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STRUCTURAL DYNAMICS OF SKIN REGENERATION AFTER THERMAL BURNS IN CONTROLLED WATER ENVIRONMENT (EXPERIMENTAL STUDY)

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Abstract
The purpose of the study was to determine the structural dynamics of healing and skin regeneration characteristics after thermal burns in laboratory rodents in an artificial local regeneration environment - the burn surface is protected by the dome of the device filled with regularly replaced sterile isotonic saline solution. The article presents characteristics of the inflammatory response reaction to steam burns and subsequent skin regeneration in the following comparative aspect: during natural healing under the eschar and in case of constant contact of the burn surface with solutions. Scientific novelty consists in the study of changes in the inflammatory and regenerative response depending on the state of state of aggregation of matter interacting with a wound. It was concluded that secondary skin alteration and fibrous changes in water environment are much lower with considerably higher degree of regeneration. These data can serve as the basis for new regenerative technologies in combustiology.

Keywords: burns, skin, regeneration, local regeneration medium, scarless wound healing, liquid wound healing environment, combustiology.

INTRODUCTION

Optimization of burn wound healing remains an urgent problem in medicine. One of approaches, used to increase the treatment efficiency, was arrangement of favourable local conditions for skin regeneration. It includes tissue protection, infection prevention and control, adsorption and removal of toxic wound fluid, normalization of microcirculation and other measures. Some studies have demonstrated, that to achieve skin restitution, it is necessary to control cellular regeneration sources, monitor their composition and intercellular matrix properties, as well as the level of cytokines (authors). These works have become a prerequisite for our development of a unified biotechnological concept of skin regeneration after burns.

The skin protects the body from aggressive environmental effects. It was assumed that skin protective function in case of its damage can be performed by the intermediate medium, close to the intercellular fluid. Therefore, in the 19th century there were reports of attempts to treat extensive burns by immersing patients in a bath with water solutions (Hebra F.V., 1861; Thiersch S., 1886). During World War II, English surgeons used seawater to treat wounds and burns (Bunyan J., 1941). The first experimental studies showed, that in a controlled water environment, wound epithelialization accelerates, skin regeneration degree increases, fibrosis severity decreases (Ivanishchuk P.P. et al., 1990; Kovalev A.V. et al., 1991; Vranckx JJ, et al., 2004; Svensjo T., et al., 2006; Eriksson E., et al., 2007). At the same time, the structural dynamics of skin regeneration after burns in a controlled water environment remains unstudied. Development of cellular technologies and skin tissue engineering require research on the development of special techniques that contribute to the optimal preparation of a burn wound for biotechnological methods of organotypic skin regeneration. Due to the lack of skin donor resources in case of extensive burns, these studies are becoming especially important. In this regard, the purpose of our study is to analyse structural features of skin reparative regeneration in the experiment after thermal steam burns in a controlled water environment.

MATERIALS AND RESEARCH METHODS

To achieve this purpose, a special chamber was developed in the form of a transparent container, tightly attached to the intact skin surface around the burn. The controlled water environment was represented by a sterile apyrogenic isotonic solution of sodium chloride that was introduced into the container through fittings in a volume of 1.5 ml. The chamber was protected from damage by the animal by a metal casing. The solution was changed 4 times a day, providing a constant water environment near the burn.

The animals were divided into the main and control groups. The main group consisted of 60 rats, their skin regenerated in the water environment. The control group included 60 rats, their wound healing occurred under the eschar without water environment. The similar non-tight devices, filled with

circulating natural air, were attached on these animals. The experiments were performed on 120 hooded rats of mature reproductive age of "August" line. The study was performed in compliance with current "Regulations for works using experimental animals" (order of the Ministry of Higher Education of November 13, 1984, No. 724). Standard burns were inflicted with steam onto the back, using the metering device, developed by us. The burn had a rounded shape, 1 cm in diameter with the lesion, corresponding to a third-degree burn. The native and histological specimens of the regenerating skin at the burn site at different observation periods re considered as the main study object.

Detailed morphological studies of the skin in animals of the main and control groups were performed on days 1, 2, 3 (every 6 hours), and then in 5, 10, 14, 30, 60 days. Using light (MBI-15 microscope), electron scanning microscopy (Hitachi device) and morphometry of histological specimens on VideoTest digital image analyzer (St. Petersburg), the structural dynamics of regenerating skin was studied. The specimens were stained with hematoxylin-eosin, as well as according to the methods of Shueninov, Mallory, Weyert and Tenzer-Unna. At this, the parameters of newly formed epidermis and granulation tissue, thickness and length of the growing epithelial wedge were determined. For cytomorphological studies of granulation tissue and skin regenerate, semi-thin sections were prepared according to the method of V.E.Sokolov. The quantitative composition of various populations of skin regenerate cells were evaluated on the specimens. The morphological criteria, determined for cell identification at the light-optical level were considered for cell counting (Yurina N.A. et al., 1990). The quantitative assessment of the microvasculature in the regenerate was performed according to the total length of capillaries by the method of S. M. Blinkov (1961). To determine parameters of the regenerate cell synthetic activity, luminescence microscopy - microspectrophotometry according to the method of A. Karnaukhova (1984) was used. The study of the skin regenerate surface microrelief and its fibre frame was performed by scanning electron microscopy of native preparations (Karaganov Ya.M. et al., 1986). Scanning electron micrographs were used for evaluation of the orientation index of connective tissue fibres in skin regenerates. Parametric and non-parametric methods were used for statistical processing of quantitative data.

STUDY RESULTS AND DISCUSSION

In 6 hours after the burn, the control group rats in the lesion focus swell and homogenization of the collagen fibres of the papillary dermis are observed. The fibres are oxyphilic. The epidermis is thinned, isolated sloughing is observed. Epithelial cells of the epidermis basal layer and hair follicles in a state of necrobiosis look as follows: elongated, vacuolated, with karyopiknosis and karyorhexis phenomena. The reticular layer and subcutaneous tissue swelling is observed. A pronounced mixed vascular congestion, marginalization of neutrophils in venules is observed. Stasis is observed in capillaries. During the first day, swelling increases, active diapedesis of neutrophils are present. By the end of the first day, the subcutaneous tissue is infiltrated by a large number of neutrophils, a moderate number of monocytes and a few fibroblastic cells. Tissue basophiles and lymphocytes are present in perivascular zones. The following necrotic processes increase in the epidermis: basal membrane destruction, cell karyolysis, epithelial cells lose their shape and merge occasionally. In animals of the main group necrotic changes are less pronounced at the same time. The depth of necrosis by the end of the first day was much less than in the control group ($p < 0.05$). The basal membrane of the epidermis is preserved on a larger area. Cell identification sites alternate with homogeneous areas with oxyphilic colouration. The nuclei of epithelial cells are hyperchromic and pycnotic. Epithelial cells maintain the normal structure and shape of the nucleus in deep-lying hair follicles. Swelling is expressed as in the control group. At the same time, the connective tissue infiltration, as compared to the control group, looks less dense, the cells are located fairly evenly, not forming clusters. In this case, neutrophils predominate On the second day after burn in the control animals, epidermal cells do not contour. The necrotic zone is separated from living tissues by the torus demarcationis, located in the reticular layer below the border of hair follicles. The necrosis depth, as compared with the first days of observation, increases and secondary necrosis forms. The torus demarcationis in control animals starts to form in 30 hours after the burn. Accumulations of neutrophils and macrophages are observed first along the burn periphery, then the torus demarcation is moves to its centre. The edge epithelialization of the wound begins in the form of migrating elongated epidermal cells wedging under the necrotic zone. With secondary necrosis formation and its delimitation, young connective tissue regeneration is in progress. On the second day, it consists of a leukocyte-necrotic layer with active neutrophils. There is an accumulation of fibroblast-like cells and macrophages that phagocytise dead tissues under this layer. The underlying skin layers are infiltrated with neutrophils, macrophages and fibroblasts.

In the main group animals, on the second day basal membrane traces are preserved, basal layer epithelial cells are contoured. The necrosis depth is considerably less, as compared to the control group ($p < 0.001$). Living tissues are infiltrated with leukocytes, but macrophages and fibroblasts are present in less quantity, as compared to the control group. The pronounced vascular congestion with adhesion of leukocytes to their inner surface is observed. Unlike the control group, the torus demarcationis is not formed yet. It starts to form later, 54 hours after the burn. The torus demarcationis contains mainly neutrophils and a small number of macrophages. During this period, the wound epithelialization starts. The epidermis in control group rats in three days from the experiment start looks completely homogeneous. Its basal membrane is not traceable along its entire length. The torus demarcationis is fully formed and delimits viable tissues from the necrotic zone. Neutrophils in the torus are partially destructed. Macrophages with pronounced phagocytosis of neutrophils predominate. With connective tissue regeneration, necrotized tissues gradually dry and eschar forms. Between the eschar and preserved dermis structures epithelial wedge grows along wound edges. Further development of newly formed connective tissue is observed. Compared with the second day, the number of macrophages and fibroblast cells increases. Macrophages are located directly under the leukocyte-necrotic layer. There are fusiform or stellate fibroblasts with large bright nuclei in the subcutaneous connective tissue re observed. Thin collagen and single elastic fibres are observed between granulation tissue cells. The number of newly formed capillaries increases. In the main group of animals, three days later, epithelial cells become elongated and get clearly contoured in intact parts of hair follicles. Further increase in necrosis, but to a lesser extent, compared to control group ($p < 0.05$). Under the necrotic tissue, along wound edges, the epithelial wedge

continues to grow in. The necrotic zone is separated from living tissues by the torus demarcationis, consisting mainly of neutrophils. The torus is located on the border of papillary and reticular layers, partially affecting hair follicles. Due to uniform neutrophil infiltration, the torus demarcationis does not have clear boundaries. However, it is less thicker than in control group ($p < 0.05$). The subcutaneous tissue remains edematous and contains neutrophils, macrophages, and fibroblastic cells, but in a smaller amount, compared to the control group. Collagen and elastic fibres are formed in the intercellular substance of the regenerating connective tissue. But the number of newly formed capillaries remains minimal.

Five days later, in control group rats, eschar rejection begins over in growing epithelium. There are wide gaps, filled with a neutrophil-free liquid between eschar and underlying granulation tissue. The newly formed connective tissue is represented by oppositely directed capillaries, large macrophages with fabaceous nuclei and collagen fibres, located parallel to the skin surface with fusiform fibroblasts are located. Compared to the previous observation period, thickening of collagen fibres continues. The amount of elastic fibres increases mainly in deep-lying layers of connective tissue regenerate. The regenerate vessels remain full-blooded.

In the main group animals, during 5 days in the water environment, the eschar was absent. After capsule has been removed, the eschar formed within a few hours. Neutrophils, large macrophages and stellate fibroblasts without specific orientation are present in the regenerating connective tissue. Pale-coloured fibre structures re located between them. The number of elastic fibres, as compared to the previous observation period, grows. The newly formed connective tissue on the histological thickness and area on the histological section is significantly less than in the control group ($p <0.001$). In ten days after the burn, epitheliazation of the defect zone is observed in the control group. The epidermis is hypertrophied, its basal layer is loosely connected to the basal membrane. The eschar connection with underlying tissues is preserved only in the centre of the wound. The connective tissue regenerate contains a large number of vessels in the centre, surrounded by many fusiform fibroblasts. In some cells, mitoses at the anaphase stage are clearly distinguishable. To the regenerate periphery, the number of vessels decreases, arrangement of fibroblasts takes a pronounced orientation parallel to the skin surface. In deeper layers, collagen fibres thicken and become brighter. The number of elastic fibres also increases. In the main group rats, after ten days, the eschar is completely absent. The defect epitheliazation, similarly to the control group, is not complete. However, the epidermis is less hypertrophied. The following cellular elements predominate in the connective tissue regenerate: in larger numbers - fusiform fibroblasts, oriented parallel to the skin surface; in smaller numbers - macrophages and other cell populations. The intercellular substance is less structured than in the control group, and the number of connective tissue fibres is increased in the regenerate lower part. Thickness of collagen fibres, compared to the fifth day of observation, continues to increase. The number of elastic fibres also increases. There is less vessels s compared to the control group, most of them do not contain red blood cells. Accumulations of adipose tissue are higher in number. Thickness and area of the connective tissue regenerate is less than that of the control group ($p < 0.001$).

After fourteen days of observation in the control group animals, a completely epitheliazed regenerate is formed. The epidermis in the damaged area is hypertrophied and contains approximately 9 layers of cells. Epitheliocytes are vacuolated and loosely located on the basal membrane. Under the epidermis, connective tissue is represented by many fusiform fibroblasts with large nuclei and basophilic cytoplasm, oriented parallel to the skin surface. The specific area of cellular elements, measured using computer morphometry, is 18.6%. The intercellular substance between them is dense and consists mainly of pale-coloured fibrous structures parallel to the skin surface. The specific area of collagen fibres is 48.9%. Subepidermally, small areas of the main amorphous substance between collagen fibres are observed. The specific area of the main amorphous substance is 32.5%. In deep layers of the regenerate, randomly located fibroblasts are stellate in shape. Collagen fibres in these areas are brighter and form thick bundles. Isolated elastic fibres are present. The hair follicles, present along the regenerate edge are hypertrophied. Root sheaths have 5-6 cell layers. As compared to the tenth day of observation, the number of capillaries decreases. There are pericyte chains and a small number of macrophages.

In the main group animals, after fourteen days, the regenerate is also completely epitheliazed. The epidermis and hair follicles in the regenerate are hypertrophied. Fibrous tissue is represented by fusiform and elongated fibroblasts parallel to the skin surface. Cellular elements account for 16% of the total regenerate area. The intercellular space is filled with fibrous elements and the main amorphous substance. Collagen fibres prevail over amorphous substance, their specific area is 46.5%. In total, amorphous intercellular substance and vascular lumen occupy 37.5% of the regenerate area. The number of capillaries is reduced, but vascularization of the regenerate zone is higher than in the control group. In general, connective tissue regenerate has a smaller area and thickness compared to the control ($p < 0.001$).

Thirty days later, the control group animals had mature fibrous tissue, covered with hypertrophic epidermis. It is represented by tightly packed bundles of hyalinized collagen fibres. The orientation index of connective tissue fibres is 83.4 3.6%, the specific area is 65.3%. Dendritic fibroblasts and fusiform fibrocytes are located between fibres. The specific area of cellular elements is 15.9%. Small areas of amorphous substance are present in the upper layers of fibrous tissue, its specific area is 18.8%. Isolated elastic fibres are present. The regenerate blood stream is poorly developed. Sebaceous glands are absent. Isolated hair follicles are present only along the regenerate edge. Thickness of root sheaths is higher than in intact skin. In main group animals, after thirty days, the epidermis regenerate is less hypertrophied, as compared to the control group ($p < 0.001$). The bundles of collagen fibres are characterized by a lower packing density and a lower degree of order. The orientation index of fibres, perpendicular to the epidermis is 53.6, 3.5%, parallel - 46.4, 3.7%. The specific area of connective tissue fibres is 43%. A considerable amount of basic amorphous substance and cellular elements, represented mainly by fibroblasts and fibrocytes, is present between fibres. The specific area of cells is 16.8%, the main amorphous intercellular substance - 40.2%. Elastic fibres in the upper layers are isolated, their number increases in the regenerate depth. Transverse and longitudinal capillaries with red blood cells are present. Hair follicles and sebaceous glands re-present in the regenerate. Thickness of root sheaths is less than in the control group ($p < 0.001$) and does not differ from that in the intact skin. Parameters of the regenerate area and thickness are less than

in the control group animals ($p < 0.001$).

After sixty days, a regenerate was formed in the main group, approaching to normal skin by its structure. At the same time, in the control group animals, the regenerate is a skin scar. Besides, the regenerate in the main group of animals has a much smaller area and thickness ($p < 00001$).

CONCLUSION

Using fluorescence microscopy and photospectrometry in the main group, we determined preservation of the cell synthetic activity on the boundary of papillary and reticular layers in the thermal effect zone during the entire observation period. It proves preservation of cell viability. In the control group, the lesion deepened, cell synthetic activity at this level was no longer observed in 6 hours after the burn. Therefore, the liquid environment promotes tissue survival in parabiosis zone. The inflammatory reaction degree is reduced by decrease in the activity of inflammation effectors and possible diffusion of low-molecular, as well as biologically active substances in a controlled water solution. These factors reduce development intensity and secondary necrosis depth (secondary alteration in inflammation).

In water environment, wound epitheliazation is accelerated, mainly due to elimination of obstacles for migration of epithelial cells from burnt tissues and increased synthetic activity of basal epithelial cells. It was determined that the volume of connective tissue neoplasms is reduced due to inhibition of fibroblastic reaction and a decrease in neoangiogenesis. This helps to preserve the organotypic structure of burned skin and development of a regenerate close to the normal skin structure.

In water environment, the restored skin is characterized by the developed vascular network due to weakened reduction of blood vessels at the stage of regenerate formation and reconstruction.

Therefore, in the water environment, a more complete skin restoration is possible with skin pattern formation and preservation of elasticity and mobility with respect to underlying tissues.

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