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Original paper

Experimental justification for prompt neutralization of traumatic action of thermal exo- and endogenous damage factors (morphological features)

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Abstract

Objective: morphological substantiation of efficiency of prompt neutralization of traumatic action of exogenous and endogenous damage factors in burn injuries in experimental conditions.

After simulating the burns on 60 rats, the traumatic hyperthermic damage factors in the main group were immediately neutralized by a gauze napkin soaked in water. Depending on its duration, the animals were divided into subgroups. Such applications were not performed on the control group. The study involved histological examination of tissues.

Animals of the main groups had the presence of histologically confirmed skin appendages with a formed scar of connective tissue without signs of inflammation. In animals of the control group there were no skin appendages, which indicates a deeper thermal damage to tissues and the impossibility of self-epithelialization of wounds.

These results confirm the importance and necessity of prompt neutralization of the traumatic effect of damage factors as the main elements of burn depth formation.

Keywords hyperthermic factors, burns, neutralization, first aid, morphological structure

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Introduction

Prompt neutralization of traumatic action of hyperthermic factors in burn injuries proved to be effective in wound healing process, but its pathogenetic justification remains an urgent task of modern combustiology [1, 2]. Neutralization of hyperthermic exogenous factor is known to be followed by activation of endogenous one.

In recent years, the incidence of terrorist attacks has increased significantly worldwide, resulting in numerous traumatic injuries with burns making from 5-8% to 50% and over. Practical experience has shown that the lower is the exposure to traumatic effect of exogenous factors of damage, the less severe is the course of burn injury and the better treatment outcomes [3, 4, 5]. Comprehensive study of specific morphological changes in the damaged tissues seems to be of great significance for better understanding of the major pathogenetic principles in giving first aid or self-care in burn injuries depending on the time of intervention [6].

Objective: to present morphologically based arguments for efficiency of prompt neutralization of traumatic action of hyperthermic exo- and endogenous damage factors in vivo experimental models of burns.

Materials and Methods

Experimental study was carried out on 60 mature Wistar rats (150 - 160 g) being fed with standard normal diet. The study conformed to international rules and principles of protection of vertebrate animals used for experimental purposes as well as ethical guidelines for animal research.

Experimental animals were divided into study (experimental) group (10 rats) and control group (10 rats in each of 5 subgroups). Modified scalding technique proposed by Pfurtscheller et al. [7] was used to produce second-degree scald burns in animals of both groups. In animals of experimental group, traumatic effect of hyperthermic exo- and endogenous damage factors was neutralized using a gauze pad soaked in water (t=18-20^{0}C) immediately after the scald modeling. According to the time of application of gauze pad on the burn region – 1, 2, 5, 10 and 15 minutes, the animals were divided into subgroups 1, 2, 3, 4 and 5, respectively. Application pads were changed at water temperature of 34 ^{0}C. No such applications were used in rats of the control group.

The study involved histological investigation of tissues taken from visible parts of the wound and adjacent tissues up to 5 mm thick at 1, 3, 7, 14 days after thermal injury modeling. After sampling the material was immersed into 10% solution of neutral formalin for fixation followed by its washing and dehydration. The tissue was then embedded in paraffin, and sections 7 μm thick were made on the microtome device. The histological specimen were stained with hematoxylin and eosin [8] and then studied under light microscope OLYMPUS BX-41 (Ministry of Health of Ukraine, State Registration Certificate No 8120/2008, code 9011800000) at magnification of 40x, 100x and 200x. Image visualization was performed using the program Quickphoto micro 2.3 (licensing agreement No 925113924).

Results and discussion

Histologic specimen of tissues taken on day 1 of the experiment (neutralization time - 1 minute) demonstrated severe alternative changes in the damaged skin of animals from group 1. The epidermis was absent due to its coagulation and desquamation. Skin appendages located close to the epidermis were absent with the signs of coagulation changes in the deeper layers of the dermis. There was marked swelling of collagen and elastin fibers of the dermis as well as their splitting. In subepithelial layers of the dermis proper, coagulation changes of collagen fibers with their compaction, homogenization and post-burn eschar formation were observed as well. Vascular response was characterized by paretic vasodilation in the burn injury zone with moderate hyperemia and hemolysis of erythrocytes. Moderate swelling of hypodermis and dermal reticular layer was visualized in the scalded area. There were foci of inflammation presented as perivascular inflammatory infiltrates and consisting predominantly of segmented leukocytes and lymphocytes (Fig. 1).

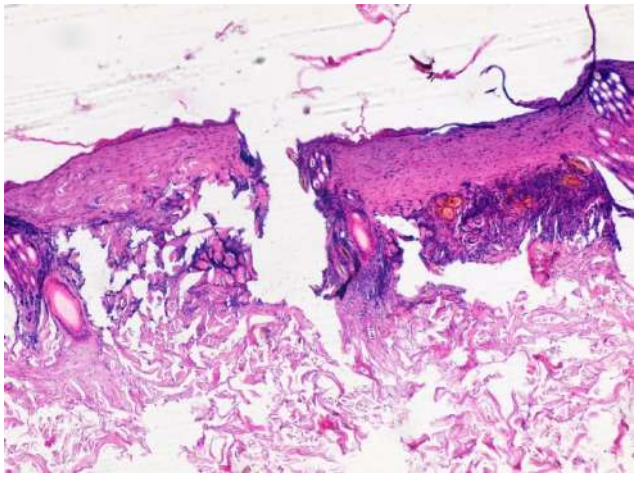

Figure 1. Microscopic changes in the skin of the animal from experimental group 1 on day 1 after thermal injury modeling. Degenerative alterations in the dermal layer with necrotic zone formation, marked perifocal edema, paretic dilation and vascular erythrostasis, perivascular lymphoid infiltration. Hematoxylin-eosin staining, x100.

Animals of experimental groups 2 and 3 (neutralization time - 5 and 10 minutes, respectively) had less severe alternative changes in the damaged skin as compared to those in experimental group 1. The epidermis was absent because of its coagulation and desquamation, and the skin appendages located close to the epidermis had less evident coagulation changes. There was moderate swelling of dermal collagen and elastin fibers, their mild splitting. In subepithelial layers of the dermis proper, coagulation changes of collagen fibers with their compaction, homogenization and formation of thin eschar were observed as well. Vascular response was presented as paretic vasodilation in the burn injury zone with moderate hyperemia and hemolysis of erythrocytes. Moderate swelling of hypodermis and dermal reticular layer was visualized in the scalded area. There were foci of inflammation presented as perivascular inflammatory infiltrates and consisting predominantly of segmented leukocytes and lymphocytes (Fig. 2).

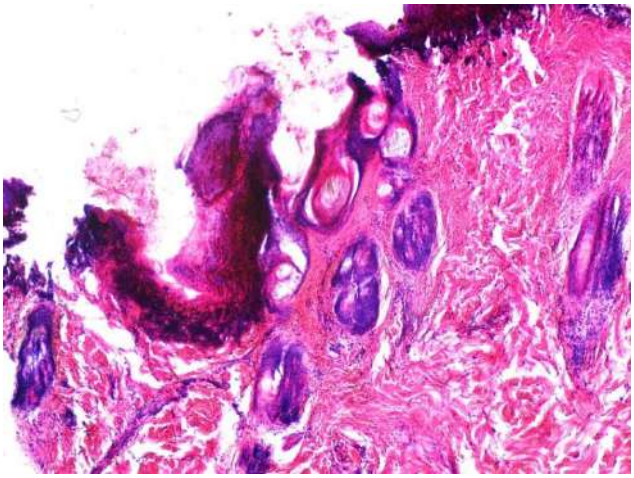

Figure 2. Microscopic changes in the skin of the animal from experimental group 2 on day 1 after thermal injury modeling. Degenerative alterations in the dermal layer with zone of necrosis formation, marked perifocal edema, splitting of dermal collagen fibers. Hematoxylin-eosin staining, x100.

Histologic investigation of injured tissues from the rats of experimental groups 4 and 5 (neutralization time - 15 and 20 minutes, respectively) found minimal alternative changes in the damaged skin as compared to those in group 1 and the control. The epidermal layer was absent due to its coagulation and desquamation, and skin appendages located close to the epidermis had minor coagulation changes. Swelling of dermal collagen and elastin fibers as well as their insignificant/slight splitting, formation of thin eschar, paretic vasodilation in the burn injury zone with moderate hyperemia and hemolysis of erythrocytes were observed. However, inflammatory changes in the injured skin tissues could hardly be visualized at this time (fig. 3).

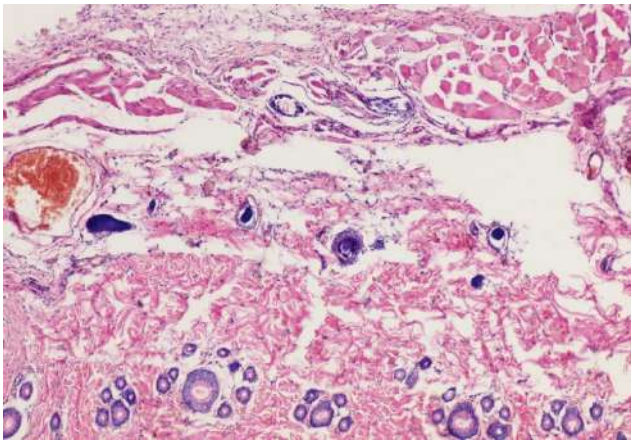

Figure 3. Microscopic changes in the skin of the animal from experimental group 5 on day 1 after thermal injury modeling. Perifocal edema, splitting of dermal collagen fibers, paretic vasodilation with hyperemia. Hematoxylin-eosin staining, x100.

The study of histological structure of wound tissues from control group animals revealed extended damaged area with no distinct margins and necrosis formation in the center. Besides, marked perivascular lymphocytic infiltration and the signs of impaired microcirculation were seen as well. The epidermis was absent because of its complete desquamation due to evident coagulation changes. The dermis consisted of bundles of collagen and elastic fibers oriented in various directions with the signs of necrosis, as well as their edema in the perifocal areas. Disturbances of underlying hypodermis structure were attributed to degenerative changes (Fig. 4).

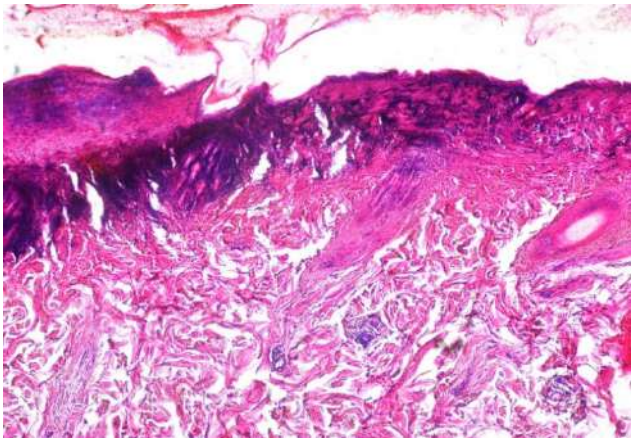

Figure 4. Microscopic changes in the skin of the animal from the control group on day 1 after thermal injury modeling. Degenerative alterations in the dermal layer with formation of coagulation necrosis zone, lack of epidermis, perifocal edema, splitting of dermal collagen fibers. Hematoxylin-eosin staining, x100.

On day 3 of observation, rats of experimental group 1 and the control group exhibited even more evident changes in the damaged skin. Inflammation involved the subcutaneous tissue and muscles. Composition of cellular infiltrate was represented

mostly by segmented neutrophils with a small number of lymphocytes and monocyte lineage cells (macrophages, multinucleated giant cells, foreign body giant cells). Vascular response to the burn was characterized by moderate hyperemia, edema of the hypodermis and reticular dermis in inflammation region. There were isolated bundles of collagen fibers in dense regular connective tissue of the dermis with the signs of edema, splitting and fragmentation. Skin appendages had the signs of dystrophic changes (Fig. 5).

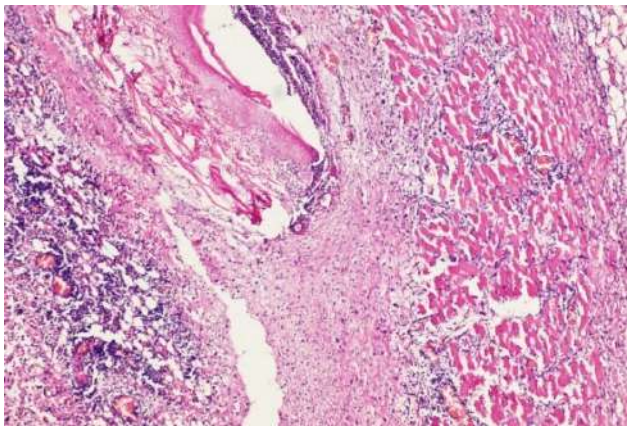

Figure 5. Microscopic changes in the skin of the animal from the control group on day 3 after thermal injury modeling. Tissue necrosis, absence of skin appendages, hyperemia, diffuse leukocyte infiltration, homogenized collagen and elastin fibers of the dermis proper, spread of inflammatory infiltrate to muscle tissue. Hematoxylin-eosin staining, x100.

In animals of experimental groups 2 and 3, less evident changes in the damaged skin were detected. There were inflammatory changes in skin tissues as well but they reached only the subcutaneous tissue. Composition of cellular infiltrate was also represented mostly by segmented neutrophils which included a small number of lymphocytes and monocyte cells, but inflammatory cell infiltration was less extensive (Fig. 6).

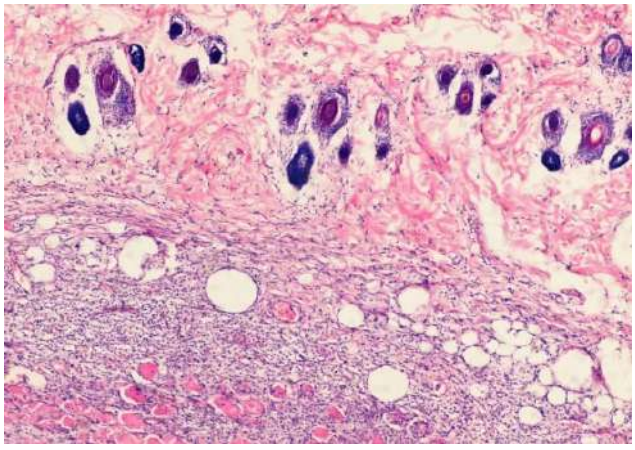

Figure 6. Microscopic changes in the skin of the animal from experimental group 2 on day 3 after thermal injury modeling. Dystrophic changes of skin appendages, spread of inflammatory infiltrate to the hypodermis, dermal edema. Hematoxylin-eosin staining, x100.

At longer thermal exposure far less pathological changes in the damaged region were found in animals of experimental groups 4 and 5. Microscopy showed isolated foci of inflammation. Inflammatory infiltrate consisted mostly of lymphocytes, mononuclear cells such as blood monocytes, and few segmented leukocytes. There were mild hyperemia, edema of the hypodermis and reticular dermis especially in the area of inflammation, isolated bundles of collagen fibers with the signs of mild edema, splitting, fragmentation of fibers. Skin appendages showed minor dystrophic changes (Fig. 7).

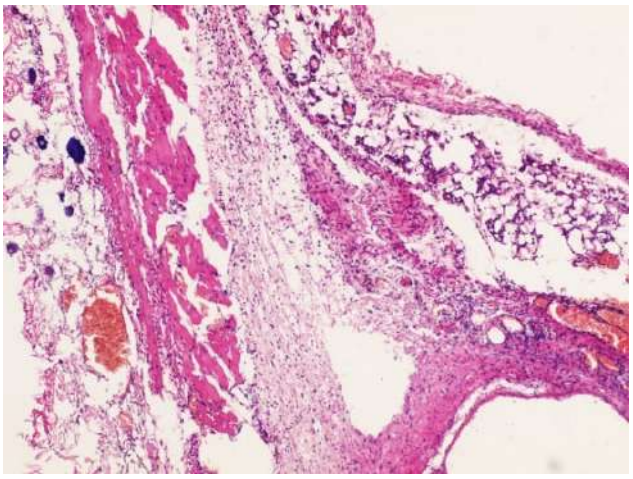

Figure 7. Microscopic changes in the skin of the animal from experimental group 5 on day 3 after thermal injury modeling. Skin appendages with minor dystrophic changes, moderate diffuse inflammatory infiltration, congestion and hyperemia. Hematoxylin-eosin staining, x100.

On day 7 post-injury, initial proliferative changes were registered in animals of experimental group 1 and the control group, namely the development of young epithelium at the edges of the wound. There was the growth of its epithelial layer on granulation tissue, covering only a quarter of the wound surface. Moderate inflammatory changes in skin tissues persisted reaching the subcutaneous layer. The initial stage of granulation tissue development at the site of dead tissue was revealed, its main components being vertical vascular loops and leukocyte-necrotic zone. The formation of new microvessels was observed along with increase in number of capillary endothelial cells (angioblasts), which first formed cellular cords and later were involved in formation of vascular tubes to receive blood. Blood corpuscles and edematous fluid were discharged from newly formed vessels into surrounding tissues. New fibroblasts were identified in inflammatory cells. Reactive inflammatory infiltration was observed in the hypodermis. Composition of cellular infiltrate was represented mostly by lymphocytes with a small number of segmented leukocytes and monocyte lineage cells. Vascular response was characterized by persistent moderate hyperemia, edema of the hypodermis and reticular dermis in inflammation region. Isolated bundles of collagen fibers in dense regular connective tissue of the dermis with the signs of edema, splitting, fragmentation of fibers were also visualized (Fig. 8).

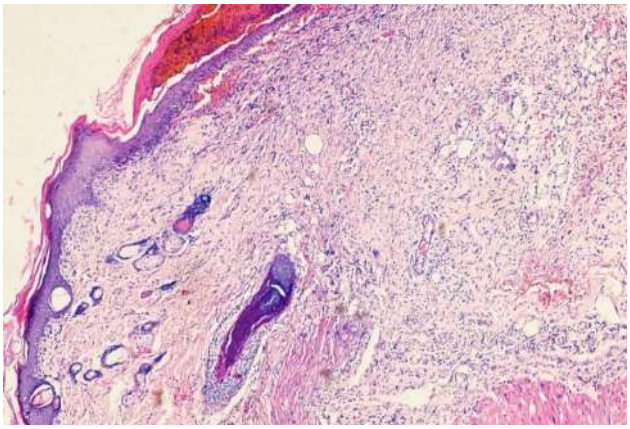

Figure 8. Microscopic changes in the skin of the animal from the control group on day 7 after thermal injury modeling. Moderate inflammatory cell infiltration, proliferation of new stratified squamous epithelium at the edges of the burn wound. Hematoxylin-eosin staining, x100.

At that time, the histological material from animals of experimental groups 2 and 3 exhibited more evident proliferative changes - growth of young epithelium on granulation tissue covering 2/3 of the wound surface area. Mild inflammatory changes of skin tissues persisted reaching the subcutaneous layer. Granulation tissue was developing at the site of dead tissue. Besides, increased number of new fibroblasts (formation of new scar tissue) was observed (Fig. 9).

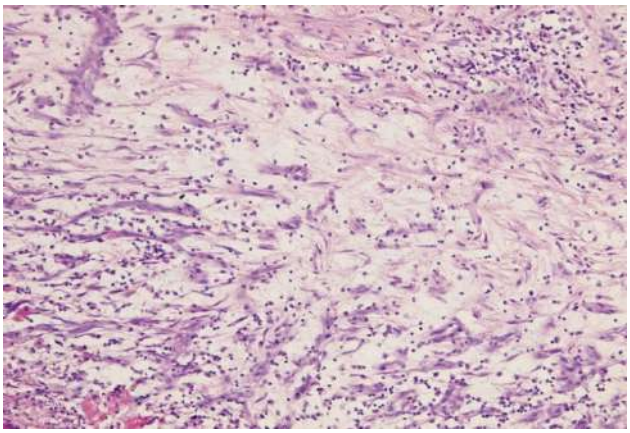

Figure 9. Microscopic changes in the skin of the animal from experimental group 2 on day 7 after thermal injury modeling. Moderate inflammatory infiltration, dermal edema, increased number of new fibroblasts. Hematoxylin-eosin staining, x 200.

In rats of experimental groups 4 and 5, the healing of burn wound with no evident signs of inflammation was registered on day 7 of observation. In the zone of previous inflammation, young connective tissue scar covered with epithelium was formed. A layer of regular fibrous tissue was revealed under the scar tissue. Scar tissue partially replaced the structural elements of the skin. Isolated dermal papillae, hair follicles, sebaceous and sweat glands were visualized. Scar tissue consisted of a large number of fibrocytes and collagen fibers. There was reduction of blood vessels and closure of their lumen in scar tissue region. Thin layer of hypodermis was observed under the fibrous tissue. However, in some cases, a small number of lymphohistiocytic elements were still detected in the scar region. In the hypodermis, there were mild signs of microhemocirculation disorder in the form of moderate hyperemia and isolated focal inflammation zones (Fig. 10).

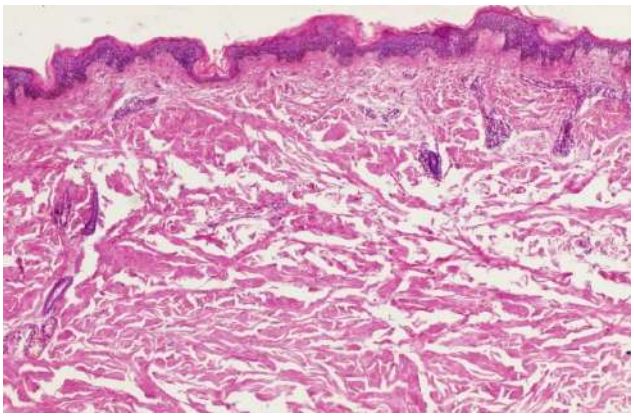

Figure 10. Microscopic changes in the skin of the animal from experimental group 5 on day 7 after thermal injury modeling. Scarring of the dermis and skin appendages, the appearance of stratified squamous epithelium on the scar surface. Hematoxylin-eosin staining, x100.

Histological investigation of thermal injury tissues from animals of experimental groups 1, 2, 3 and the control group on day 14 post-injury demonstrated burn wound healing. In the area of previous inflammation, young cellular connective tissue scar was formed with the signs of epithelialization. A layer of regular fibrous tissue was seen under the scar tissue. Scar tissue was partially replaced by structural elements of intact skin. Dermal papillae, hair follicles, sebaceous and sweat glands were still absent. Scar tissue consisted of a large number of young fibroblasts, fibrocytes and collagen fibers. There was reduction of blood vessels and remodeling of the scar. A thin layer of hypodermis with mild lymphohistiocytic infiltration was defined under the fibrous tissue of animals from the control group (Fig. 11).

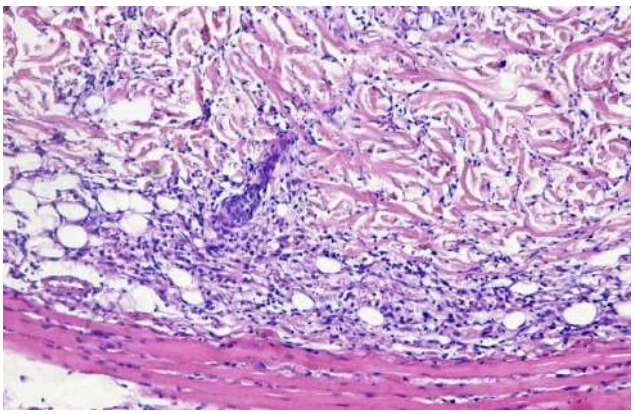

Figure 11. Microscopic changes in the skin of the animal from the control group on day 14 after thermal injury modeling. Scarring of the dermis with no skin appendages, hypodermis with mild lymphohistiocytic infiltration. Hematoxylin-eosin staining, x 200.

In animals of experimental groups 4 and 5, histological specimens exhibited the healing of burn wound on day 14 after thermal injury evidenced by formation of connective tissue scar with no signs of inflammatory reaction. A layer of regular fibrous tissue was determined under the scar tissue. There were isolated hair follicles, sebaceous and sweat glands. Scar tissue consisted of a large number of fibrocytes and collagen fibers (Fig. 12).

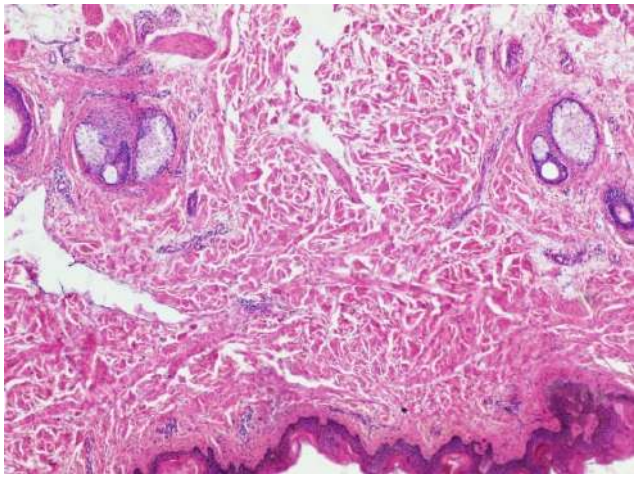

Figure 12. Microscopic changes in the skin of the animal from experimental group 5 on day 14 after thermal injury modeling. Epithelialized scar formation, developed skin appendages with no signs of inflammation. Hematoxylin-eosin staining, x100.

Conclusion

The results of obtained morphological studies of tissues after burn injury have confirmed the beneficial effect of rapid neutralization of traumatic action of hyperthermic exo-, endogenous damage factors as the major elements of burn depth and necrotic tissue formation.

Characteristic microscopic changes of the skin after thermal injury modeling are consistent with clinical and laboratory data obtained in previous studies emphasizing the significance of prompt neutralization of traumatic effect of hyperthermic exo-, endogenous damage factors. The procedure should begin immediately after the injury using room temperature water and continue until disappearance of pain in the wounds.

Conflict of interest

The authors declares that there is no conflict of interest.

Acknowledgment

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