mast cells to skin wounds and enhances their degranulation activity [46]. In this context, high levels of IL-10 cytokines were found, which exert an anti-inflammatory effect and reduce macrophage activity [46].

After the influence of CSS, wound healing was notably slower, with disruptions in the inflammatory, proliferative, and remodeling phases. Moreover, all layers of the epidermis and dermis in the periwound area were significantly reduced compared to the control group. These deviations in the reparative processes resulted from the disruption of the aforementioned regulation of the central and regional axes.

It is now well-established that immune cells play a significant role in regulating morphogenetic processes in histogenesis and the regeneration of non-lymphoid structures, including skin [27, 31, 33, 47]. A high level of stress hormones negatively affects immune cell activity, leading to dysfunction in the synthesis of interleukins and other regulatory factors, which results in delayed and reduced reparative processes. There is evidence that CSS is accompanied by an increased synthesis of anti-inflammatory cytokines (IL-4, IL-5, IL-10, IL-13, etc.), with IL-10 being the most prominent in this case. These cytokines greatly reduce the production of pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-8, gamma interferon, TNF-alpha, etc.), thereby delaying the development of vascular-cellular reactions in the inflammation stage and infiltration of immune cells (neutrophils, eosinophils, macrophages) into the inflammatory zone, thus lowering their immunological activity [1, 3, 27, 31, 33, 47]. In turn, the delayed onset of the inflammatory stage leads to the chronicization of subsequent stages of wound healing.

Previously, in this experiment, we established that wound healing at all stages in this group was accompanied by reduced mast cell numbers but with enhanced degranulation, even to the point of apoptosis, as a marker of functional strain in an attempt to resolve regenerative process disruptions [42, 43]. These data support the findings of other researchers who suggest that reparative processes in the wound proceed under greater metabolic stress, ending with apoptotic reactions and a significant deficit in the recirculation of new cellular elements from the blood [16, 17, 44, 45].

The above findings are further corroborated by the primary functions of GCs, which play a key role in reparative processes. In addition to their primary metabolic function in regulating carbohydrate metabolism, prednisone regulates migration, proliferation, differentiation, and apoptosis of tissue cells. Each of these functions is dose-dependent on glucocorticoids, increasing in intensity in the order listed [16, 46, 47].

Thus, along with the already disrupted local regulatory system, the reparative processes in the experimental group of animals proceeded at a delayed rate. Visual wound healing was significantly delayed. By the end of the proliferative period, the relative area of the wound was 53.3%, compared to 17.5% in the control group. By the end of the experiment (on day 30), a defect was observed (9.1%). The main reparative phases also proceeded with a significant delay: inflammation, proliferation, and remodeling. In the inflammation phase, the vascular-cellular responses of this phase were delayed, ultimately leading to a reduction in epidermal and dermal thickness on days 1 and 3 of healing. The delayed clearance and vascular-cellular responses in the inflammation phase also led to delays in subsequent reparative stages.

Proliferative reactions of tissue precursors lagged significantly behind the control group: the thickness of the epidermis and dermis on day 7 was significantly lower than in the control group. The formation of the primary reparative granulation tissue was delayed. On day 7 of observation, only small islands of this tissue were present. Differentiation processes in the tissues surrounding the wound were also suppressed, as evidenced by the inhibition of the initiation of remodeling processes in the skin layers by day 14. By day 30 of observation, the morphometric parameters of the skin in the periwound had not returned to baseline (prewound) values, nor had they reached the control level, indicating that both remodeling and com-

pensatory processes, induced by emotional stress, had not been completed.

In our previous study, within the same experiment, by day 30 of observation, we identified a second peak in the increase of mast cells and their degranulation activity [42, 43]. Therefore, considering their function, we interpreted this as evidence of compensatory reparative responses to the chronic prolongation of regenerative processes, aimed at restoring their migratory and proliferative activity at the deposition site for the subsequent completion of remodeling processes in the wound [43].

The maintenance of a high mast cell count was accompanied by an incomplete remodeling process, which may also indicate residual effects of chronic social stress. Indeed, there is evidence that blockade of glucocorticoid receptors in the skin wound with the Ru408 drug (mifepristone) did not affect the elevated levels of glucocorticoids and catecholamines in the plasma of animals experiencing chronic stress, while the drug had a positive effect on wound healing [48].

In light of these findings, ongoing stimulation by hormones leads to the deposition of mast cells in the incompletely healed wound, promoting the synthesis of pathognomonic anti-inflammatory cytokines during this period, including IL-10. These cytokines shift the immune response predominantly towards T-helper regulation, activating the reparative reaction and fostering a predominance of cell differentiation processes over proliferation in granulation tissue, leading to its replacement with original mature skin cell lineages [45, 49–51].

Given these results, future studies should focus on the molecular and cellular disruptions caused by emotional stress and how they affect the coordination between the immune, endocrine, and nervous systems in regenerative processes. Investigating potential pharmaceutical interventions that modulate stress mechanisms may offer new therapeutic pathways for optimizing wound healing under stress conditions, particularly those aimed at mitigating vascular-cellular disturbances during the early stages of the inflammatory response to injury.

Conclusions

Thus, we have shown that chronic aggressive-dominant social stress is a powerful damaging factor for susceptible animals, leading to a disruption of the structural homeostasis of skin tissues, accompanied by high levels of hormones from systemic and local neuroimmune-humoral regulatory networks. This leads to disturbances in physiological and reparative tissue regeneration, manifested in the thinning of all skin layers, the chronicity of wound healing, and the incompleteness of reparative remodeling by the end of the study. In cases of impaired recovery processes, cellular reactions at all stages of skin wound healing are delayed. However, the primary factor is the suppression of the early inflammatory response, the success of which determines the reparative processes during the proliferation/differentiation and remodeling stages. Therefore, based on the results obtained, future research should focus on the molecular and cellular disruptions caused by the chronic effects of emotional stress. Special attention should be given to the inhibition of vascular-cellular reactions at the earliest stage of inflammatory response development, as this is the most vulnerable phase in reparative situations.

Pharmacological stimulation of local blood circulation appears to be the most promising therapeutic approach in this case, improving trophic, recirculatory, and thereby remodeling processes.

Interests disclosure

The authors have no conflicts of interest to declare.

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МОРФОМЕТРИЧНІ ЗМІНИ КРАЙОВОЇ ЗОНИ РАН ШКІРИ ЩУРІВ ПІД ЧАС ЗАГОЄННЯ РІЗАНИХ РАН ПІСЛЯ ВПЛИВУ ХРОНІЧНОГО СОЦІАЛЬНОГО СТРЕСУ

Проблематика. Хронічний стрес є найбільш поширеним системним фактором, який негативно впливає на загальну резистентність організму, в т.ч. і на загоєння ран. Добре визначені низка аспектів відновлення шкіри після нанесення хірургічної рани, однак залученість клітин крайової ділянки ран вивчена не достатньо.
Мета. Визначити морфологічні особливості крайової ділянки ран шкіри на різних етапах процесу загоєння після впливу хронічного соціального стресу.
Методика реалізації. Моделювали хронічний соціальний стрес шляхом тривалого психоемоційного впливу на лабораторних щурів лінії Wistar експериментальної групи. Тваринам контрольної та експериментальної груп наносили різану рану в міжлопатковій ділянці. Забирали матеріал крайової ділянки ран на 1, 3, 7 ,14 та 30-й дні загоєння ран і обробляли за стандартною гістологічною методикою.
Результати. Перенесений хронічний соціальний стрес ще до нанесення рани призводить до потоншення шарів шкіри. Візуальне загоєння рани йшло з великим запізненням. З достовірною затримкою відбувалися й основні репаративні процеси за фазами: запалення, проліферація, ремодуляція.

Висновки. Агресивно-домінантний соціальний стрес є досить сильним ушкоджуючим фактором для сприйнятливих тварин, що призводить до порушення фізіологічної регенерації в шкірі, яке виражалось у витонченні шарів у гістологічних зразках шкіри, ще до нанесення рани за рахунок зниження процесів проліферації та диференціації. Негативні наслідки хронічного соціального стресу далі виявлялися і в загоєнні хірургічної рани: затягувалися процеси репарації на основних фазах, зокрема запаленні та проліферації, що зрештою призвело до хронізації репаративної регенерації.

Ключові слова: загоєння ран; хронічний соціальний стрес; крайова зона ран; епідерміс; дерма; підшкірна клітковина.