ISSN: 2476 - 2415

Research Article

International Journal of Clinical & Experimental Dermatology

Cell technology for experimental ulcerative lesions

Mavlyanova Shahnoza Zakirovna, Obidov Sardor Zafarovich

[1]*Dermatology, Head of the Experimental Scientific Laboratory, Head of the Scientific Group Dermatology*

[2]*Basic Doctoral Student*

***Corresponding author**
Mavlyanova Shahnoza Zakirovna, 1Dermatology, Head of the Experimental Scientific Laboratory, Head of the Scientific Group Dermatology

Submitted: 30 Jan 2021; **Accepted**: 04 Feb 2021; **Published**: 15 Feb 2021

Citation: *Mavlyanova Shahnoza Zakirovna (2021) Cell technology for experimental ulcerative lesions. International Journal of Clinical & Experimental Dermatology 6(1): 1-4.*

Abstract

The article presents experimental studies on the use of cell technology in experimental ulcerative pyoderma. The results of the study showed that the introduction of embryonic cultured allofibroblasts in the treatment of experimental ulcerative pyoderma is associated with the activation of fibroblasts in the dermis and promotes significant epithelization of ulcerative lesions on the skin in experimental animals.

Lately, in dermatological practice, there has been an increase in the frequency of chronic, long-term non-healing ulcerative foci of the skin and subcutaneous tissue, which make up a pressing issue in terms of choosing the appropriate therapy. Etiological factors in the development of ulcerative foci of skin lesions are endocrinopathy (primarily diabetes mellitus, obesity), disorders of the vascular system, as well as the nervous system.

Keywords: Ulcerative Pyoderma, Cell Technology, Fibroblasts, Pathomorphology

Such an effective and safe method for the treatment of TU is the use of fibroblast cultures. Currently, there is a large number of studies of their use in the treatment of burns, the main goal of which is the rapid closure of the lesion with minimal scar formation [1-3]. When used in medical practice, fibroblasts are divided into autologous (own) and allogeneic (obtained from another organism). A significant acceleration of epithelialization after fibroblast transplantation is associated with the fact that these cells release components of the extracellular matrix (type 1, 2, 3 and 4 collagens, laminin, nidogen, fibronectin, etc.) and growth factors - regulatory peptides with the ability to activate cell proliferation. The latter include, for example, FGF-β - the main growth factor of cell fibroblasts, stimulating the proliferation of all types of cells in the wound and the production of components of the extracellular matrix by them [4].

The aim of the research was to assess the effectiveness of fibroblasts in the treatment of experimental ulcerative pyoderma.

Research materials and methods

The experiments were carried out on 30 white mongrel rats weighing 180-200 g. with ulcerative pyoderma. To assess the cell technology, the animals were divided into groups: Group I - healthy animals, Group II (control) - with ulcerative lesions receiving no treatment, Group III - rats with ulcerative lesions, which underwent cell therapy with an embryonic allofibroblasts culture.

Ulcerative lesions of the skin in rats were induced by the one-time subcutaneous application of 0.7 ml solution of 70 ° acetic acid on the inner surface of the thigh [5].

The progress of ulcer healing was assessed by measuring its maximum size, which was determined by applying a sterile transparent film to the wound. The contour of the lesion was outlined with a marker, after that the film was laid over a sheet of linear graph paper, and the area of the ulcerous surface was calculated.

Method for obtaining allofibroblasts

In our studies, we used a developed method for treating burns and wounds (Urazmetova M.D. 1998), which includes obtaining a primary culture of allogeneic fibroblasts from embryonic tissues with subsequent application of a cell suspension to the wound surface.

Primary culture of fibroblasts was obtained by fermenting rat embryonic tissues, subjecting fibroblasts to repeated subculturing in RPMI or 199 culture medium containing 10-15% fetal bovine serum, 2% α-glutamine, Hepes medium and antibiotics. The last subculturing was performed in Heraeus dishes. The final product was a suspension of cultured allogeneic fibroblasts in a culture medium, with cell concentration of up to 200,000-300,000 per ml of culture media, with a high percentage of living fibroblasts (up to 90% of cells). The use of this technique for culturing fibroblasts has the following advantages:

1. obtaining fibroblasts does not require expensive nutrient media, growth stimulants, which reduces the cost of cultivation;
2. fibroblasts are easily passaged, which makes it possible to obtain a large volume of cell cultures in a short time.

Cell therapy using fibroblasts was carried out by one-time injection of fibroblasts into the lesion at a dose of 3,000,000 cells in 1 ml.

To transplant allofibroblasts onto the wound, we used disposable syringes (5, 10 ml) with a puncture needle. The use of this needle made it possible to achieve a more even distribution of the suspension on the wound surface. After transplantation of allofibroblasts onto the wound, we waited for 15-20 minutes to ensure fixation of the transplanted fibroblasts. Then the wound was covered with a napkin, moistened abundantly with saline and antibiotic solution (80 mg of gentamicin was added to 500 ml of saline). A retentive gauze bandage was applied.

To objectify the results of treatment, in order to control the healing of ulcers, clinical histological and microbiological examination methods were carried out.

Clinical judgement of the effectiveness of treatment was carried out on the basis of visual observation of the course of the wound process, with particular attention paid to the presence of pyoinflammatory phenomena, the nature of granulations and the timing of epithelialization.

Microbiological examination was carried out by examining a biopsy specimen from the lesion before the introduction of fibroblast cells on the 7th, 14th days of experimental observation. The material was collected on aseptic basis. Material taken with one of the swabs was used to prepare a Gram smear. The microscopy-based assessment of the qualitative composition of the wound microflora includes the findings of microscopic and bacteriological studies [6].

Histological examination was carried out on the marginal area of the ulcerative focus on the 3rd, 7th and 14th day of experimental observation after applying allofibroblasts to the wound.

Statistical processing of the data obtained was carried out using the Student's t-test using the Microsoft Office Excel and Biostatistics 4.03 programs. The criterion for statistical significance was $p < 0.05$.

Results and their interpretation

The experimental TU after the injection of the toxin was represented by the formation of an ulcerative focus of 1.5x2 and 2x3 cm diameter on average. On the 2nd day of the experimental study, 8 out of 10 rats developed serous crusts in the ulcerative foci. On the 3rd day of the study, the lesion was characterized by severe hyperemia and infiltration with exudative-purulent discharge. On the 4th day of observation, the group III rats were injected with allofibroblast cells in the lesion foci in an appropriate dose of 1 ml [7].

Table: Comparative characteristics of the ulcerative focus epithelialization against the background of the cell technology introduction (in days)

Group	Ulcer formation	Development of skin crust	Epithelialization			Resorption of the purulent-inflammatory process
			Marginal	Central	Full	
Group II (control) N=10	2,9±0,06	19,8±0,4	24,3±0,4		30,3±0,8	20,1±0,1
Group III N=10	2,9±0,06	3,8±0,1*	3,9±0,1*	5,4±0,3	17,6±0,8*	13,9±0,2*

Note: * - statistical significance index compared to the control group (P <0,05).

Histological studies of skin biopsies from lesions in animals were carried out before and after cell technology introduction. (fig. 1, 2, 3).

Pathomorphological studies of the skin of laboratory rats after a trophic ulcer experimental induction on the 1st day showed the following: on the lesion focus, the horny layer is absent, destruction and necrotic changes are observed in the epidermis: the granular layer of the epidermis is destructed, its nuclei are disrupted, the boundaries between the cells are not visible; there are pronounced phenomena of vacuolar degeneration and spongiosis in the spinous and basal layers. In some areas, the epidermis is absent [8]. In some places, the dermis is covered with a serous-hemorrhagic crust. In the papillary layer of the dermis, there is pronounced edema, the vessels are dilated, surrounded with perivascular lymphohistiocytic infiltrates with a large amount of mainly granulocytic leukocytes and fibroblasts. There are also foci of extravasates. Collagen fibers are edematic, loosened and frayed. Skin appendages: there are destruction and necrosis of some hair follicles, surrounded with similar lymphohistiocytic infiltration is also noted.

Thus, the study of morphological changes in the skin 1 day after the chemical exposure to acid (under Falco's method) indicates a trophic ulcer. (fig. 1).

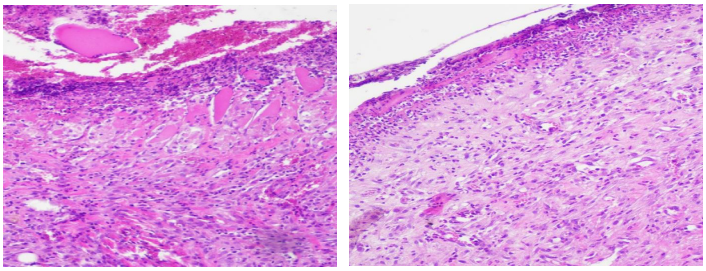

Figure 1: Skin biopsy from the ulcerative foci in white rats on the first day of ulceration (staining with hematoxylin and eosin, 40-fold increase)

Pathomorphological studies of the skin of laboratory rats after a trophic ulcer experimental induction on the 7-th day showed the following: against the background of treatment with cell technology introduction, in some places, the lesion focus is characterized by the absence of horny layer and orthokeratosis. In some preparations, a sweeping crust is visible and underneath it, there are areas of epithelial cells growth. In one of the animals, a layer of squamous epithelial cells is well visualized, which is located under the serous-hemorrhagic crust and is separated from it by a layer of infiltration. In the areas of the preserved epidermis, the epidermis is not firmly connected to the dermis; a layer of loosely located collagen fibers is determined subepithelially, imitating the papillary layer of the skin. The epidermis is less acanthotic, the granular layer is thinned, vacuolar dystrophy and spongiosis persist in the spinous layer, but are less pronounced than in the control groups.

The border of the basal layer is mostly restored, in some areas cell exocytosis and vacuolization are observed. In the papillary layer of the dermis, there are edema and sometimes fibrosis of collagen fibers. Inflammatory infiltration is somewhat less pronounced: the number of lymphocytes has decreased; while the cellular response from mononuclear cells and fibroblasts is enhanced. The histiocytic response increased compared to the control. The vessels extension and blood filling are preserved (fig. 2).

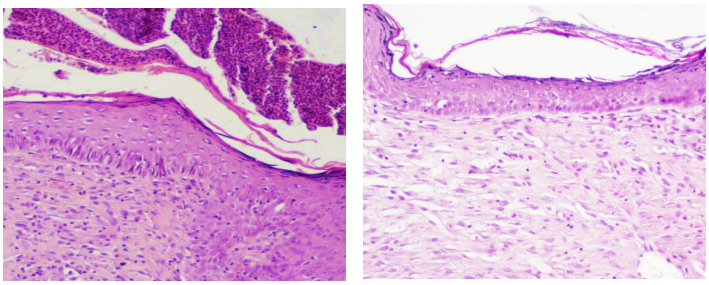

Figure 2: Skin biopsy from the ulcerative foci in white rats on the 7th day of ulceration (staining with hematoxylin and eosin, 40-fold increase)

Pathomorphological studies of the skin of laboratory rats after a trophic ulcer experimental induction on the 14-th day showed the following: against the background of treatment with cell technology introduction, the horny layer recovered in the lesion, in some places ortho-parakeratosis was noted. In the epidermis, all layers and structures completely recovered. Granular and spinous layers have no pathological phenomena. The basal layer is normal, the basal membrane is thickened in some places. Slight edema remains in the dermis, there are a plethora of blood vessels of the dermis and hypodermis, edema, which may indicate inflammation that has developed in the course of healing; inflammatory infiltration around the vessels and collagen fibers significantly reduced. The enhanced cellular response from mononuclear cells and fibroblasts is preserved. Collagen fibers are edematic and fibrously changed in some places due to the transferred inflammation. Skin appendages are almost absent, there are isolated remnants of hair follicles in the deep layers of the dermis. (fig. 3).

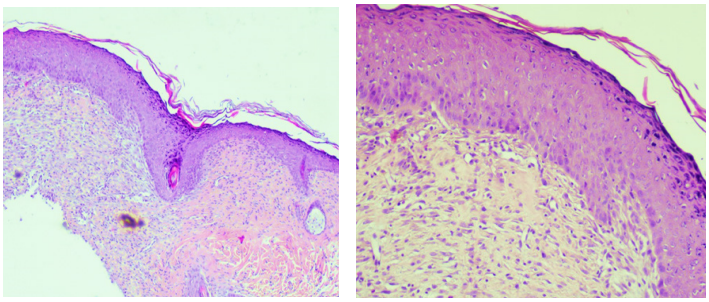

Figure 3: Skin biopsy from the ulcerative foci in white rats on the 14th day of ulceration (staining with hematoxylin and eosin, 10-fold increase)

Thus, our studies have shown that the introduction of embryonic cultured allofibroblasts in the treatment of experimental ulcerative pyoderma is characterized by the activation of fibroblasts in the dermis and promotes enhanced epithelialization of ulcerative skin lesions in experimental animals [9,10].

Trophic ulcers healed in all experimental animals; the healing time in animals of group III averaged 17.6 ± 0.8 days, while in the control (II) group it was 30.3 ± 0.8 observation days. The results obtained were statistically significant. ($P < 0.05$).

The use of cultured embryonic alloofibroblasts is an effective method for the treatment of ulcerative epidermis.

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